Investigating the molecular mechanisms of the interactions between *Lactobacillus reuteri* strains and intestinal mucus

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To

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Abstract

Mucus is the first point of contact between the gut microbiota and the host. Here we used the gut symbiont *Lactobacillus reuteri* to investigate the molecular mechanisms underlying the interactions between gut bacteria and mucus.

Firstly, the mucus binding ability of a collection of *L. reuteri* strains from different vertebrate hosts was assessed *in vitro* against mucus extracted from mouse and porcine gastrointestinal tracts. The adhesion profile was strain-specific showing the highest binding phenotype for strain ATCC 53608 (a pig isolate) and binding ability for a number of *L. reuteri* human isolates. Genome sequencing of the ATCC 53608 strain and comparative genomics was carried out to gain novel insights in the strain-specific determinants of *L. reuteri* adhesion to mucus.

The second part of this work investigated the occurrence at the genetic and protein level of two specific cell surface proteins, MUB and Lar_0958, expressed by the ATCC 53608 strain and human strains, respectively and their role in mediating mucus binding and autoaggregation ability of *L. reuteri* strains, as determined by flow-cytometry and *in vitro* mucus binding assays with recombinant and native proteins.

Finally a quantitative proteomic approach, stable isotope labelling with amino acids in cell culture (SILAC), was used to identify novel mucus binding protein candidates. This was achieved through detection of increased cell surface protein expression when *L. reuteri* was grown in presence of mucins, resulting in induced mucus binding phenotype. Collectively, these results shed new light on the nature and distribution of strain-specific surface proteins mediating *L. reuteri* adhesion to mucus.

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Preface

The present Ph.D. thesis entitled, “Investigating the molecular mechanisms of the interactions between *Lactobacillus reuteri* strains and mucus” summarises my work carried out under the supervision of Dr Nathalie Juge, under the Gut Health and Food Safety Institute Strategic Programme, at the Institute of Food Research, for the period of October 2008 to September 2012. This study was supported by the Biotechnology and Biological Sciences Research Council.

This work has resulted in the following publications:

MacKenzie D.A., **Jeffers F**., Parker M.L., Vibert-Vallet A., Bongaerts R.J., Roos S., Walter J., and Juge N. *Strain-specific diversity of mucus-binding proteins in the adhesion and aggregation properties of Lactobacillus reuteri*. Microbiology (2010) 156, 3368-3378.

Heavens D., Tailford L.E., Crossman L., **Jeffers F**., MacKenzie D.A., Caccamo M. and Juge N. *Genome sequence of a vertebrate gut symbiont Lactobacillus reuteri ATCC 53608*. Journal of Bacteriology (2011) 193, 4015-4016.

In addition, I am the co-author of several publications listed in Appendix 3.

Abbreviations

A_{280} = Absorbance measured at 280nm
ABC = adenosine-triphosphate binding cassette
AFM = atomic force microscopy
AgPAGE = agarose/polyacrylamide gel electrophoresis
AP = alkaline phosphatase
ATCC = American Type Culture Collection
BCA = bicinchoninic acid
BCIP/NBT = 5-bromo-4-chloro-3-indolyl phosphate/ nitroblue tetrazolium
BSA = bovine serum albumin
cF = carboxyfluorescein
cFDA = carboxyfluorescein diacetate
CnBP = collagen binding protein
ConA = concanavalin A
CPS = capsular polysaccharide
CSP = cell surface proteome
CWE = cell wall extract
CWP = cell wall proteome
DTT = dithiothreitol
EBI = European bioinformatics institute
ECM = extracellular matrix
EDTA = ethylenediaminetetraacetic acid
EF-Tu = elongation factor thermo unstable
EPS = extracellular polymeric substances
ER = endoplasmic reticulum
FCGBP = Fc gamma binding protein
FCM = flow cytometry
FDR = false discovery rate
FPLC = fast protein liquid chromatography
FS = forward scatter
GalNAc = N-acetyl galactosamine
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
GH = glycoside hydrolase
GI = gastrointestinal
GlcNAc = N-acetyl glucosamine
GuHCl = guanidine hydrochloride
HEWL = hen egg white lysozyme
H/L = heavy to light ratio
IAA = iodoacetamide
IBD = inflammatory bowel disease
IFR = Institute of Food Research
Ig = immunoglobulin
IgA = immunoglobulin A
IgG = immunoglobulin G
IgG-AP = immunoglobulin G – alkaline phosphatase
LC-MS = liquid chromatography – mass spectrometry
LDM = *Lactobacillus* defined medium
LDS = lithium dodecyl sulphate
LiCl = lithium chloride
LPS = lipopolysaccharide
LTA = lipoteichoic acids
LTL = *Lotus tetragonobolus* lectin
LysM = lysine motif
MALDI = matrix assisted laser desorption ionisation
MapA = mucus adhesion promoting protein
MCM = mouse colonic mucus
MCM-C = crude mouse colonic mucus
MDM-S = solubilised mouse colonic mucus

MRS = de Man, Rogosa and Sharpe
MS = mass spectrometry
Msa = mannose specific adhesin
MSIM = mouse small intestinal mucus
MUB = mucus binding protein
MubR5 = mucus binding protein repeat 5
MUB^{trunc} = truncated MUB
MUC = mucin gene in humans
Muc = mucin gene in mammals
MucBP = mucus binding protein domain
MurNAc = N-acetylmuramic acid
MW = molecular weight
MWCO = molecular weight cut off
NCBI = National centre for biotechnology information
OD_{600} = optical density measured at 600nm
PBS = phosphate buffered saline
PBST = phosphate buffered saline with tween
PCR = polymerase chain reaction
PG = peptidoglycan
PGM = porcine gastric mucin
PGMIII = type III porcine gastric mucin
PI = propidium iodide
PIS = preimmune serum
pNPP = p-nitrophenol phosphate
PSIM = pig small intestinal mucus
PVDF = polyvinylidene difluoride
RAPD = random amplification of polymorphic DNA
RAST = rapid annotations using subsystems technology
RT = room temperature
SCE = soluble cytoplasmic extract
SDP = sortase dependent proteins
SDS = sodium dodecyl sulphate
SDS-PAGE = sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SEM = scanning electron microscopy
SILAC = stable isotope labelling with amino acids in cell culture
S-layer = surface layer
SLU = Sveriges lantobruksuniversitet: Swedish University of Agricultural Sciences
SM = spent media
SNA = *Sambucus nigra* agglutinin
SP = secreted proteome
SPR = surface plasmon resonance
SPSS = statistical package for social sciences
SS = side scatter
SWGA = succinylated wheat germ agglutinin
TAE = Tris-base, acetic acid and EDTA
TEMED = tetramethylethylenediamine
TGAC = The Genome Analysis Centre
ToF = time of flight
UEA = *Ulex europaeus* agglutinin
UV = ultraviolet
WGA = wheat germ agglutinin
WHO = world health organisation
WTA = wall teichoic acids

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To the 11am coffee table crowd (you know who you are), thanks for the chats, cakes and kindness – you make the world a better place.

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1 Introduction

1.1 Physiology of the Vertebrate Gastrointestinal (GI) Tract

The vertebrate gastrointestinal (GI) tract has a large surface (~400 m^2 in human adults [1]) covered in mucus which is colonised by bacteria after birth/hatching. These mucosal surfaces become the focal point of host-bacterial interactions, providing not only the first line of defence against invading pathogens, but also a habitat for mutualistic bacteria [2]. There are seven classes of extant vertebrates, mammals, birds, reptiles, amphibians, jawless fish, cartilaginous fish and bony fish [3]. The GI tract of vertebrates can be grouped into three fundamental variations, ruminant, cecal or straight tube (also called simple gut). In ruminants, the stomach is enlarged and compartmentalised and acts as a fermentation vat, whereas in the cecal adaptation, there are blind sacs extending from the intestinal canal at the end of the small intestine/beginning of large intestine [4]. Both of the adaptations functionally delay the transit of fibrous food in order to expose it to microbial degradation for longer. Chickens, rats and mice show cecal adaptations (Figure 1.1). Birds have a relatively short midgut and hindgut with a straight colon and paired ceca with the highest cecal length to total intestinal length ratio, whereas in rodents the single cecum is large and highly developed [3]. For pigs and humans with their straight tube variation, a haustrated colon (pouches), enables maximal surface area exposure for water absorption (Figure 1.1).

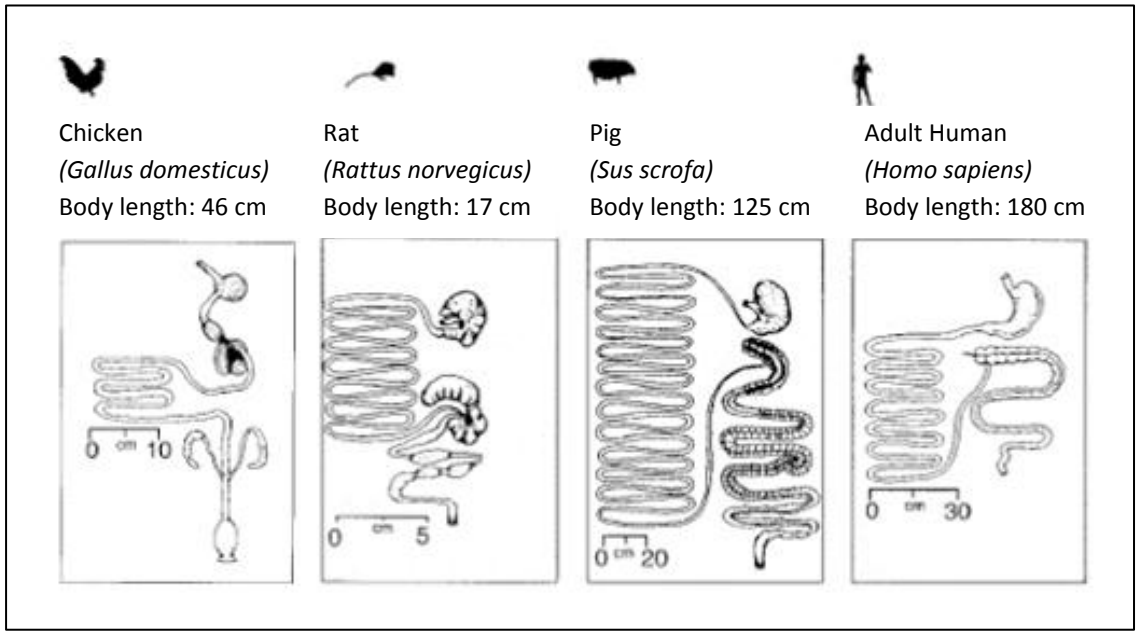


Figure 1.1 Anatomy of vertebrate GI tracts. Chicken and rat with cecal adaptations, pig and human with straight tube physiology. The colon of pigs and humans is haustrated throughout its entire length [3].

The anatomy of the human GI tract can be described in terms of foregut, (esophagus and stomach), midgut (small intestine, comprising of duodenum, jejunum and ileum) and hindgut (cecum and large intestine/colon) (Figure 1.2). The function of the foregut is to degrade large food particles, the midgut to absorb nutrients from the food and the hindgut to absorb water.

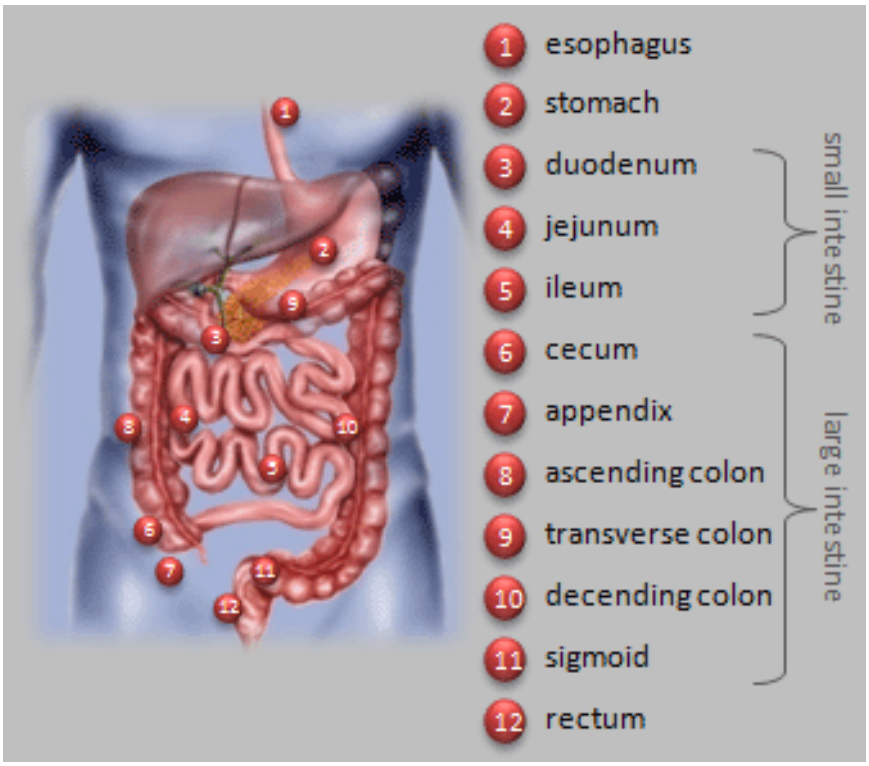


Figure 1.2 Anatomy of the human GI tract.

The GI tract is a highly dynamic environment including, food digestion, immune secretions (including mucus and anti-microbials), epithelial cell shedding, along with complex interactions between the host and the collection of resident microbes called the microbiota. However, some parameters remain relatively consistent such as body temperature, the normal range for the human body is 36.4 - 37.5 °C, while it is 37.5 – 38.5 °C in rats, 38 - 40 °C in pigs and 40.6 – 41.7 °C in chickens.

Other consistent parameters within the GI tract may be more regional, for example peristaltic movements, pH and microbial biomass (Figure 1.3). Peristaltic movements in the small intestine are continuous, creating a significantly different environment to the large intestine where transit time is greatly reduced. In the human stomach the pH is highly acidic (1.0-2.5), whereas it rises to 6.6 in the proximal intestine, and 7.5 in the terminal ileum, before a drop to 6.4 in the cecum, then rising progressively in the colon from right to left to 7.0 [5-7]. As a consequence, the microbial biomass is low (10^{2-3}

cells ml^{-1} of luminal contents) in the stomach, before rising in the small intestine to 10^{3-8} cells ml^{-1} with the highest concentration of microbes found in the colon with up to 10^{11}cells ml^{-1} (Figure 1.3) [8].

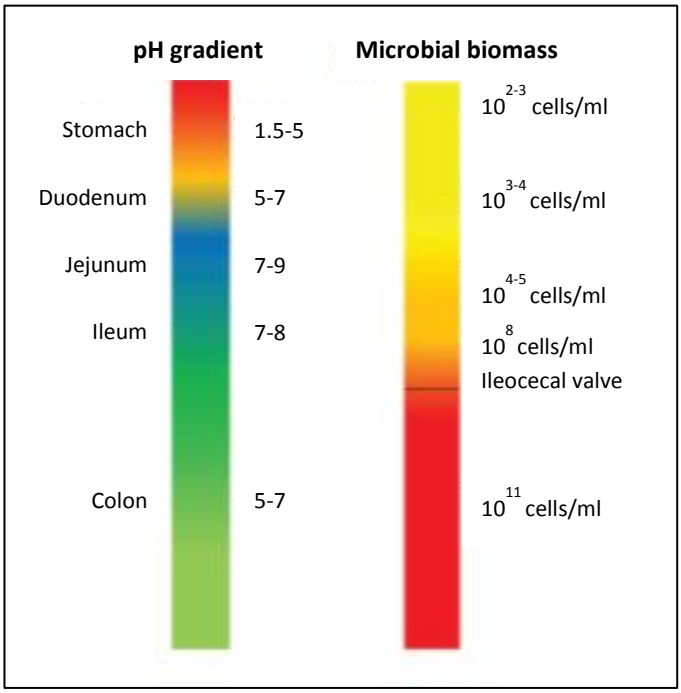


Figure 1.3 Schematic representation of pH and microbial biomass along the length of human GI tract [8].

1.2 **Mammalian GI Mucus**

The epithelium in the GI tract is covered by a layer of mucus which protects the host from the luminal contents and provides a habitat for the microbiota. Mucus is secreted by goblet cells which are one of the four main types of cells constituting the intestinal mucosal epithelium, the others being, absorptive enterocytes, Paneth cells and enteroendocrine cells. The proportion of goblet cells increases from 4 % in the duodenum to 16 % in the distal colon [9], correlating to the variation in mucus thickness along the length of the GI tract (Figure 1.4) [10]. Mucus plays an important role in the stomach (corpus and antrum) where it protects the underlying epithelium from harsh acidic digestion processes. However, the colon is the location of the greatest depth of mucus [10], which also corresponds to the highest number of permanent bacterial residents [11, 12]. Colonic mucus exists in two distinct layers, a firm and a loose layer, as shown in rats (Figure 1.4) [10] mice [13], and humans [14].

The firm mucus layer (50 µm thick in the mouse colon) is well organised, stratified in appearance and impenetrable to bacteria, thereby excluding direct contact between the gut microbiota and the colonic epithelium [15]. The loose mucus layer (100 µm thick in the mouse colon) which is easily aspirated and has a less defined outer border, is an expansion (4-5 fold in volume) of the firm layer due to endogenous proteolytic activity [13], and provides the habitat for the resident microbiota. The situation is thought to be equivalent in the human colon, however only the thickness of the firm mucus layer has been measured using histological methods with results varying from 70 to 155 µm [16, 17].

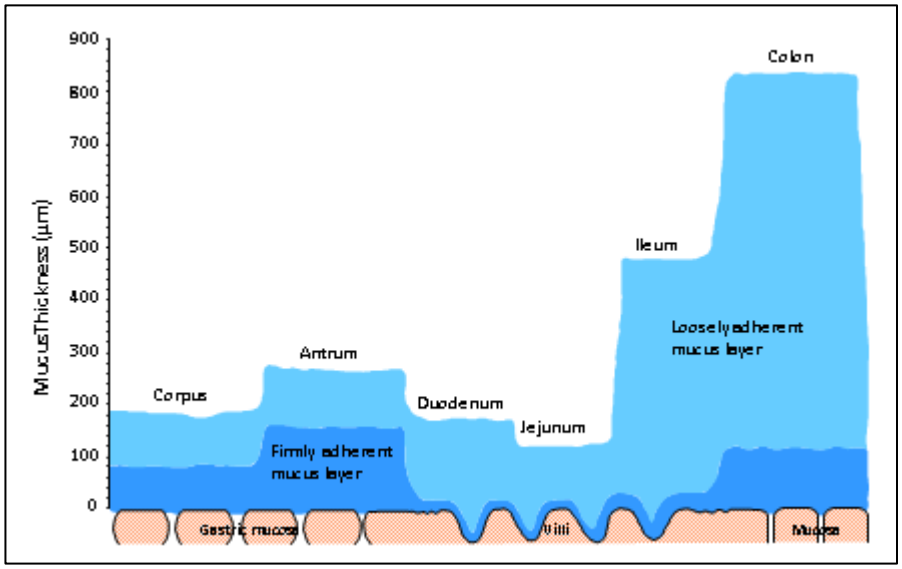


Figure 1.4 Mucus thickness varies along the length of the rat GI tract [10].

The conditions in the small intestine are different to those in the colon (Figure 1.5). Since the primary function of the small intestine is the absorption of nutrients, the structure of the epithelium reflects this with villi and crypts to enlarge the surface area and only one, thinner, loosely adherent mucus layer [18]. Although the bacterial load is greatly reduced in the small intestine, there is a need to manage the proximity of the bacteria to the epithelium. This is believed to be controlled by the host secreting antimicrobial peptides into the mucus, for example the secretion of RegIIIγ, by multiple cell types including enterocytes and Paneth cells, controls the proximity of Gram-positive bacteria to the epithelium [19].

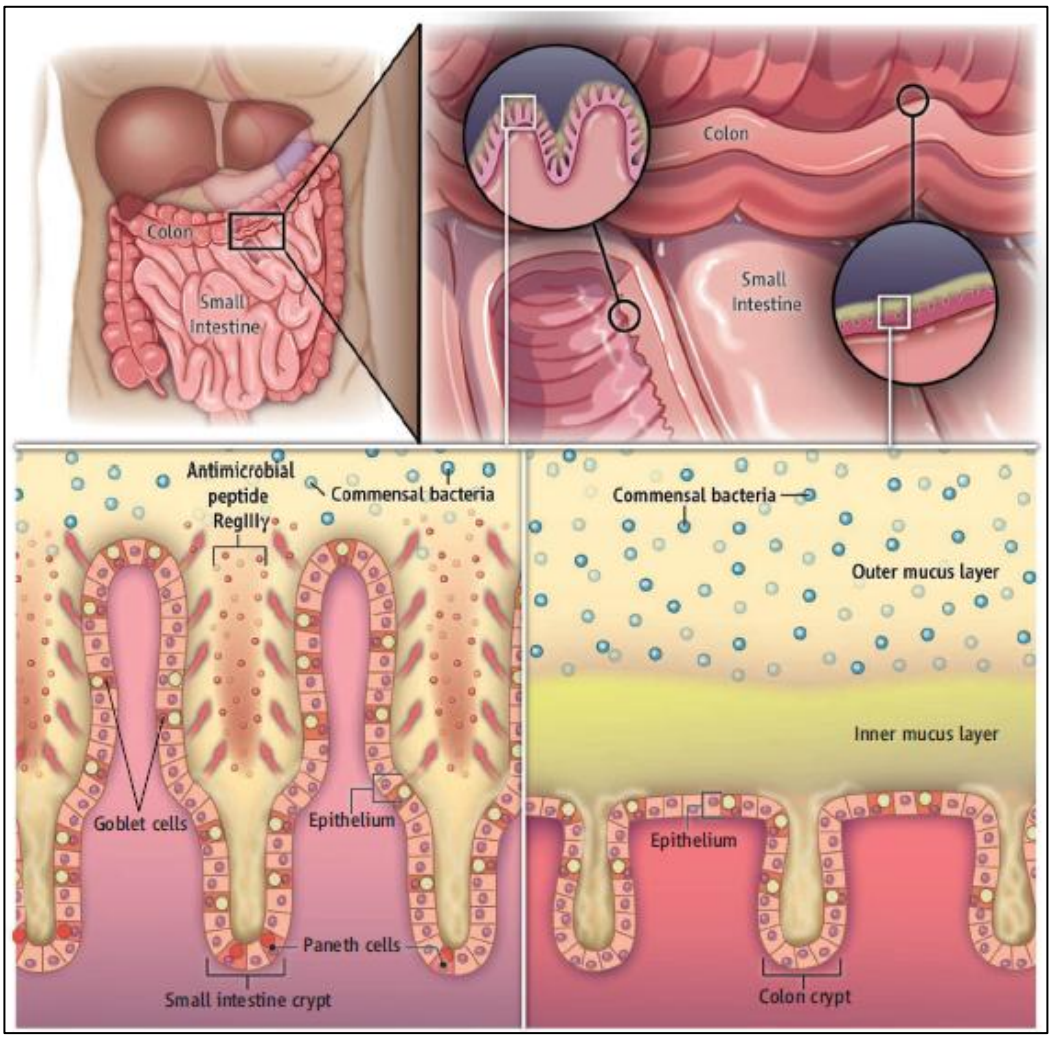


Figure 1.5 Structure of human small intestine and colon [20].

The principal constituents of mucus are large, highly glycosylated proteins called mucins [21, 22]. The protein backbone (apomucin) is highly decorated with mainly O-glycan structures. Mucins can be classified as either secreted or membrane bound (Figure 1.6), and it is the secreted gel-forming mucins which make up the bulk of the

mucus and provide its viscous properties [18]. The secreted mucins form homo-oligomers via the cysteine rich domains found at the amino and carboxyl termini, which form disulphide bonds with other mucin molecules creating a stable structure.

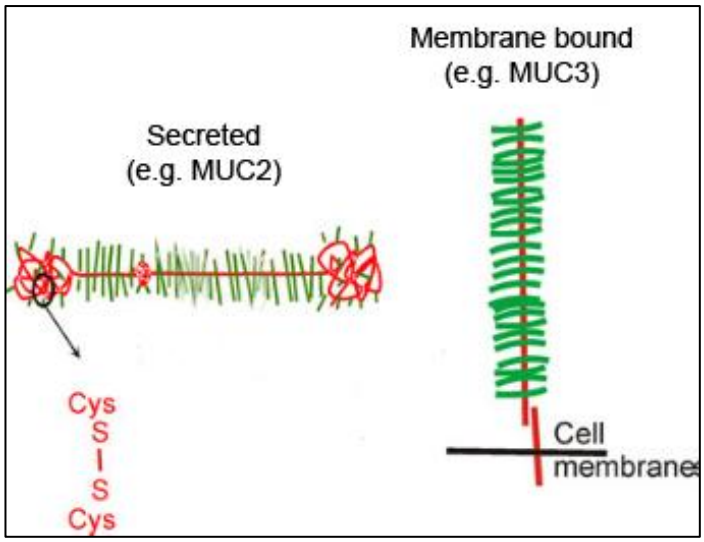


Figure 1.6 Schematic of mucin structures both secreted and membrane bound [23]. Cys S-S Cys represents the disulphide bonds of the cysteine rich apomucin.

In humans 17 different mucin genes (MUC), have been identified, however the predominant mucin expressed in the small intestine and colon, is the secreted gel-forming mucin MUC2 [24-26]. The mammalian homologue of human MUC2 is designated Muc2, and is also the predominant mucin expressed in small intestine and colon of mice, rats and pigs, while lower expression of the membrane bound mucins occur (Table 1.1) [27-29].

Mammal	Stomach	Small Intestine	Cecum & Colon
Human	**MUC5AC, MUC6**, MUC5B, MUC1, MUC13, MUC16	**MUC2,** MUC1, MUC3, MUC4, MUC5B, MUC6, MUC12, MUC13, MUC15, MUC17	**MUC2,** MUC1, MUC3, MUC4, MUC12, MUC13, MUC15, MUC17, MUC19, MUC20,
Mouse/Rat	**Muc5ac**	**Muc2**, Muc1, Muc3(17),	**Muc2**, Muc1, Muc3(17),
Pig	**Muc6**	**Muc2**, Muc4, Muc12, Muc13, Muc20	**Muc2**,

Table 1.1 Mucin expression in human, rodent and pig GI tract [30-32].

MUC2 forms large multimeric net-like structures, by first forming dimers in its C-terminus in the endoplasmic reticulum (ER) (Figure 1.7A), and then in the Golgi apparatus, the apomucin backbones are decorated with O-glycan structures before N-terminal oligomerisation and being tightly packed into secretory vesicles. During

secretion, these multimeric mucin structures combine to form large sheets (Figure 1.7B), which are further stabilised by crosslinking by both covalent and non-covalent mechanisms (Figure 1.7C) [25, 33].

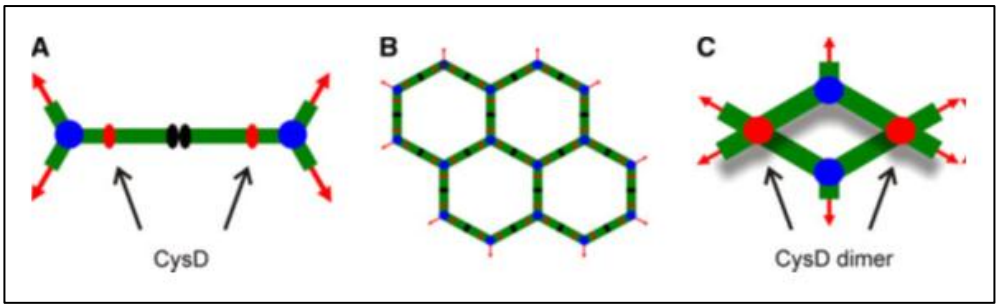


Figure 1.7 Schematic representation of MUC2 multimeric structures, A) dimerization, B) trimerisation forming net, C) stabilisation of the large sheets [23].

Glycosylation accounts for between 50-90 % of the mass of the mucin molecule (ranging from 200 - 40,000 kDa) and each glycan chain can consist of up to 12 monosaccharides, the exact oligosaccharide structure of which is influenced by specific glycosyltransferases, blood group of individual, diet, and bacteria [32, 34, 35]. The carbohydrates commonly found in mucins are N-acetylgalactosamine (GalNAc), N-acetylglucosamine (GlcNAc), fucose, galactose and sialic acid with traces of mannose and sulphate [36]. The mucin O-glycan chain is initiated with a GalNAc attachment to serine or threonine residue and consists of three structural regions, the core, the backbone and the peripheral region (Figure 1.8).

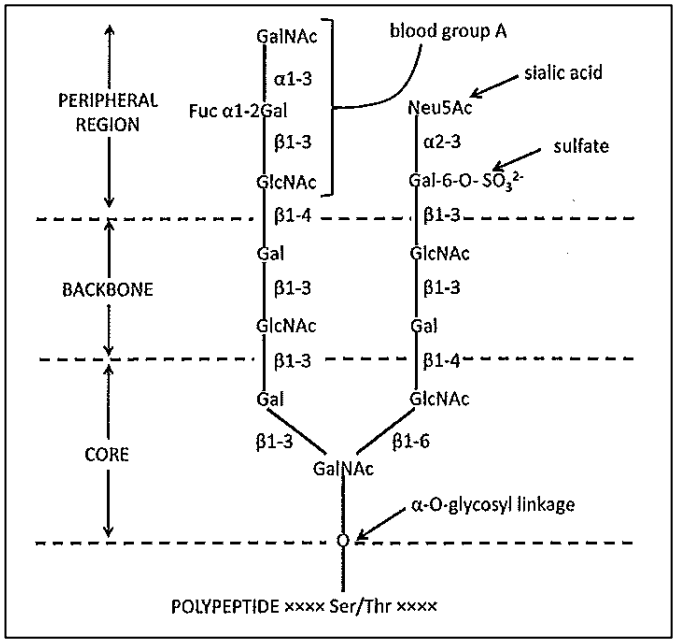


Figure 1.8 Schematic of mucin O-glycan structure, with core, backbone and peripheral regions [37].

There are eight reported core structures (Figure 1.9) [38], and in the human colon the mucin glycans are mainly built on core 3 structures [39], although core 1, 2, 4 and 5 are also present [40]. Core structures 1, 2, 3 and 4 have also been found on mucins in rat small and large intestines [41], pig stomach [42].

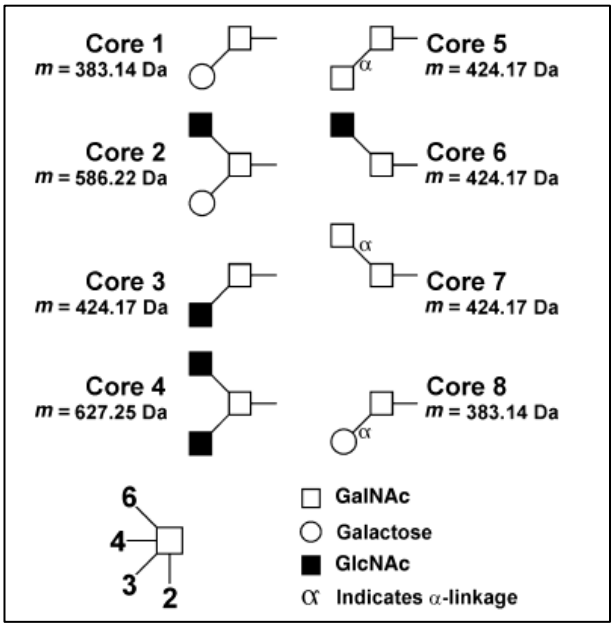


Figure 1.9 The eight core structures of mucin O-glycans. Linkage positions are illustrated by the line connecting the monosaccharides with α linkages labelled and β-anomers unlabelled [38].

The continued addition of glycans to the core structures gives rise to a large number of possible glycan structures. Over 100 different O-glycan structures, both linear and branched, are associated with colonic mucin in humans. Grouped in terms of acidity, 10 different neutral structures were described, 19 monosialylated, and 11 monosulphated structures, while the vast majority of structures present multiple acidic residues [39].

Analysis of the O-glycan structures associated with Muc2 in small and large intestines of rats, showed that the large intestine was enriched for sulphated residues whereas the small intestine was enriched in sialylated residues [41]. This region specific glycosylation was found to also occur in humans with a decreasing gradient of fucose from ileum to rectum and a corresponding increased gradient of sialic acid [43]. The acidic gradient was shown to be acquired after birth, perhaps due to bacterial colonisation or initiation of digestive and absorptive functions of GI tract [44].

These terminal mucin O-glycans have been proposed to serve as preferential binding sites for gut bacteria [45], which in the case of mouse colonic Muc2 were shown to

include fucose, GalNAc, GlcNAc, galactose, sialic acid and sulphated residues [46] whereas pig gastric mucin includes fucose, GlcNAc, GalNAc, galactose and sialic acid [47]. Furthermore, early work by Hoskins *et al* showed ~1 % of the fecal flora could degrade mucin [48], often viewed as a harmful bacterial activity it has become clearer more recently that commensal bacteria engage in mucin degradation and mucin could be designated as endogenous prebiotic [49]. Complete mucin degradation requires proteases which are produced by both bacteria and the host and a combination of saccharolytic enzymes including sialidases, α-glycosidases, β-D-galactosidases and β-N-acetylglucosaminidases [50], which are only produced by bacteria. Multiple genera have members which produce one or more mucin degrading enzymes, including, *Streptococcus*, *Akkermansia*, *Bacteroides*, *Bifidobacterium* and *Ruminococcus* [51, 52]. The mechanisms of mucin degradation in the human GI tract has recently reviewed in Derrien *et al* [49].

Mucins are not the sole constituents of mucus, a detailed proteomic analysis of the two mucus layers from mouse colon reported 1057 proteins identified within this complex mixture [53]. A large proportion of the proteins identified were predicted to be of cellular origin as a result of epithelial cells shedding into the mucus layer. Plasma proteins were also identified, although it is unclear whether these are normally found within the mucus or due to contamination during experimental manipulation of the tissue.

There are a wide variety of non-mucin proteins specifically secreted into the mucus, for example lysozyme, secretory immunoglobulin A (IgA), growth factors, defensins and β-galectins which all have well defined roles in the defence of the host. A number of proteins secreted by goblet cells are thought to interact with mucins to support the maintenance of the gel and/or contribute to the defensive functions of the mucosal barrier. These include Fc gamma binding protein (FCGBP), which may help stabilise and crosslink the net-like structure of the Muc2 mucin [53], calcyclin, a calcium binding protein which may have a role in mucus secretion [54], and intestinal trefoil factor, which may have a role in altering physiochemical properties of mucin through crosslinking [55]. Other proteins secreted into mucus include PEC-60 an inhibitor of insulin secretion [56] and Gob5 with sequence similarity to an epithelial chloride channel [57]. However the interactions of these proteins with mucin have not been well defined.

1.3 **Human Gut Microbiota**

1.3.1 Establishment of the microbial community

Vertebrates evolved around 500 million years ago from the multicellular eukaryotes which have existed for at least 1.2 billion years, which were preceded by the evolution of microbial communities. The co-evolution of vertebrates with associated microbial communities has shaped host biology [3, 58].

Defined as, “the community of microbes that lives in an individual’s GI tract” [59], the gut microbiota is composed of bacteria, archaea, eukarya and viruses. Metagenomics of 22 human fecal samples indicated that the archaea constitute 0.8 %, eukarya 0.5 % and viruses 5.8 %, while the vast majority (92.9 %) are of bacterial origin [60].

It is generally understood that from the sterile environment of the womb, the process of microbial colonisation begins at birth [61], although recent studies showed the presence of bacteria (*Enterococcus* and *Staphylococcus*) in the meconium of healthy new born infants [62], indicating interactions with microbes prior to birth. Studies suggest that colonisation in mammals is influenced by the type of delivery (vaginal vs. caesarean section) [63] and diet (formula vs. breast) [64].

The colonisation process in humans begins with aerobes and facultative anaerobes as they have the capability for oxidative metabolism, which is necessary in an environment which starts with a positive oxidation reduction potential. The colonisation is therefore initiated by *Escherichia coli* and other enterobacteriaceae (*Klebsiella*, *Enterobacter* and *Citrobacter* species), *Enterococci* and *Staphylococci* species. Other groups of aerobes such as *Aeromonas*, *Pseudomonas*, *Acinetobacter* may be found transiently in the first few weeks of life but disappear rapidly as the oxygen and redox potential is reduced to negative values, leading the way for anaerobes, *Bacteroides, Bifidobacteria* and *Clostridia* to colonise (Table 1.2) [4, 65, 66].

Age	1 week	1 month	6 months	1-4 yrs.	Adult
Ratio Anaerobe:Aerobe	1:1	10:1	100:1	100:1	500:1

Table 1.2 Colonisation of human GI tract ratios of anaerobe: aerobe corresponding to age.

The complexity of the microbiota increases gradually through childhood [67], until the community found in human adults reaches a relatively stable climax. The highest microbial load is found in the large intestine of the GI tract, where the flow rate of the contents does not exceed the doubling rate of the microbial populations [4], and where

commensals are found to inhabit the thick outer mucus layer. Changes to both the microbiota and to gut health generally occur in elderly adults, due to an increased frequency of antibiotic usage and changes in the diet [68, 69]. The stability of the adult microbiota is under question, for example the degree to which the unique bacterial communities of an individual are re-established after antibiotic treatment is unclear [70].

1.3.2 Composition and ecology of the human gut microbiota

The microbial composition of the human GI tract shows geographical distribution both along the length and cross-sectionally between lumen, mucus and epithelial layers [71]. The microbial composition in the small intestine of adult humans includes *Bacteroidetes* and members of *Clostridiales* clusters XIV and IV and *Enterobacteriaceae* [72-74]. Whereas the colonic microbiota achieves the highest cell densities recorded for any ecosystem [12, 75] and is made up of ~ 800 different species corresponding to >7000 different strains (Table 1.3). Bacteria from the Cytophaga-Flavobacterium-Bacteroides and the Firmicutes [75] divisions account for >98 % of all 16S rRNA [61], although bacteria have been identified from 9 of the 55 known bacterial divisions.

Small Intestine	**Colon**
10^8 cells ml^{-1}	10^{11} cells ml^{-1}
Escherichia coli, Klebsiella, Enterococcus, Bacteroides, Ruminococcus, Dorea, Clostridium, Coprococcus, Weissella, Lactobacillus, Granulicatella, Streptococcus, Veillonella.	Five major phyla: *Firmicutes, Bacteroidetes, Actinobacteria, Verrucomicrobio* and *Proteobacteria* – Hundreds of species.

Table 1.3 Comparison of microbial composition and load of small intestine vs. colon.

Recently, phylogenetic composition analysis of human fecal samples using metagenomic techniques revealed three clusters referred to as enterotypes, *Bacteroidetes*, *Prevotella* and *Ruminococcus* based on abundance, indicating that variability between humans is stratified rather than continuous [60]. Long term diet has been linked to enterotype partitioning [76]. However, at the species and strain level the microbiota is as individual as a fingerprint which has shifted the focus of some research from which strains are present to what they are doing functionally, since metabolic pathways are found to be evenly distributed and prevalent across individuals [77]. The stability of the system in terms of functionality can be achieved with fluctuating populations of bacteria [61] adapting as necessary to the stochastic factors, meaning that complex communities of quite different composition can have similar functional

characteristics. The microbial variability indicates that not all factors influencing the composition are predictable and that gut microbial communities are governed by a different set of ecological rules than free living communities [58].

Stochastic niche theory [78], which was first applied to the microbiota by Dethlefsen *et al* 2006 [79], combines the concepts that both predictable factors (e.g. genome of host and diet), and random factors (e.g. microbial exposure and disease), influence the composition of the microbiota, suggesting that the colonisation patterns outlined above are probabilistic rather than predictable. Predictable factors for vertebrate microbial community composition have been well studied for example, it was shown that the microbiota associated with hindgut and foregut herbivores form two distinct groups, confirming the importance of diet and/or gut physiology [58]. The random factors are less well understood and include historical contingencies occurring during the community assembly and temporal dynamics from complex microbe-microbe and microbe-host interactions [79].

Microbe-microbe interactions may involve multiple species and strains in complex food webs, where the metabolic product from one microbial strain becomes the substrate for another. These metabolic webs have the potential to be inconceivably complex and changeable when made of >7000 different strains of bacteria, able to adjust transcriptome and metabolomes to the variations in substrate availability [61]. As well as these positive interactions, the microbes may also compete and produce antimicrobials such as bacteriocins in order to gain competitive advantage. Microorganisms can compete and cooperate simultaneously, for example *Bacteroides thetaiotaomicron* and *Methanobrevibacter smithii* facilitate each other's growth by complementary energy metabolism, while competing for nitrogen [80].

Microbe-host interactions may also involve more than one microbial partner, for example lactate released by some *Bifidobacterium* species, is used by lactate fermenters such as *Eubacterium hallii*, to produce butyrate, which can then be metabolised by the host to maintain gut health [81]. Another example of these complex relationships is the one between mucin degrading bacteria e.g. *Bacteroides fragilis* which degrade sulpho-mucins present on the host, to release sulphate which is used by sulphate reducing bacteria (e.g. *Desulfovibrio desulfuricans*) to reduce to the highly toxic hydrogen sulphide [82].

The profound importance of the gut microbiota to host health was first revealed by germ free mice models, which provided evidence that intestinal bacteria are involved in a number of key physiological functions including nutrient absorption [83, 84], epithelial renewal [85], angiogenesis [83, 86], energy harvest, use and storage [87], development, expansion and function of the immune system [88], and the development of neural systems [89].

The increasing prevalence of chronic and degenerative diseases in industrialised countries has been linked to the disruption of the co-evolved mutualism between humans and their microbiota [59]. Some diseases are associated with a specific gut microbe for example peptic ulcer disease and *Helicobacter pylori* and *Streptococcus gallolyticus* (previously named *Streptococcus bovis*), has a well-known association with the pathology of colorectal cancer [90-92]. Whereas changes in composition of microbiota have been associated with obesity, with a lower proportion of Bacteroidetes and a higher proportion of Actinobacteria found when comparing obese to lean individuals [93]. The aetiologies of the inflammatory bowel diseases (IBD), (which include Crohn's disease and ulcerative colitis) are not associated with a specific species of gut microbe but chronic microbial infections are implicated in the pathology [94-96]. With antibiotic and probiotic use for the treatment of pouchitis [97, 98], an increase in bacterial translocation in Crohn's disease [99] and a colitogenic microbial flora has been described which can induce colitis in a mouse model [100].

Colonising gut microbes can be defined as either specialists, bacteria which show host specific evolutionary genetic adaptations such as *Lactobacillus reuteri* [101, 102] or generalists, with larger genomes and therefore the ability to adapt to variations in the environment, for example the ability to utilise a wide range of host and dietary carbohydrate moieties in *Bacteroides thetaiotaomicron*, *B. vulgatus* and *Parabacteroides distasonis* with their extensive array of carbohydrate active enzymes [8, 103].

1.3.3 *Lactobacillus reuteri* in the vertebrate GI tract

Gram-positive lactobacilli which belong to the Firmicutes phylum are natural mutualistic inhabitants of the GI tract of mammals [104, 105]. Within the genus *Lactobacillus*, there are 145 recognised species, although many members of which form part of the ecosystem in the GI tract, lactobacilli are also found in many other niches which may impact the GI environment, including plant, dairy, meat products and sourdough bread

[106]. *L reuteri* belongs to the Bacilli class, a major group in the Firmicutes, one of the two dominant phyla of the GI microbiota (Figure 1.10).

Phylum : Firmicutes

Class : Bacilli

Order : Lactobacillales

Family : Lactobacillaceae

Genus : *Lactobacillus*

Species : *L. reuteri*

Figure 1.10 Phylogenetic classification of *L. reuteri.*

L. reuteri strains are ideal candidates for studying the adaptation of bacteria to their host due to the evidence of host-driven diversification with host specific clades. This was determined using phylogenetic analysis, consisting of principle component analysis using multi-locus sequence analysis of house-keeping genes. This is supported not only by their ubiquitous nature, inhabiting many species of mammals and birds including, human, pig, chicken, turkey, mouse and rat. but also by rodent isolates preferentially colonising *Lactobacillus* free mice *in vivo* [101].

L. reuteri strains are found in stomach, duodenum, jejunum, ileum, cecum and recto-sigmoidal colon of humans [72, 107]. The type strain DSM 20016 was detected in a human subject over several months and is considered autochthonous [108, 109], however the organism is not detected in every human subject [110]. In contrast, *L. reuteri* strains are commonly found in GI tract of pigs [111-113], rodents [114, 115] and chickens [116]. It is well documented that *Lactobacillus* species are able to adhere directly to the squamous epithelium in the forestomach of rodents, the stomach of pigs and the crops of chicken [116], however this does not exclude their colonisation of the lower GI tract.

A number of *L. reuteri* strains have had their genomes sequenced, a complete genome sequence is available for DSM 20016 (Genbank accession number CP000705.1), and JCM 1112 (Genbank accession number AP007281.1). Both strains have been considered as the type strains and are derived from the same original human faecal isolate, *L. reuteri* F275 [117]. Their genome sequences are very similar apart from the presence of two large unique regions in *L. reuteri* JCM 1112 that result in a 40-kb

difference between the strains. The draft genomes of seven other *L. reuteri* strains are also available (see www.ncbi.nlm.nih.gov/genome/438), including MM4-1a (human isolate), 100-23 (rat isolate), CF48-3A (human isolate), SD2112 (human isolate), MM2-3 (human isolate), lpuph (rodent isolate) and mlc3 (rodent isolate).

Colonisation is a multifactorial process linked to cell surface properties and the ability of the bacteria to adapt to its environment. Persistence is often used as a measurement of colonisation and is reported to be strain specific [118]. Additionally, binding to the intestinal mucosa is considered a prerequisite for successful colonisation [119]. The ability of *L. reuteri* to colonise the GI tract has been attributed to a number of colonisation factors, including adherence to epithelial cells [120], binding of fibronectin [121], collagen binding ability [122, 123], ability to bind to mucus [124], autoaggregation [125], production of antimicrobial substances [126], presence of lipoteichoic acids (LTA) on the bacterial cell surface [127] along with extracellular polymeric substances (EPS) [128].

Probiotics are defined by the World Health Organisation (WHO) as, “Live microorganisms which when administered in adequate amounts confer a health benefit on the host” [129]. The criteria for selecting probiotic micro-organisms include the ability to adhere mucus and/or human epithelial cells and cell lines. Some *L. reuteri* strains are considered as probiotics especially since the discovery of their ability to produce an antimicrobial substance called reuterin [130]. However, a recent literature review on the subject reported that only one *L. reuteri* strain (human isolate ATCC 55730) presented substantial evidence for its probiotics properties in humans (Table 1.4) [131]. *L. reuteri* ATCC 55730 is used in humans for the prevention and cure of colitis, diarrhoea, gastroenteritis, rotavirus, and is also noted for its hypocholesterolemic effects [96, 132-138]. Other *L. reuteri* strains are effective in animal models and are widely used in livestock industries [112, 139, 140] .

Other health related properties of *L. reuteri* strains are yet to be fully exploited, including the production of vitamin colbalamin (vitamin B12) by *L. reuteri* DSM 20016, a lack of this vitamin is characterised by anaemia and macrocytosis of red blood cells [141]. Furthermore, *L. reuteri* ATCC 53608 has the potential to be used to produce D-Mannitol (important in food, pharmaceuticals, medicine and chemistry) through the action of a mannitol-2-dehydrogenase [142].

Strain of *L. reuteri*	Probiotic action	Reference(s)
ATCC 55730	Prevention of diarrhoea and treatment of gastroenteritis in humans	[133, 134, 136, 143]
ATCC 55730	Reduction of colicky symptoms in 95% of human infants	[144, 145]
ATCC 55730	Reduction of IgE associated eczema in 2 yr old humans	[146]
ATCC 55730	Immune stimulation in humans – CD4 lymphocytes	[107]
SD2112	Tolerated in humans with HIV	[147]
DSM 12246 R2LC JCM 5869 ATCC PTA 4659 ATCC 55730 6798	Prevention of experimental colitis using animal models	[138, 148-151]
100-23	Immune stimulation and regulation in mice	[152, 153]

Table 1.4 Strains of *L. reuteri* with reported probiotic actions.

1.4 **Bacterial mechanisms of adhesion**

General mechanisms used by bacteria to adhere within the GI tract (including to mucus) involve biofilms, aggregation and mucosal recognition mediated by both specific and non-specific bacterial cell surface factors.

1.4.1 Biofilm formation, aggregation and mucosal recognition

Biofilms are mono or polymicrobial aggregates surrounded by a mixture of polysaccharides, proteins and nucleic acids which form a matrix, creating localised conditions and a lifestyle entirely different to the planktonic state. The matrix protects bacteria from desiccation, oxidising agents, antibiotics, ultraviolet radiation and in the GI tract against host immune defences [154]. The bacteria within the biofilm may express a different metabolic phenotype when compared to free-living counterparts [155]. Biofilms have been characterised in the squamous epithelium in the stomach of mice [156], however the situation in the rest of the GI tract is less well understood [157, 158]. The formation of biofilms requires adhesion and aggregation of bacterial cells. Bacterial aggregation to self (autoaggregation), or between mixed bacterial strains (coaggregation) is the initiation step for numerous functions to occur including conjugal pairing, biofilm formation, colonisation of the GI tract, and clearance of pathogens [159-162].

The classical theory of adhesion between bacteria and a solid substrate involves five types of forces with three stages in the process; at a distance > 50 nm van der Waals' attractive forces are at work, at 10-20 nm there is an addition of electrostatic interactions, salvation (hydration) and steric forces and at less than 1.5 nm, specific interactions become involved [163] (Figure 1.11). Both bacteria and the biological surfaces to which they adhere usually have a negative zeta potential. The liquid layer surrounding the bacteria exists as two parts, an inner region where ions are strongly bound and an outer region where they are less firmly associated. The potential at the boundary of inner and outer regions is the zeta potential (Figure 1.12). When the bacteria have a large negative zeta potential they will tend to repel each other.

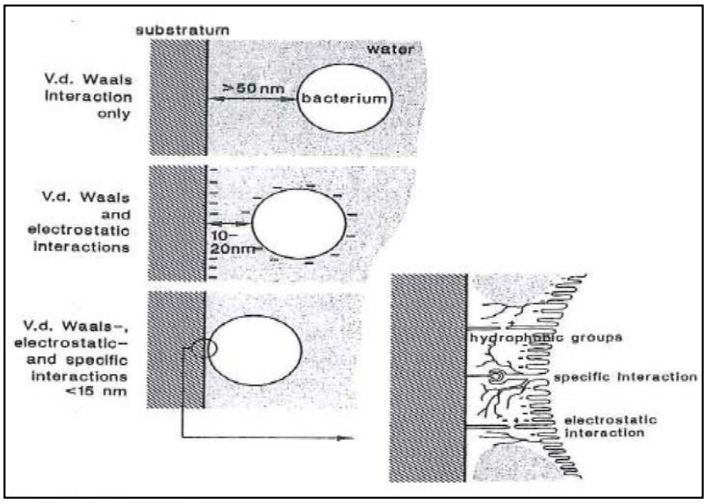


Figure 1.11 Schematic representation of the interactions involved in bacterial adhesion to solid substrate [163].

During the second stage of the interaction, hydrophobicity and hydrophobic surface components on bacteria (e.g. S-layers) probably have a dehydrating effect on the water film, enabling the third stage of direct contact of specific interactions between protuberant parts of the cell surface and the substratum to occur [163, 164]. Some *Lactobacillus* strains (*L. acidophilus* ATCC 4356 and *L. casei* ATCC 393), can alter their hydrophobicity in response to increasing ionic strength in the surrounding liquid [165], giving them an advantage in adhesion/ colonisation, although this is yet to be confirmed.

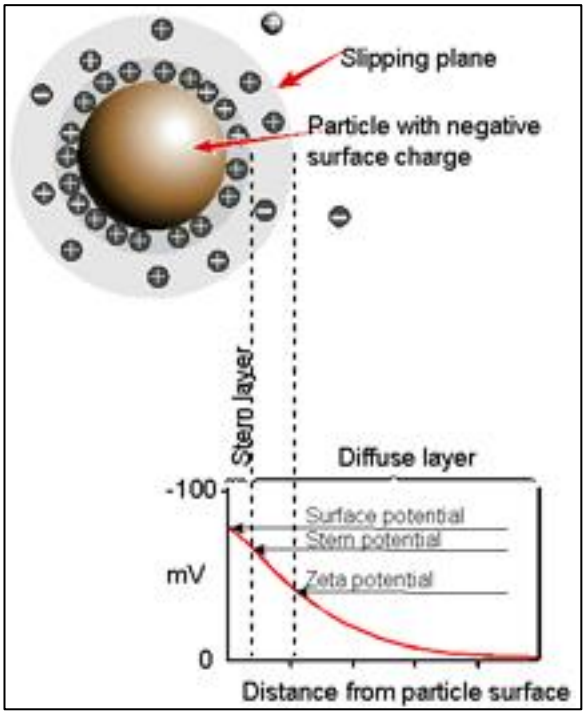


Figure 1.12 Zeta potential surrounding negatively charged bacteria.

1.4.2 Bacterial cell surface structures

Cell surface structures are used by bacteria to interact with their environment. Gram-positive and Gram-negative bacteria have different cell wall structures (Figure 1.13 and Figure 1.14).

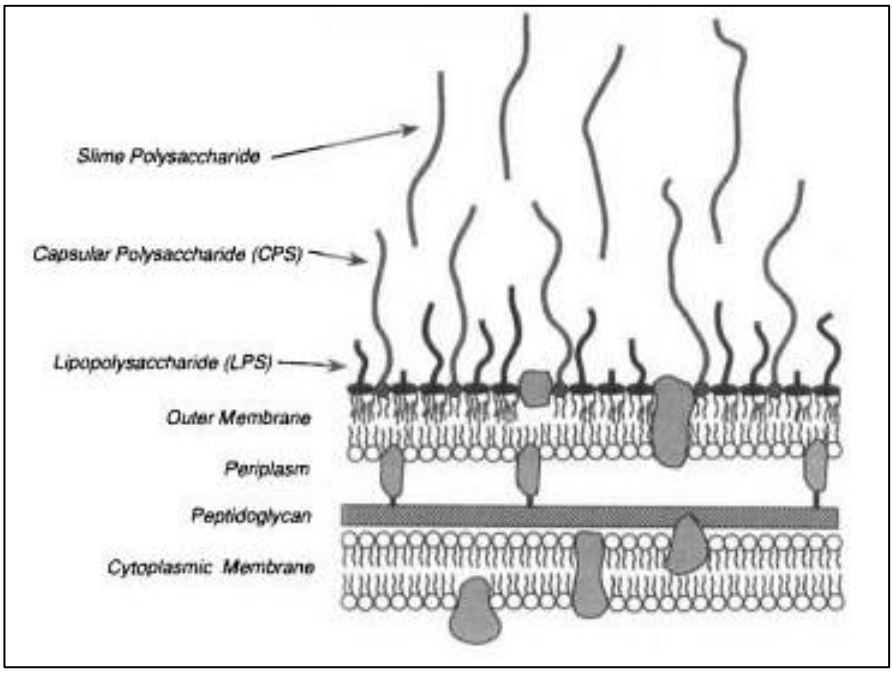


Figure 1.13 Schematic representation of the cell surface architecture of Gram-negative bacteria. Components include; slime and capsular polysaccharides (CPS), lipopolysaccharides (LPS), outer membrane, periplasm, peptidoglycan (PG), and cytoplasmic membrane [166].

The primary difference is the outer membrane which is present in Gram-negative bacteria but absent in Gram-positive bacteria. The peptidoglycan (PG) layer is thinner in Gram-negative bacteria and usually represent <10 % dry weight of the cell wall, whereas for Gram-positive bacteria the PG layer is thicker usually representing 10 % dry weight of the cell wall. Lipid content of the cell walls shows the reverse trend with higher values between 11-22 % in Gram-negative bacteria compared to 4 % in Gram-positive bacteria. In Gram-negative bacteria, the outer membrane contains lipopolysaccharides whereas unique to Gram-positive bacteria are the teichoic acid and lipoteichoic acid structures [167].

In certain species of both Gram-positive and Gram-negative bacteria, the cell is surrounded by an outer shell of proteins packed in a paracrystalline surface layer (S layer). The attachment site of the S-layer is the outer membrane in Gram-negative bacteria and the PG layer for Gram-positive bacteria. S-layers are generally monomolecular crystalline arrays exhibiting a morphologically similar, oblique lattice

structure and representing 10–15 % of the total protein of the bacterial cell wall [168-170]. However, the biological functions of most of these S-layer proteins remain to be defined [171].

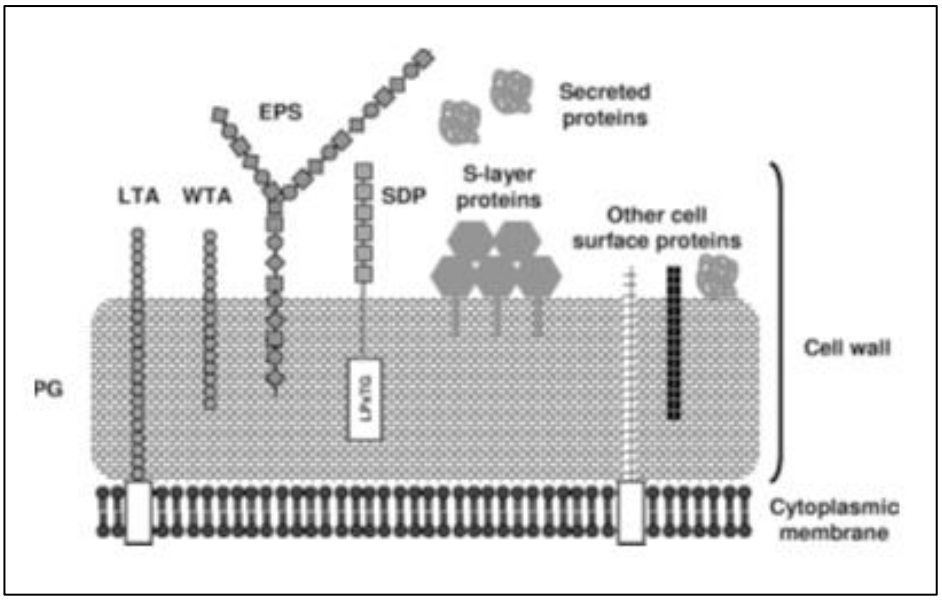


Figure 1.14 Schematic representation of the cell surface architecture of Gram-positive bacteria. Components include; peptidoglycan (PG), lipoteichoic acids (LTA), wall teichoic acids (WTA), extracellular polymeric substances (EPS), sortase dependent proteins (SDP), surface layer proteins (S-layer proteins) and secreted proteins [33].

Another possible feature of both Gram-positive and Gram-negative bacteria is a thick shell composed of capsular polysaccharides, often a characteristic of pathogenic bacteria thought to help mask the bacteria from an immune response [172]. Extracellular appendages such as pili/fimbriae and flagella are found in both Gram-positive and Gram-negative bacteria and are well studied in pathogens, although pili have recently been identified on the surface of probiotic *L. rhamnosus* GG [173]. The proteinaceous pilus fibre is composed of one major pilin that forms the pilus backbone and two minor pilin subunits [174-177]. These slender, elongated, surface appendages often mediate adherence between microbes and their host cell targets [178]. Flagella are substantially larger than pili and are composed of several thousand copies of flagellin subunits. Although the primary role of flagella is locomotion, they also have a role in adhesion [179].

EPS were previously known as exopolysaccharides, defined as long-chain polysaccharides composed of branched, repeating units of sugars loosely attached to the cell surface or secreted into the environment by certain Gram-positive bacteria [180]. They were later re-named as extracellular polymeric substances, when it

became clear that the matrix also contained proteins, nucleic acids, lipids and other biopolymers such as humic substances [154, 181, 182]. EPS form the bulk of many biofilms and can also play a role in adhesion via non-specific interactions by contributing to the physico-chemical properties of the cell surface. Being negatively charged, they have the potential to interfere electrostatically with the binding of the bacteria to the receptor on the mucus. For example, EPS extracted from *Bifidobacterium longum* NB667, reduced binding of *L. rhamnosis* GG to mucus in a dose dependent manner [183].

Bacterial adhesins are often large proteins consisting of repeating modules, anchored to the cell surface of Gram-positive and Gram-negative bacteria by various different mechanisms [184], such as single N- or C-terminal transmembrane anchors, lipoprotein anchors, LPxTG-type anchors, or other repeated cell wall-binding domains including Lysine Motif (LysM) domains or glycine-tryptophan dipeptide motifs [185, 186]. Other proteins are secreted in the surroundings, some of which have been shown to re-associate with the cell surface by electrostatic interactions [170, 185].

1.5 **Specific interactions between lactobacilli and mucus**

Many of the bacterial-mucus adhesion studies have been carried out with *Lactobacillus* species, since mucus binding is often studied in potential probiotic bacterial strains, with the aim to select probiotics based on their ability to persist in the gut [178, 187-201]. Bacterial adhesion to mucus has also been studied in pathogenic bacteria, as part of the infection process, *Helicobacter pylori* for example, has been shown to have at least four mechanisms of mucin adhesion [202]. Furthermore, mucus binding has been studied in the context of competitive exclusion, the preferential adhesion of probiotic bacteria, thereby reducing the virulence of pathogens [105, 111, 203-205]. The biochemical mechanisms for sensing host environments, interacting with host surfaces and even communicating with the host are often the same in human pathogenic, commensal and mutualistic organisms [70].

The majority of studies on *Lactobacillus* adhesion to mucus have been carried out using *in vitro* methods, where mucus is immobilised on microtitre plate wells and the adhesion of bacteria detected in a variety of ways, predominantly radioactive labelling or cell counting. Recently, more sensitive techniques have begun to be developed, surface plasmon resonance (SPR) has been used to determine binding of lactobacilli to mucin [195, 196], flow cytometry (FCM) used to determine the binding of *Bacteroides fragilis* to mucin [206] and atomic force microscopy (AFM) was used to determine the binding of *Lactococcus lactis* to mucin [207], these more sensitive techniques give further information on the kinetics of the interactions and can provide insights on the contributions of specific and non-specific interactions. However these labour intensive and costly methodologies are not generally used for screening.

Mucus glycoproteins are generally postulated to act as the molecular receptors of bacterial binding. The mucus and mucins used in these assays are from a variety of sources; extracted from intestines, faeces, from mucus-secreting cell lines such as HT29-MTX or from a commercial source, which may have an impact, since the identification of region-specific glycosylation [40, 43]. However only indirect evidence has been gathered for adhesion to mucin glycans in *Lactobacillus* species [208, 209], and further work is needed to confirm the role of mucin carbohydrates in adhesion.

Although EPS [118, 183, 210-212], LTA [213], S-layer proteins [168, 179, 214-217], undefined secreted factors [111, 218, 219], autoaggregation ability, and hydrophobicity [220] have been suggested as potential bacterial adhesion factors, little biochemical

evidence supporting their role in adhesion is available. In contrast, several bacterial cell surface proteins have been shown to act as mediators of specific *Lactobacillus* adhesion to mucus [124, 190, 216, 218, 219, 221, 222]. At present only a few mucus adhesins have been functionally and structurally characterised (Table 1.5).

Bacterial Strain	Bacterial Adhesin	Mucus Receptor	Reference
L. reuteri 1063	Mucus binding protein (MUB) (GenBank AF120104)	Sigma porcine gastric mucin, mucus from pig or hen intestines, fractionated mucus from pig or hen intestines. Immunoglobulin (non-immune Fab-dependent)	[124, 223]
L. reuteri 104R (previously named *L. fermentum* 104R)	Mucus adhesion-promoting protein (Map A) (Previously MAPP) (GenBank AJ293860)	None identified Binds to purified Sigma porcine gastric mucin, small intestinal porcine mucus. Also binds to epithelial cells, specifically ANXA13 and PALM from Caco2 cells	[203, 222, 224-227]
L. johnsonii La1 NCC 533	Elongation Factor Tu (La1 - EF-Tu) (GenBank NP 964043)	None identified, binds to purified mucus from HT-29MTX cells containing MUC 5AC and human colonic mucus extract containing MUC2, also binds epithelial cells	[228]
L. johnsonii La1 NCC 533	Heat shock protein of GroEL class (La1 - GroEL) (GenBank NP 964487)	None identified, binds to purified mucus from HT-29MTX cells containing MUC 5AC, also binds epithelial cells	[229]
L. plantarum LA 318	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (GenBank AL935254)	Binds to human colonic mucus.	[230]
L. rhamnosus GG	Pilin subunits SpaC, SpaF, SpaB (GenBank YP003170190, YP003172118, YP003170189)	Binds to human colonic mucus, SpaC and SpaF specific. SpaB non-specific electrostatic interactions	[173, 174]

Table 1.5 Functionally characterised mucus adhesins from *Lactobacillus* species.

Mucus binding protein (MUB), a 358 kDa protein from *L. reuteri* 1063, was the first functionally and structurally characterised mucus adhesion in commensals [124]. MUB, shows the typical structure of many Gram-positive cell surface proteins, with a YSIRK signal peptide for translocation across the cytoplasmic membrane and a C-terminal LPxTG anchor motif, recognised by a family of enzymes called sortases, which

covalently attach the protein to the PG. The 14 Mub repeats found in MUB, can be divided into six type 1 and eight type 2 domains based on sequence homology. The six type 1 repeats show 15-85% amino acid (aa) sequence identity, whereas the eight type 2 domains show higher conservation with >91% aa sequence identity [124] (Figure 1.15).

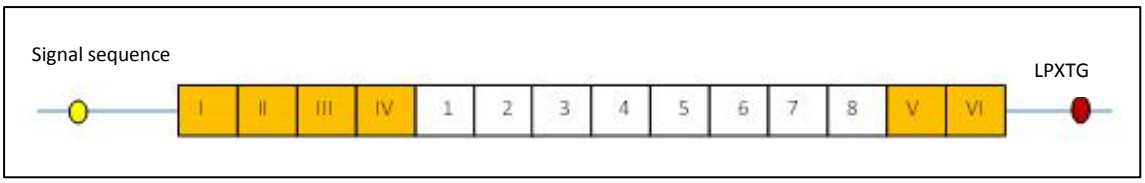


Figure 1.15 Structure of mucus binding protein MUB, with YSIRK signal sequence, Mub repeats (type 1 in orange and type 2 in white) and LPXTG cell wall anchor.

Mub repeats are believed to mediate binding to mucus, since fusion proteins consisting of different Mub repeats and the maltose-binding protein (MBP) were shown to adhere to pig mucus components, pig gastric mucin and hen intestinal mucus. The binding of Mub-MBP to mucus components could be partly inhibited by the glycoprotein fetuin, which suggests that the receptor of Mub adhesion is a sialylated glycan structure, possibly those attached to mucins.

By searching protein databases, Boekhorst *et al* [231], identified 48 proteins with at least one Mub domain in nine different lactic acid bacterial species, mostly lactobacilli from GI tract. More recently an *in silico* analysis of extracellular proteomes of lactobacilli revealed 47 proteins from six *Lactobacillus* genomes contained mucus binding domain(s) [232], strongly suggesting that the Mub repeat is a functional unit that could play an important role in host-microbe interactions. Many of these proteins contain multiple Mub repeats which raises interesting questions about the role and requirement of each repeat unit.

Furthermore, each Mub repeat, approximately 200 aa in length, consists of two domains B1 and B2. The B1 domain of Mub repeat 5 (MubR5), has structural similarity to repeat unit of Protein L, an immunoglobulin (Ig) binding protein from *Peptostreptococcus magnus*, and was shown to bind to mammalian Igs including secretory IgA [223]. Further work by Coic *et al* [233] showed that the chemically synthesised MUB_{70} which corresponds to the B1 domain has binding affinity for colonic mucus from humans, guinea pig and rabbits but not mice or rabbit ileal mucus, and specific recognition of Muc2.

A distinction needs to be made between MUB domains which have been characterised at both biochemical and structural level and the putative mucus binding MucBP domains found in the Pfam database (PF06458 http://pfam.sanger.ac.uk/). The MucBP domains are predicted to be smaller than the Mub domains and roughly correspond to the B2 domain, but show a much wider phylogenetic distribution than the Mub domains (Figure 1.16) [231, 234]. Spr1345 from *Streptococcus pneumonia* is one of the few MucBP domains to have been functionally characterised [235], binding to mucin and polysaccharides hyaluronan and chondroitin sulphate, suggesting specificity for the carbohydrate moiety of mucins [236].

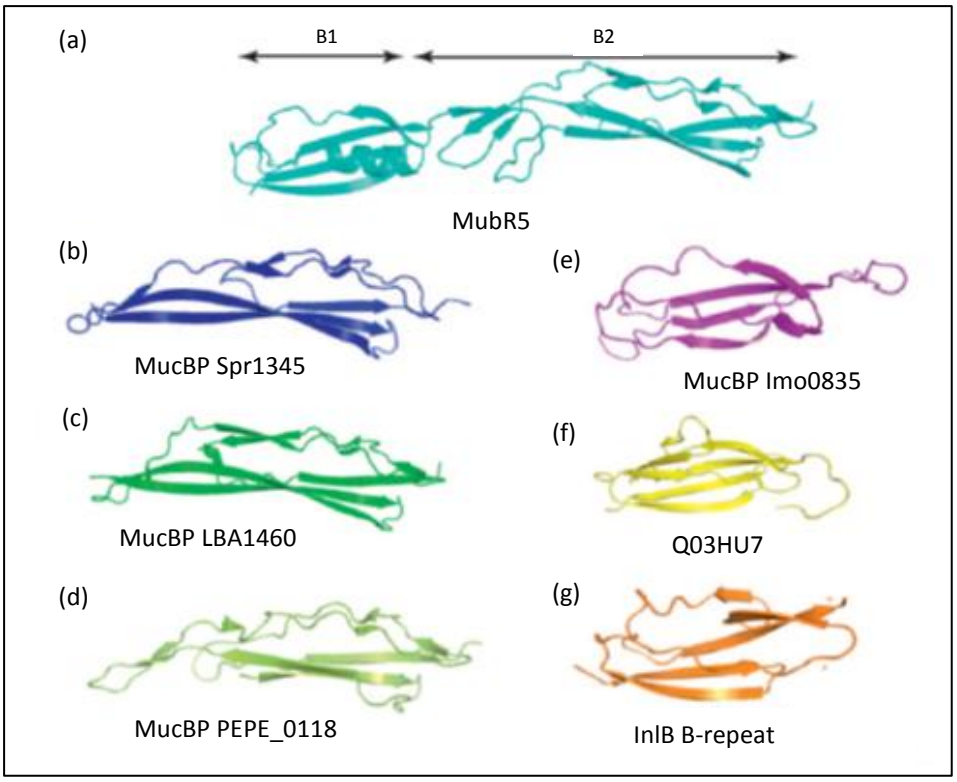


Figure 1.16 Crystal structures of Mub repeats, MucBP domains and related proteins. a) *L. reuteri* MubR5 (PDB 3I57); b) Spr1345 from *Streptococcus pneumonia* (PDB 3NZ3); c) LBA1460 from *L. acidophilus* (PDB 3O69); d) PEPE_0118 from *Pediococcus pentosaceus* (PDB 3LYY); e) Imo 0835 from *Listeria monocytogenes* (PDB 2KT7); f) related adhesion protein from *P. pentosaceous* (PDB 2KYW) and g) InlB B repeat from *L. monocytogenes* (PDB 3NZ3) [234].

Another *L. reuteri* mucus adhesion protein (Map A) was purified, identified, and characterised (Table 1.2.2) from strain 104R [224]. After lithium chloride (LiCl) extraction from the cell surface, proteins were purified by either gel filtration or mucin affinity purification, and MapA was detected in the fractions by dot blot assays with horseradish peroxidase-labelled porcine intestinal mucus and commercial porcine gastric mucin. Map A was also found to inhibit adhesion of *L. reuteri* 104R to porcine

intestinal mucus. Identification was performed by, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and Western blotting. Map A is part of the ATP-binding cassette (ABC) transporter family, which include adhesins specific to other GI tract receptors e.g. collagen, and epithelial cells.

Some of these adhesins are multifunctional molecules with at least two independent functions for example, in the cytoplasm protein elongation factor thermo unstable (EF-Tu) is part of the mechanism to synthesise new proteins as part of the ribosomal complex whereas on the cell surface EF-Tu binds to mucus and epithelial cells [228]. EF-Tu and a heat shock protein from Hsp60 class (GroEL), were recently identified as surface molecules and functionally characterised as mucus binding proteins in *L. johnsonii* NCC533 La1 (Table 1.2.2) [228, 229]. *L. johnsonii* NCC533 La1 EF-Tu was identified from cell surface proteins extracted with LiCl. Proteins which adhered to epithelial cells, were run on SDS-PAGE, transferred to nitrocellulose membrane, and identified using antibodies against cell surface proteins whereas the presence of GroEL at the bacterial surface was demonstrated using a whole-cell enzyme-linked immunosorbent assay. Recombinant proteins of both EF-Tu and GroEL were produced and shown to bind to mucins extracted from human colon and HT29-MTX cells. Although present at the cell surface, EF-Tu and GroEL, do not contain a signal sequence, LPxTG peptidoglycan anchor motif or lipoprotein motif and are considered as intracellular proteins. Interestingly the GroEL of *Salmonella typhimurium* induced aggregation of bacteria in the presence of mucus [229].

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) isolated from the cell surface of *L. plantarum* LA 318 has also been functionally characterised as a mucus binding protein [230] (Table 1.2.2). *L. plantarum* LA 318 cells washed in PBS had reduced ability to bind to human colonic mucin as detected by SPR and GAPDH was identified as a protein in the PBS wash fraction, by SDS-PAGE followed by N-terminal aa sequencing. Purified with a two-step chromatographic procedure GAPDH was then shown to bind to human colonic mucin. GAPDH is common on the surface of many pathogenic bacteria, and the presence of adhesins on both pathogenic and mutualistic bacteria reinforces the theory of competitive exclusion.

L. rhamnosus GG has recently been shown to possess pilin structures [173]. Prototypically, the pilus fibre is composed of 3 pilin subunits only one of which shows adhesive ability, the other two contribute to structural integrity [174-177]. *L. rhamnosus*

GG has two pilus gene clusters, spaCBA and spaFED and the pilus subunits SpaC, SpaF and SpaB bind *in vitro* to human intestinal mucus [173, 174] (Table 1.2.2). Using recombinant proteins, the two adhesive pilin subunits SpaC and SpaF, showed specific mucus binding ability, whereas the binding between pilin subunit SpaB and mucus was shown to be mediated by electrostatic interactions.

Adhesion exhibited by these mucus binding proteins is not solely restricted to mucus, Map A, EF-Tu and GroEL also bind to epithelial cells whereas GAPDH from *L. crispatus* ST1 binds to plasminogen [237] and MUB to Igs [223].

Other functionally characterised adhesins are considered as mucus binding proteins (Table 1.6), although a careful inspection of the literature shows that these have been tested either indirectly, against epithelial cells or in a denatured form so no direct biochemical evidence of binding to mucus is available.

Bacterial Strain	**Bacterial Adhesin**	**Receptor**	**Reference**
L. reuteri NCIB11951	Collagen binding protein (CnBP)	Collagen	[123]
L. plantarum WCFS1 *L. plantarum* 299v	Mannose specific adhesin (Msa)	Mannose	[221, 238-240]
L. acidophilus NCFM	*La*-MUB	None stated	[216]
L. reuteri JCM 1081	29 kDa protein (*Lr* W1)	Porcine gastric mucin and epithelial cells	[241]

Table 1.6 Functionally characterised adhesins of *Lactobacillus*, with putative mucus binding function.

The adhesion of Collagen binding protein (CnBP) isolated from *L. reuteri* NCIB11951 to collagen was inhibited by both porcine intestinal mucin and by a lectin with specificity for α-D-galactose [123], indicating a possible lectin-like adhesion to mucus although this has not been studied further. CnBP is a MapA homologue and part of the ABC transporter family, which include other types of adhesins.

Mannose specific adhesin (Msa) proteins from both *L. plantarum* WCFS1 and *L. plantarum* 299v show similar structure to MUB proteins, with N-terminal signal peptide, sortase dependent LPxTG anchor motif and the presence of Mub repeats [216, 221, 242]. Msa contributes to *L. plantarum* to bind to mannose but no experimental work has been carried out to assess its binding to mucus.

One of the MUB homologues from *L. acidophilus* NCFM (*La*-MUB), was shown to be involved in adhesion to epithelial cells as observed by the 65% reduction in binding with a MUB mutant strain, however no binding experiments to mucus were carried out [216].

A 29 kDa protein (named *Lr* W1) isolated from *L. reuteri* JCM 108 and separated on an SDS-PAGE was shown to bind to mucus and epithelial cells [241]. However the protein was in a denaturated form and no experiment was performed using the native form of the protein. *Lr* W1 shows 71.1% sequence similarity to protein Lr0793 from *L. reuteri* ATCC 55730 which is a CnBP homologue. *Lr* W1 N-terminal aa sequence shows similarity to CnBP and like Map A and CnBP, *Lr* W1 is part of the ABC transporter family [122, 241, 243]. Together this information indicates that the role played by Map A, CnBP and *Lr* W1 in mucus adhesion may be similar.

A review on the extracellular biology of lactobacilli by Kleerebezem *et al* highlighted the prediction of 2451 putative extracellular proteins from 13 lactobacilli genomes. The largest exoproteomes were found in *L. casei* (306 proteins representing 11.1% of all proteins encoded in genome) and *L. plantarum* (313 proteins 10.4 %), whereas the smallest exoproteomes were found in *L. fermentum* (128 proteins 6.9 %) and *L. reuteri* (117 proteins 6.1 %). On average the function of up to 60 % of these exoproteins are unknown [232], revealing large numbers of potential cell surface proteins for the interaction of bacteria with the GI tract.

1.6 **Aims and objectives of the research project**

1.6.1 Aim

The overall aim of the project is to investigate the mechanisms by which mutualistic gastrointestinal (GI) microbes bind to intestinal mucus, assessing the contributions of specific molecular interactions involved in the adhesion process.

1.6.2 Objectives

The first objective is to screen mutualistic *Lactobacillus reuteri* strains from different host origin for mucus adhesion and autoaggregation properties, using *in vitro* adhesion and autoaggregation assays.

The second objective is to investigate the role played by specific mucus binding proteins of representative *L. reuteri* strains, in bacterial autoaggregation and mucus adhesion.

The third objective is to identify novel *L. reuteri* cell surface factors potentially involved in binding to mucus using a quantitative proteomics approach

2 Materials and Methods

2.1 General Materials

The water used in this study was deionised and ultrapure to resistance 18.2 MΩ cm^{-1}, (Barnstead Nanopure Diamond, Barnstead Thermolyne Corporation, New Hampshire, USA). All chemicals used in this study were from Sigma-Aldrich (Poole, Dorset, UK) unless otherwise specified. Phosphate buffered saline (PBS) with the following composition, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.7 mM Kill, 137 mM NaCl (pH 7.4) was used throughout the study.

Porcine gastric mucin (PGM) from Orthana (Biofac A/S, Kastrup, Denmark), was used for the standard curve in protein concentration assays, and type III porcine gastric mucin (PGMIII) from Sigma (Sigma-Aldrich, Poole, Dorset, UK) was used for media supplementation and as a control throughout the optimisation of the mucus characterisation methods.

Blocking Buffer used in this study was Protein Free Blocking Buffer in PBS (Thermo Scientific, Waltham, USA). Fluorescein labelled lectins, Wheat germ agglutinin (WGA), succinylated wheat germ agglutinin (SWGA), *Sambucus nigra* agglutinin (SNA), *Ricinis communis* agglutinin I (RCA), concanavalin A (ConA), *Lotus tetragonobolus* lectin (LTL) and *Ulex europaeus* agglutinin (UEA) were all from Vector Labs (Peterborough, UK).

Rabbit polyclonal antibodies used in Western blotting, flow cytometry (FCM), immunogold labelling, competition assays, purification of native proteins and protein adhesion assays were produced by BioGenes (Berlin, Germany) to titres of > 200 000 (Table 2.1). Preimmune serum (PIS) was provided for each rabbit.

Antibody	Raised against	AA sequence*
Anti-MubR5	Recombinant type 2 Mub repeat 5	G2105……D2289
Anti-MubRI	Recombinant type 1 Mub repeat I	V550…….T742
Anti-Lar	Recombinant Lar repeat 1	K577…….D672

Table 2.1 Polyclonal antibodies used throughout study. * The aa positions given are those on the full-length protein before removal of the secretion signal.

2.2 **Microbiology**

2.2.1 Bacterial strains, media and culture conditions

L. reuteri strains used in this study are listed in Table 2.2. Bacterial culture media, de Man, Rogosa and Sharpe (MRS) and two variations of *Lactobacillus* defined media (LDM) were used in this study. The MRS is composed of 10 mg ml^{-1} peptone, 8 mg ml^{-1} "Lab-Lemco", 4 mg ml^{-1} yeast extract, 20 mg ml^{-1} glucose, 2 mg ml^{-1} dipotassium hydrogen phosphate, 5 mg ml^{-1} sodium acetate $3H_2O$, 2 mg ml^{-1} triammonium citrate, 0.2 mg ml^{-1} magnesium sulphate $7H_2O$, 0.05 mg ml^{-1} Manganese sulphate $4H_2O$ and 1 ml sorbitan mono-oleate. The composition of LDM II and LDM III is reported in Appendix 1.

The cultures were maintained as frozen stocks held at -80 °C in MRS with 20-50 % (v/v) glycerol. *L. reuteri* strains were grown from frozen stocks to stationary phase in MRS broth at 37 °C for 20 h. Cells were subcultured to 0.2 % by volume (20 µl into 10 ml) in MRS or prewarmed (37 °C) LDM II and grown at 37 °C to exponential phase (9.5 h) or to early stationary phase (16 h). For the induction assay, bacterial cells were grown from frozen stocks as above then subcultured to 0.2 % by volume (20 µl into 10 ml) in either MRS or prewarmed (37 °C) LDM II, supplemented with 0.1 % (w/v) Type III porcine gastric mucin (PGMIII) and grown at 37 °C to early stationary phase (16 h). For growth curves, OD_{600} values were measured every hour for ~80 h. Three biological replicates were performed.

2.2.2 Carboxyfluorescein (cF) labelling of bacteria

L. reuteri strains were grown to early stationary phase in MRS, cells were collected by centrifugation (1342 x*g*, 5 min, 15 °C), washed twice with PBS and resuspended in PBS. Cell suspensions were adjusted to an optical density at 600 nm (OD_{600}) of 0.5, representing approximately 2 x 10^8 cells ml^{-1}. The bacterial cells were then labelled by incubation with 10 µM carboxyfluorescein diacetate (cFDA) (Sigma-Aldrich, Poole, Dorset, UK) at 37 °C for 40 min. Carboxyfluorescein (cF) labelled cells were then centrifuged, washed and resuspended in PBS.

Strain	Source	Comments
ATCC 53608	Pig	Purchased from the American Type Culture Collection (ATCC; Lab of the Government Chemist, LGC Standards, Teddington, UK), derived from strain 1063
DSM 20016	Human	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
MM4-1a	Human	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
MF2-3	Human	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
CF4-6g	Human	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
FJ1	Human	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
MF14-C	Human	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
LMS11-3	Human	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
sr11	Human	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
JW2015	Pig	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
Lp167-67	Pig	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
20-2	Pig	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
3c6	Pig	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
CR	Rat	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
One-One	Rat	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
ATCC 55739	Rat	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
DSM 17509	Rat	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
N2D	Rat	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
R2LC	Rat	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
ML1	Mouse	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
#20	Mouse	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
r13	Mouse	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
Lr4020	Mouse	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
LB54	Chicken	Kindly supplied by Jens Walter, University of Nebraska, Lincoln, USA
MM4KO	Human	Full name MM4-1a Lar_0958 K/O was kindly provided by Stefan Roos, University of Agriculture Science (SLU), Uppsala, Sweden.
1063N	Pig	Isolated by Donald MacKenzie at the Institute of Food Research (IFR), Norwich, UK, was a spontaneous mutant derived from strain 1063 (isolated from pig) supplied by Jens Walter.

Table 2.2 *L. reuteri* strains used in this study.

2.3 **Biochemistry**

2.3.1 Mucus preparation

For preparation of the solublised mouse colonic mucus (MCM-S), mouse large intestines (from wild type C57BL/6 mice) were washed with cold sterile PBS additionally containing a cOmplete Mini Protease Inhibitor Cocktail Tablet per 10 ml PBS (Roche, Basel, Switzerland). The mucosal surface was scraped with a glass slide to remove the mucus layer. Mucus scrapings were collected into 1 ml of ice-cold, PBS containing 4 M guanidine hydrochloride and protease inhibitors. The tissue was kept on ice and homogenised with both an Ultra-Turrax polytron (IKA, Staufen, Germany) and a Status 70 ultrasonicator set to 3 x 30 s pulses at 45 % power (Philip Harris Scientific, Lichfield, UK), with intermediate incubation steps on ice for 30 s, then the sample was centrifuged (17 000 x*g*, 1 h, 4 °C) to remove cell debris. The sample was stored at -20 °C. For preparation of the crude mouse colonic mucus (MCM-C), mouse small intestinal mucus (MSIM) and pig small intestinal mucus (PSIM), mouse small and large intestines (from wild type C57BL/6 mice) were rinsed with ice cold sterile PBS + 0.05 % (w/v) Tween 20 (PBST 0.05 %). The mucosal surface was scraped with a scalpel and the mucus layer placed into ~1 ml ice cold PBST 0.05 %. The tissue was rinsed using ice cold PBST 0.05 %, and material collected in the solution was combined with the scraped material. Fresh porcine small intestine, obtained from the local slaughterhouse, was rinsed with 67 mM phosphate buffer (pH 6.7) containing 0.02 % w/v sodium azide and a mix of protease inhibitors (Roche Diagnostics GmbH, Mannheim, Germany; 1 tablet per 50 mL buffer). Mucus was removed by scraping the epithelial surface of the jejeunal segment of the intestine with a plastic scraper (Corning, NY). After mixing by vortex (Genius 3- IKA, Staufen, Germany) the mucus extracts were centrifuged (17 000 x*g*, 1 min at room temperature (RT)). The supernatant was stored, 1 ml of fresh ice cold PBST 0.05 % was added to the pellet and mixed. The samples were sonicated (Branson 2110, Danbury, Connecticut, USA) for 15 min at RT then centrifuged (17 000 x*g*, 1 min at RT). The supernatant was combined with the saved supernatant and the sample was stored at -20 °C. Mucin concentrations were estimated using the bicinchoninic acid (BCA) assay (see section 2.5.1) with PGM for the standard curve.

2.3.2 Mucus characterisation: agarose/polyacrylamide composite gel electrophoresis (AgPAGE) and Western blot

Composite gels were made by mixing two heated solutions, solution A (1.2 % agarose, 0.375 M Tris-HCl (pH 8.1), 12 % glycerol, 7 % acrylamide/bis-acrylamide w/w ratio

19:1) and solution B (0.5 % agarose, 0.375 M Tris-HCl (pH 8.1)). The 0.5-1.2 % agarose gradient, 0-7 % polyacrylamide gradient, 0-12 % glycerol gradient gels were cast in the mini-Protean gel casting apparatus (Bio Rad, Hertfordshire, UK), at 60 °C, after adding tetramethylethylenediamine (TEMED) 0.03 % and ammonium persulphate 0.01 % to both solutions. The gel was allowed to set for 3 h at RT, then kept at 4 °C overnight in a humidified environment.

Samples were reduced and alkylated in sample loading buffer to a final concentration 0.23 M Tris-HCl (pH 8.1),18 % glycerol, 0.003 % bromophenyl blue, 0.6 % SDS, 200 mM dithiothreitol (DTT), for 3 h at 37 °C, and stored at -20 °C until needed. Immediately prior to loading on the gel, samples were boiled at 92 °C for 5 min. Gels were run at 300 V for 3 h at 4 °C in 192 mM Tris-borate (pH7.6), 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 % SDS. Gels were stained using Alcian Blue following the protocol in Schulz *et al* [244] and Colloidal Blue (Life Technologies, Ltd., Paisley, UK), following manufacturer's instructions.

Prior to Western Blotting, Colloidal Blue stained gels were destained with 10 % acetic acid overnight, incubated in 50 mM Tris-HCl pH 7.5, 1 % SDS for 1 h. Proteins were transferred at 150 V for 4 h at 4 °C to HybondP 0.45 µm polyvinylidene difluoride (PVDF) membrane (GE Healthcare, New Jersey, USA) using 3 mm filter paper (Whatman, Maidstone, UK), and following the procedure from Life Technologies blotting handbook (Life Technologies, Ltd., Paisley, UK), with blotting buffer 25 mM Tris-HCl, 192 mM glycine, 0.04 % SDS, 20 % methanol. Membranes were blocked with Blocking Buffer, incubated overnight with 1/1000 dilution in PBS of fluorescein labelled lectins. Membranes were washed twice in PBS before fluorescent signals were detected using a Pharos-FX Plus Molecular Imager (BioRad, Hemel Hempstead, UK).

2.3.3 Adhesion (Bacterial, Protein and Induction assays)

2.3.3.1 Bacterial Adhesion Assay

Bacteria were grown to early stationary phase and cF labelled as above (section 2.2.2), mucus prepared as above (section 2.3.1) was diluted in PBS (1 mg ml^{-1} for adhesion to MCM-S, or 0.1 mg ml^{-1} for adhesion to PSIM and MSIM) and 200 µl of the solution was loaded into high binding, black, polystyrene microtitre plate wells (Greiner Bio-One Ltd., Stonehouse, UK) and incubated for 20 h at 4 °C. The wells were washed three times with 200 µl PBST 0.05 % using a plate washer (Applied Quality Systems, Tonbridge, UK). Then, 200 µl of Blocking Buffer was added to each well and the plates incubated

at RT for 1 h. The wells were washed as above, before cF-labelled bacteria (200 µl) were added to each well and incubated at 37 °C for 4 h. The wells were washed as above and adhered bacteria were lysed by incubation at 37 °C for 1 h with 200 µl of 1 % (w/v) SDS in 0.1 M NaOH. The released cF fluorescence was measured in a FLUOstar OPTIMA plate reader (BMG Labtech, Offenburg, Germany) at 485 and 520 nm as excitation and emission wavelengths, respectively. Negative controls of bovine serum albumin (BSA) coated wells were included. Each assay was performed in triplicate and on at least three separate occasions. Standards were included on each occasion also in triplicate consisting of 200 µl of SDS lysed cF-labelled bacteria in order to evaluate the % adhesion.

Competitive bacterial binding assays were performed as described above but with the following modifications, prior to addition of 200 µl of labelled bacterial suspension to each well, the bacterial suspensions were incubated at 37 °C for 1 h with 1 % (v/v) of one of the following, anti-Mub-R5, anti-Mub-RI, anti-MubR5 and anti-MubRI combined, 0.5 % (v/v) of anti-Lar or the preimmune serum from each corresponding rabbit as a negative control.

2.3.3.2 Protein adhesion assay

Mucus prepared as above (section 2.3.1) was diluted in PBS to 0.1 mg ml^{-1} and 200 µl of the obtained solution was loaded into high binding polystyrene microtitre plate wells (Greiner Bio-One Ltd., Stonehouse, UK) and incubated for 20 h at 4 °C. In order to remove unbound mucus components, the wells were washed three times with 200 µl PBST 0.05 % using a plate washer (Applied Quality Systems, Tonbridge, UK). Then, 200 µl of Blocking Buffer was added to each well and the plates incubated at RT for 1 h. The wells were washed as above, then, 100 µl of protein (native MUB, recombinant MubR5, recombinant MubRI-II-III or recombinant Lar repeat) at a concentration of 0.1 mg ml^{-1} was added per well and the plates incubated at RT for 2 h. The wells were washed as above to remove unbound protein. Primary antibody either anti-MubR5, anti-MubRI or anti-Lar or negative control PIS from the appropriate rabbit was added to the wells, (100 µl at 1/20 000 dilution in PBS) and incubated at RT for 1.5 h. The wells were washed as above. Secondary antibody anti-rabbit IgG alkaline phosphatase (AP) (100 µl at 1/30 000 dilution in PBS) was added to the wells and incubated at RT for 1.5 h. Substrate SIGMAFAST p-nitrophenol phosphate (pNPP) (100 µl of 1 mg ml^{-1} pNPP in 0.2 M Tris 5 mM $MgCl_2$, pH 9.6-10.5; Sigma) was added to the wells and absorbance was measured at 405 nm after overnight incubation at RT. Each assay was performed

in triplicate and the data were analysed in Excel (Microsoft, Washington, USA) see section 2.3.5.

2.3.3.3 Induction assay

Following the method by Jonsson *et al* 2001 [191], *L. reuteri* strains were grown to early stationary phase in MRS or LDM II broth either in the presence or absence of mucin supplementation at 0.1 % (w/v) PGMIII. Bacterial cells were collected by centrifugation (9000 x*g*, 6 min, RT), washed once with PBST 0.05 % and resuspended in PBST 0.05 % to an OD_{600} of 0.5.

Mucus samples prepared as described in section 2.3.1 were diluted to 0.1 mg ml^{-1} in PBS and 100 µl was loaded into sterile, flat bottom, 96-well microtitre plates (Greiner Bio-one, Stonehouse, UK) and incubated for 20 h, at 4 °C. In order to remove unbound mucus components, the wells were washed with 200 µl PBST 0.05%. Then, in order to block the wells, 200 µl of PBST 1 % was added per well and the plates incubated at RT for 1 h. The wells were washed twice as above with PBST 0.05 %. Then, 100 µl of PBST 0.05 %-washed bacteria was added per well and the plates incubated at RT for 2 h. The wells were washed four times with PBST 0.05 % to remove unbound bacteria, and 100 µl PBST 0.05 % was added to the wells before adhered bacteria were examined under an inverted microscope with an Olympus U-PMTVC 1514899 UC30 camera (Olympus, Southend on Sea, UK) to capture images. The PBST was removed from the plate, the wells allowed to dry for 20 h at RT and the dry plate was read at 460 nm using the FLUOstar OPTIMA plate reader (BMG Labtech, Offenburg, Germany). Each assay was performed in triplicate and on at least three separate occasions. Negative controls of BSA coated wells were included on each occasion also in triplicate. Data were analysed in Excel (Microsoft, Washington, USA), see section 2.3.5.

For the protease treatment of bacterial cells, the induction assays were performed as outlined above with the following changes, the PBST washed bacteria were diluted to OD_{600} 1 and incubated for 1 h at 37 °C with 1 ml of either PBS, shaving buffer (20 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 M D-arabinose, 10 mM CaCl2), or 1.7 µg ml^{-1} trypsin in shaving buffer. After the incubation cells were washed once (5000 x*g* for 5 min) and then resuspended to OD_{600} 0.5 in PBST 0.05 % and 100 µl of cell suspensions were added to the coated wells.

2.3.4 Spectrophotometry autoaggregation assay

L. reuteri strains were grown to early stationary phase in MRS broth, cells were collected by centrifugation (1342 x*g*, 5 min, 15 °C), washed twice with PBS and resuspended in PBS to an OD_{600} of 0.5. Samples (1 ml) were analysed by spectrophotometry using a U-3010 Spectrophotometer (Hitachi, Tokyo, Japan) by recording OD_{600} values every 10 min for 5 h at 37 °C.

2.3.5 Statistical Analysis

For bacterial adhesion assay (section 2.3.3.1), protein adhesion assay (section 2.3.3.2), and induction assay (section 2.3.3.3) the data were analysed in Excel (Microsoft, Washington, USA), statistical analysis using Student's *t*-test.

For the spectrophotometric autoaggregation assay (section 2.3.4), the data were analysed using Excel and One-way ANOVA statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) (IBM, New York, USA) on normalised data (inversed).

2.3.6 Flow cytometry (FCM)

FCM was carried out using bacteria were grown in filtered (0.2 µm) MRS broth to early stationary phase. Aliquots (1 ml) were harvested (17 000 x*g*, 2 min 4 °C) and washed once with filtered (0.2 µm) PBS buffer and the OD_{600} value was adjusted to 2.5 with PBS. Bacterial cells were analysed in a FC500 flow cytometer using CXP software (Beckman Coulter, High Wycombe, UK). At least 20,000 events per sample were acquired at low flow rate.

To determine bacterial cell numbers, the number of events (corresponding to cells) was counted for 1 min at a constant flow rate of 10 µl min^{-1}. To determine bacterial autoaggregation ability, the forward scatter (FS) and side scatter (SS) signals of PBS-washed cells were recorded. To determine the cell surface expression of the MUB and Lar_0958 proteins, the following additions to the above method were made. Prior to FCM analysis, aliquots (50 µl) of PBS-washed cells were incubated with polyclonal anti-MubR5 or anti-Lar antibody (1:100 final dilution in PBS) in the dark on ice for 30 min. Following a wash with 1 ml PBS, the cells were resuspended in 25 µl PBS containing goat anti-rabbit IgG (whole molecule)-fluorescein conjugated antibody (1:26 final dilution in PBS) and incubated in the dark at RT for 15 min. Finally, 20 µl of this

treated cell suspension was diluted in 0.6 ml PBS, (for ATCC 53608) or 1 ml (all other strains). The level of protein on the cell surface was quantified by measuring the green fluorescent signal in FL1 channel (530/30 nm). Data were analysed using FlowJo (Tree Star Inc., Ashland, USA), Excel (Microsoft, Washington, USA) and SPSS (IBM, New York, USA).

2.3.7 Immunogold scanning electron microscopy (SEM)

In order to visualise MUB and Lar_0958 proteins on the surface of whole bacteria by immunogold SEM, bacteria were grown to stationary phase in MRS broth (16 h incubation at 37 °C), washed once with PBS and resuspended in PBS to OD_{600} 3.0 (~ 1 $\times$ 10^9 cells ml^{-1}). The PBS-washed bacteria were air dried on to carbon-coated nickel grids and fixed by exposure to 25 % (v/v) glutaraldehyde vapour. Residual free aldehydes were blocked with PBS buffer (pH 7.4) containing 50 mM glycine for 15 min. The grids were incubated in goat blocking solution (Aurion, Wageningen, The Netherlands) for 1 h, washed for 3 $\times$ 5 min with incubation buffer (PBS buffer containing 0.1 % (w/v) BSA-c™ (Aurion, Wageningen, The Netherlands)), then incubated overnight at 4 °C with anti-MubR5/anti-MubRI combined or singly, or anti-Lar polyclonal antibody (1:1000) in incubation buffer. Grids were washed 6 $\times$ 10 min with incubation buffer and then incubated for 2 h at RT with goat anti-rabbit IgG-15 nm gold particle conjugated antibody GAR-15 (Aurion, Wageningen, The Netherlands), 1:50 in incubation buffer). Following 6 $\times$ 5 min washes with incubation buffer and 3 $\times$ 5 min washes with PBS, the bacteria were re-fixed in PBS containing 2 % (v/v) glutaraldehyde. After 1 $\times$ 5 min wash with PBS and finally 2 $\times$ 5 min washes with ultrapure water, the grids were examined and photographed in a FEI Tecnai G2 20 Twin transmission electron microscope. Control grids treated with preimmune serum (PIS) or treated only with the secondary antibody were included.

2.4 **Molecular Biology**

2.4.1 Genomic DNA extraction

Bacteria were grown to early stationary phase in MRS (37 °C, 16 h) and the equivalent of 1.5 ml of an OD_{600} value of 3 (approximately 2 x 10^9 bacteria) was used for extraction. Genomic DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Crawley, West Sussex, UK) following the manufacturer's instructions for Gram-positive bacteria and increasing the lysis incubation time at 37 °C to 1 h. DNA

was eluted in buffer “AE” (200 µl per extraction) (Qiagen, Crawley, West Sussex, UK) was stored frozen at -20 °C.

2.4.2 General bioinformatics tools

SignalP v4.0 was used for the prediction of secretion signals [245] (www.cbs.dtu.dk/services/SignalP/). Protein domain predictions were performed using the Conserved Domain search tool from the National Centre for Biotechnology Information (NCBI) website, (www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

2.4.3 Polymerase chain reaction (PCR) of *mub* and *lar_0958* genes

Bacteria were grown to early stationary phase in MRS broth, cells were collected by centrifugation (1342 x*g*, 5 min, 15 °C), washed twice in ultrapure water, resuspended to OD_{600} 5.5 in ultrapure water. Whole cell PCR was performed in 50 µl reaction volumes consisting of 10 pmol each primer, 125 U HotStarTaq (Qiagen, Crawley, West Sussex, UK), and 10 µl of washed bacterial cell suspension. For detection of the *mub* gene, primers MucB1-RVIf and MucB2-RVIr were used and for the *lar_0958* gene, primers lar0958-f1 and lar0958-r1 were used (see Table 2.3). The reactions were carried out in a thermocycler (Veriti 96 well - Applied Biosystems, Carlsbad, California USA) with the following programme: 15 min of initial denaturation and Taq activation at 95 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at either 50 °C (*mub*) or 58 °C (*lar_0958*) and either 60 s (*mub*) or 40 s (*lar_0958*) at 72 °C and a final extension step for 10 min at 72 °C. The resulting PCR products were analysed by agarose electrophoresis gel (section 2.4.4) and DNA sequencing.

Primer	Sequence	Tm
MucB1-RVIf	5’-ATGCAAGAAGCTCAAGCCATC-3’	66 °C
MucB2-RVIr	5’-TTAATCAAGCTTCTTGTAGGT-3’	55 °C
lar0958-f1	5’-AAAGTTACCTATAGTGGTAGTG-3	60 °C
lar0958-r1	5’-AATCCCTAGTGGATTAATCGTG-3	62 °C

Table 2.3 Primers used for *mub* and *lar_0958* gene detection by PCR.

2.4.4 Agarose gel electrophoresis

Agarose gels (1.5 %) were used for the separation of PCR amplification products. All gels were prepared using TAE buffer (20 mM Tris-base, 10 mM acetic acid, 5 mM EDTA, pH8). Samples were loaded into the gel after addition of the Orange G loading dye (New England BioLabs Inc., Massachusetts, USA) according to manufacturer’s

instructions. Electrophoresis was carried out using the Horizon58 system from Biometra (Goettingen, Germany) with a constant voltage of 100 V. The gels were incubated in 0.5-1 µg ml^{-1} ethidium bromide solution for 30 min before PCR products were visualised on a shortwave UV transilluminator. Gel images were taken using the Alphaimager (Protein Simple, Santa Clara, California, USA).

2.4.5 Random amplified polymorphic DNA (RAPD) analysis

RAPD analysis was performed in 50 µl reaction volumes consisting of 100 pmol primer, (see Table 2.4) 125 U HotStarTaq in x1 PCR buffer and approximately 40 ng of genomic DNA (see section 2.4.1 for genomic DNA extraction method).

Primer	Sequence 5'-3'	Tm °C	GC%	Reference
BF2	CGGCCCCTGT	29	80	[246]
1247	AAGAGCCCGT	25	60	[246]
1254	CCGCAGCCAA	27	70	[246]
XD8	CAAGGCCATCC	29	63.6	[246]
XD9	GAAGTCGTCC	25	54.5	[246]
M13	GAGGGTGGCGGTTCT	60	66.7	[246]
M14	GAGGGTGGGGCCGTT	65	73.3	[246]
Coc	AGCAGCGTGG	27	70	[246]
OPL-04	GACTGCACAC	25	60	[247]

Table 2.4 List of primers tested in RAPD-PCR analysis

The reactions were carried out in a thermocycler (Veriti 96 well - Applied Biosystems, Carlsbad, California USA) with the following programme: 15 min of initial denaturation and Taq activation at 95 °C, followed by 45 cycles of 30 s at 94 °C, 30 s at either 33 °C (primers BF2, 1247,1254, XD8, XD9, Coc, and OPL-04) or 45 °C (primers M13 and M14) and 1 min at 72 °C and the final extension step for 10 min at 72 °C. The amplification products were analysed by agarose gel electrophoresis (see section 2.4.4).

2.5 **Proteomics**

2.5.1 Protein Quantitation Assays

Protein concentrations were estimated either using the BCA assay (Sigma-Aldrich, Poole, Dorset, UK) according to manufacturer's instructions, or by NanoDrop ND-1000 (Thermo Scientific, Waltham, USA), when an absorbance value of 1 at 280 nm was equivalent to 1 mg ml^{-1} unless otherwise specified.

2.5.2 Preparation of cell wall extracts, soluble cytoplasmic extracts and spent media extracts

The bacteria were grown to early stationary phase in MRS broth, cells were harvested by centrifugation at 1342 x*g* for 10 min. The spent growth media (2 ml) was retained and concentrated using Vivaspin-2 ultrafiltration spin columns with a 100,000 Da molecular weight cut off (MWCO) (Sartorius Stedim Biotech, Aubagne Cedex, France) at 4 °C, following the manufacturer's instructions. The concentrated samples were then buffer-exchanged with PBS and centrifuged to give approximately 50-70 fold concentration, this sample was named spent media (SM) extract. Soluble cytoplasmic extracts (SCE) and cell wall extracts (CWE) were prepared from PBS-washed bacteria following the method described in [173] with modifications. Briefly, cell pellets from each 20 ml culture were weighed and the cells were disrupted by vortexing with an equal volume of 425-600 µm diameter glass beads (Sigma-Aldrich, Poole, Dorset, UK) and 17 ml PBS g^{-1} wet weight of cells for 3 $\times$ 2 min pulses at full speed with 2 min intervals on ice between pulses. Undisrupted cells were removed by centrifugation at 1000 x*g* for 1 min, the supernatant was recovered and recentrifuged at 16 000 x *g* for 30 min at 4 °C to pellet the cell wall material. The supernatant from the latter spin was kept as the SCE. The cell walls were resuspended in 50 µl of digestion buffer (50 mM Tris-HCl, 5 mM $MgCl_2$, 5 mM $CaCl_2$, 10 mg ml^{-1} hen egg white lysozyme (HEWL) and 150 U ml^{-1} mutanolysin) and incubated for 3 h at 37 °C, to release the cell wall proteins. The CWE was clarified by centrifugation at 17 000 x*g* for 30 min at 4 °C, and the supernatant was recovered and stored at -20 °C.

2.5.3 SDS-PAGE and Western Blotting

NuPAGE® 4-12 % Bis-Tris gels were used with 3-(N-morpholino)propanesulphonic acid (MOPS) SDS running buffer (50 min at 200 V constant voltage) and HiMark™ Unstained High Molecular Weight Protein Standard as marker. Samples were prepared for SDS-PAGE by adding lithium dodecyl sulphate (LDS) and Reducing Agent, all according to manufacturer's instructions (Invitrogen, Life Technologies Ltd., Paisley, UK).

Gels were stained with the Colloidal Blue staining kit (Life Technologies Ltd., Paisley, UK) and scanned in a GS-800 calibrated densitometer (Bio-Rad, Hertfordshire, UK). The software TL120 v2006e from Nonlinear Dynamics Ltd (Newcastle-upon-Tyne, UK) was used for analysis of apparent molecular weight (MW).

For Western Blot, proteins were transferred electroblotted onto Immobilon^{TM}-P membrane with 0.45 µm pore size, (Millipore, Watford, UK) using the XCell II blot module and NuPAGE transfer buffer (Life Technologies Ltd., Paisley, UK), following the manufacturer's instructions. Membranes were blocked with Blocking Buffer for 1 h and proteins detected with either anti-MubR5 or anti-Lar polyclonal antibody (1:1000 dilution in PBS), followed by goat anti-rabbit IgG (whole molecule)-alkaline phosphatase conjugate (1:30,000 dilution in PBS) and incubated with 100 µg ml^{-1} Nitroblue tetrazolium, 50 µg ml^{-1} 5-bromo-4-chloro-3-indolyl phosphate-toluidine in 4 mM $MgCl_2$, 100 mM Tris-HCl (pH 9.6) as alkaline phosphatase substrate for 20 min. All washing steps between incubations were performed in PBST 0.05 %.

2.5.4 Cell surface proteome extraction

Bacteria were grown to early stationary phase in MRS broth, cells (50 ml) were harvested at 12 000 x*g*, 15 min, 4 °C and washed with shaving buffer (20 mM Tris-HCl, 150 mM NaCl pH 7.4). Cell pellets were resuspended in 0.5 ml of trypsin solution (10 µg ml^{-1} trypsin, 1 M D-arabinose, 10 mM $CaCl_2$, 20 mM Tris-HCl, 150 mM NaCl pH 7.4) and incubated in at 37 °C for 30 min with gentle shaking (10 rpm, Innova 44, New Brunswick Scientific, Enfield, Connecticut, USA). Control samples were incubated in the solution without trypsin. Cells were then centrifuged at 17 000 x*g*, 20 min at RT, and the supernatant collected, filtered (0.2 µm), quick frozen on dry ice and stored at -20 °C. The protein concentration of samples was estimated using the standard BCA assay (see section 2.5.1) using BSA for the standard curve.

Shaved protein extracts were further digested with trypsin or chymotrypsin as follows, samples (50 µg) were diluted in digestion buffer 1 (DB1: 8 M Urea 100 mM Tris, pH 8, 5 mM DTT) to a volume of 50 µl and incubated at RT for 2 h before 389 µg iodoacetamide (IAA) was added to each sample, followed by an incubation in the dark at RT for 20 min. Ammonium bicarbonate (50 mM) was added to reduce the urea concentration to 1 M, and trypsin or chymotrypsin was added to the samples to make an enzyme: protein ratio of 1:50. Samples were incubated at 28 °C, 16 h and then adjusted to 0.1 % (w/v) with trifluoracetic acid using a 2.5 % stock solution. The solution was filtered by OMIX Varian c18 tips (Walnut Creek, California, USA) following the manufacturer's instructions. The samples were dried using a speed vac (Thermo Scientific, Waltham, USA) and frozen at -80 °C. Samples were reconstituted with 80 µl, 0.5 % (v/v) formic acid, sonicated for 5 min, then 45 µl loaded into the LTQ-Orbitrap

plate (to enable two injections of 20 µl), with blanks of 50 % (v/v) acetonitrile in between each sample.

Samples were analysed by LTQ-Orbitrap, the resulting RAW files were converted to Mascot generic files using Proteome Discover version 1.1.0.263 (Thermo Scientific, Waltham, USA) prior to protein identification using the MS/MS ion search with an in-house version of Mascot search engine (Matrix Science Ltd., London, UK) against an in-house *L. reuteri* ATCC 53608 proteomic database.

2.5.5 Purification of MUB and Lar_0958 proteins

Bacteria (*L. reuteri* ATCC 53608 or MM4-1a) were grown to early stationary phase in LDM II media, cells were removed from the spent medium by centrifugation at 10 458 x*g* for 15 min at 4 °C. The medium was then filtered through a 0.22 µm filter before concentration using the Vivaflow tangential flow filtration system at 4 °C, with 50 and 200 cassettes run in parallel (Vivascience AG, Hannover, Germany). The concentrated medium extract was then dialysed in PBS using Spectra/Por membrane MWCO 2500 Da, before a final filtration step using 0.45 µm PVDF membrane spin columns (Millipore Ultrafree-CL Centrifugal devices). The proteins in the medium extract were then separated using a prepacked gel filtration Superpose 6 PC 3.2/30 column on an AKTA Fast Protein Liquid Chromatography (FPLC) system (GE Healthcare, New Jersey, USA), with PBS as the eluent, detecting protein content of the fractions at 280 nm. The fractions were analysed by SDS-PAGE and Western Blot (see section 2.5.3).

2.5.6 Preparation of stable isotope labelling with amino acids in cell culture (SILAC) samples

Bacterial cells were grown from frozen stocks overnight in MRS, then spun at 1342 x*g* for 6 min at RT, spent MRS was removed and cells were resuspended in LDM III without arginine or mucin then subcultured to 2 % by volume (3 ml into 150 ml) in LDM III, with one of four variations, a) heavy arginine b) heavy arginine supplemented with 0.1 % (w/v) PGMIII c) light arginine or d) light arginine supplemented with 0.1 % (w/v) PGMIII and grown at 37 °C to early stationary phase (16 h), the bacteria were subcultured again into the same media, grown at 37 °C to an OD_{600} 0.4. The heavy arginine and light arginine pairs of cultures, 100 ml each (one with mucin, the other without) and the reciprocal pair were mixed in equal amounts to give 200 ml at OD_{600} 0.4. Three biological replicates were performed.

Cells were harvested using the Avanti J-26XP (Beckman Coulter, High Wycombe, UK) high performance centrifuge at 10 000 x*g*, for 30 min, at 4 °C. The spent media supernatant was concentrated x8-fold through an Amicon Ultra 4 3000 Da MWCO cellulose spin column (Millipore, Watford, UK), as described in 2.4.6 and the sample was designated secreted proteome (SP).

Cells were treated with shaving buffer as described in section 2.3.8, but with 2.7 µg ml^{-1} trypsin. Samples were centrifuged at 17 000 x*g*, 20 min at RT, and the supernatant collected, filtered (0.2 µm), quick frozen on dry ice and stored at -20 °C. This sample was designated cell surface proteome (CSP).

Cell pellets were further processed to extract cell wall proteins. Samples were re-suspended in 1 ml PBS and ultrasonicated on ice (Status 70 ultrasonicator 6 x 30 s at 55 % power with 30 s cooling intervals) (Philip Harris Scientific, Lichfield, UK). Samples were centrifuged at 17 000 x*g*, 30 min, 4 °C, and the supernatant discarded. The cell wall pellets were incubated with 1.8 ml g^{-1} wet weight pellet of digestion buffer (50 mM Tris-HCl, pH 9.6, 5 mM $MgCl_2$, 5 mM $CaCl_2$, 10 mg ml^{-1} HEWL at 93 000 U mg^{-1}, 150 U ml^{-1} mutanolysin) for 3 h at 37 °C. The extract was clarified with centrifugation at 17 000 x*g*, 4 °C, 30 min, after which the cell wall proteome (CWP), was removed carefully and stored at – 80 °C until use.

The protein concentration of samples was estimated using the NanoDrop (see section 2.5.1). All samples were further digested with trypsin, following the digest protocol described in section 2.5.4 for the CSP samples while the SP and CWP samples were first separated by SDS-PAGE, (as described in section 2.5.3) and the lanes cut into five sections before an in-gel digestion as described below.

Gel slices were cut into ~1 mm^3 pieces and washed twice with 1 ml Solution A (0.2 M ammonium bicarbonate in 50 % (v/v) acetonitrile) with 15 min incubations in the solution before removal with a pastette. Then gel pieces were washed with 100 % (v/v) acetonitrile, before a 10 min incubation in 100 % (v/v) acetonitrile, the liquid was removed by pastette and samples air-dried. DTT solution (1 ml of 10 mM DTT in 50 mM ammonium bicarbonate) was added to the samples and they were incubated for 30 min at 60 °C. DTT solution was removed by pastette before 1 ml IAA solution (100 mM IAA in 50 mM ammonium bicarbonate) was added and samples incubated for 30 min at RT in the dark. IAA solution was removed with a pastette and gel pieces washed twice

with Solution A, with 15 min incubations in between washes. Gel pieces were then treated with 100 % (v/v) acetonitrile, as described above and air-dried for 16 h. Trypsin solution (30 µl of 10 µg ml^{-1} trypsin in 10 mM ammonium bicarbonate) was added to all samples. After the trypsin solution was fully absorbed, ammonium bicarbonate (10 mM) was added to cover the gel pieces. Samples were incubated at 37 °C for 3 h. An equal volume of 1 % (v/v) formic acid was added to the tubes which were vortexed then allowed to stand for 10 min. Samples containing the digested proteins were then recovered from the tubes by pastette, the gel pieces were incubated for a further 3 min with a further equal volume of 50 % (v/v) acetonitrile. Both samples were pooled together, dried using a speed vac (Thermo Scientific, Waltham, USA) and frozen at -80 °C.

2.5.7 Mass spectrometry of SILAC samples and data analysis

Prior to analysis, 90 µl 0.5 % (v/v) formic acid was added to the SILAC samples above (section 2.5.6), samples were sonicated for 5 min, then 22 µl loaded onto the LTQ-Orbitrap plate in duplicate with blanks of 50 % (v/v) acetonitrile in between samples from each lane of the gel.

All samples were analysed by LC-MS/MS, performed on an Nanoflow-HPLC system (NanoAQUITY) (Waters, Manchester, UK), coupled to the LTQ-Orbitrap MS (Thermo Scientific, Waltham, USA) using the following settings. Samples (20µl) of the digested peptides were trapped off-line to a Symmetry C18 Trap (5 µm 180 µm x 20 mm) which was then switched on-line to a UPLC BEH C18 column (1.7 µm 75 µm x 250 mm) and held at 45 °C. Peptides were eluted by a gradient of 0-80 % acetonitrile in 0.1 % formic acid over 80 min at a flow rate of 250nl min^{-1}. The MS was operated in positive ion mode with nano spray source at a capillary temperature of 200 °C. The LTQ-Orbitrap was run with a resolution of 60 000 over the mass range m/z 300-2000 and an MS target of 10^6 and 1 s max scan time. The MS/MS was triggered by a minimal signal of 2000 with an automatic gain control target of 30000 ions and max scan time of 150 ms. For MS/MS events selection, a charge state of 2+ and 3+ was used. Dynamic exclusion was set to 1 count and 30 s exclusion time with an exclusion mass window of ± 20 ppm.

Raw MS spectra were processed using MaxQuant software suite v 1.2.2.5 [248] (consisting of MaxQuant, Andromeda and Perseus programs from Max Planck Institute, Martinsried, Germany) and peak lists were searched using the Andromeda

search engine [249], for identification of proteins against forward and reversed protein database of *L. reuteri* downloaded from NCBI (http://www.ncbi.nlm.nih.gov/protein/?term=lactobacillus%20reuteri) containing 37,307 protein sequences. The default database search criteria were used, which included carbamidomethylation as a fixed modification, heavy arginine Arg 10 and oxidation (M) as variable modifications, two missed cleavages were allowed, mass tolerance was 0.5 Da at the fragment ion level. Search results were parsed at FDR 1 % at both the peptide and the protein level. The following modifications were made: under the modification and labels tab, Lys8 was removed from the heavy labels, the enzyme was changed to trypsin and the main search ppm to 5; under the identifications and quantification tab, the box for kept low-scoring versions of identified peptides was ticked and also the between parameter groups and match between runs boxes with a time window of 2 min. In Perseus, for SILAC ratios to be considered significantly different, there must be a ratio present in at least three out of the five samples, all going in the same direction (i.e. all positive or all negative), and at least one shown to be significantly different using the Significance B test with Benjamini Hochberg FDR set to 0.05.

For comparison, data were also analysed in Mascot Distiller v 2.4.3.1 (Matrix Science Ltd., London, UK). For quantification purposes the proteins had a minimum of two peptides, with ion score significance threshold of <0.05, a correlation threshold of >0.9 and a standard error threshold of <0.05. For SILAC ratio analysis, Scaffold Q + S v3.4.3 (Proteome Software, Portland, USA) [250] was used to distinguish protein up- or down-regulation displaying a $\log_2$ fold change. These proteins were investigated for subcellular localisation using a combination of bioinformatics tools, SignalP v4.0 (http://www.cbs.dtu.dk/services/SignalP/) [245] for secretion signal prediction, SecretomeP v2.0 (http://www.cbs.dtu.dk/services/SecretomeP/) [251] for non-classical secretion pathway prediction, TMHMM v2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/) [252] for transmembrane helix prediction, LipoP v1.0 (http://www.cbs.dtu.dk/services/LipoP/) [253] for lipoprotein prediction, and PSORTb (http://www.psort.org/psortb) [254] for subcellular localisation prediction.

3 *L. reuteri* adhesion to mucus

Gastrointestinal (GI) mucus is the first point of contact between the gut microbiota and the host, providing the host with defence against pathogens and also a habitat for mutualistic bacteria. It is likely that bacteria which have evolved to reside in the mucosal niche will have developed mechanisms to specifically interact with mucus. Here we investigated autochthonous bacteria-host interactions, by testing the mucus binding ability of a large collection of strains of the model gut symbiont *L. reuteri*, isolated from different vertebrate species, (human, pig, rat, mouse and chicken).

3.1 Extraction and characterisation of GI mucus

Mucus was chosen in this study rather than commercially available mucin, as it represents a more native substrate for binding. Mucus was extracted from mouse colons (MCM), mouse small intestines (MSIM), and pig small intestines (PSIM). Crude mucus extracts were prepared using the method described in Roos *et al* 2000 [255], for PSIM, MSIM and MCM (referred to as MCM-C), resulting in material in a native form. For comparison, solubilised mucus extracts of MCM were also prepared using a GuHCl based extraction method (referred to as MCM-S).

A basic characterisation of the mucus samples was carried out using the agarose/ polyacrylamide composite gel system based on the work of Schulz *et al* 2002 [256], with modifications (see Materials and Methods section 2.3.2). The use of AgPAGE composite gels allows the electrophoretic separation of very high molecular mass mucin glycoproteins, the nature of which make mucus samples unsuitable for traditional electrophoresis and analysis techniques. Commercially available type III pig gastric mucin (PGMIII) was used as a positive control during the optimisation of the method.

The BCA assay was first used to provide an approximate value of the protein concentration in mucus. The loading of the mucus samples onto the AgPAGE gel was further adjusted in order to obtain comparable Colloidal Blue signals from all samples, corresponding to 0.31 mg of MCM-S, 1.12 mg of MCM-C, 0.38 mg PSIM and 2.36 mg MSIM as estimated by BCA assay, using PGM for the standard curve. Mucus samples in the gel were initially probed with Alcian Blue stain (Figure 3.1A). Alcian Blue (copper phthalocyanine), stains acid mucopolysaccharides and glycosaminoglycans, through electrostatic forces with the negatively charged macromolecules.

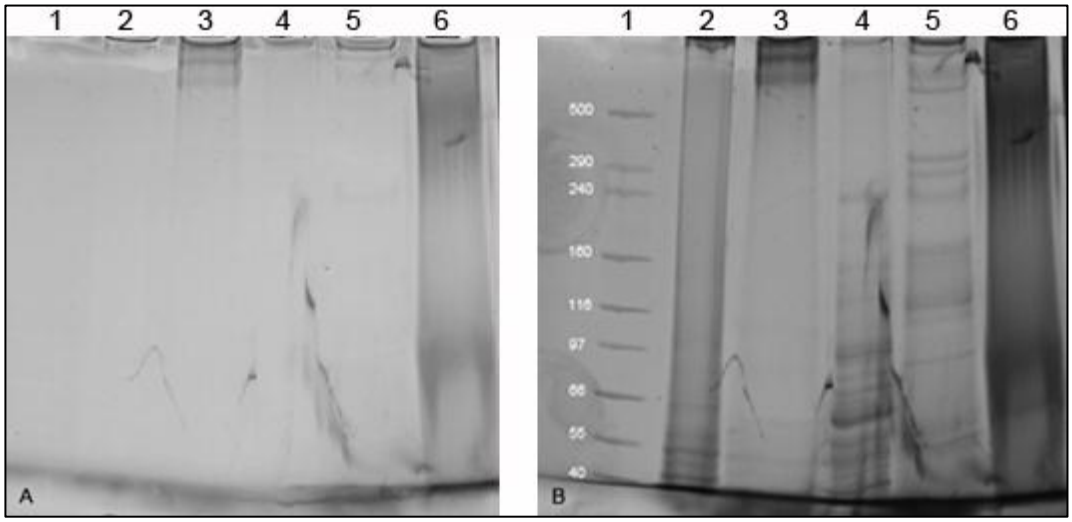


Figure 3.1 Electrophoresis of mucus samples using composite gel stained with A) Alcian Blue and B) Alcian Blue and Colloidal Blue. Lane 1, Hi MW markers; lane 2, MCM-S; lane 3, MCM-C; lane 4, PSIM; lane 5 MSIM and lane 6, mucin control.

At high apparent molecular mass > 500 kDa, strong Alcian Blue staining was observed for the MCM-C mucus sample, with fainter staining for the MCM-S and MSIM samples, indicating the presence of acidic mucins in these samples. The commercial mucin preparation showed strong Alcian Blue staining with a smeared appearance. Subsequent Colloidal Blue staining confirmed the presence of other proteins in the samples (Figure 3.1B), with discrete bands corresponding to apparent molecular mass between 40 and 66 kDa were observed across all samples. The small intestinal mucus samples showed discrete bands between 66 – 290 kDa, whilst bands above 500 kDa were observed across all samples. In order to further characterise the mucus samples with regard to their carbohydrate moieties, the proteins separated on AgPAGE were transferred onto a PVDF membrane and probed with fluorescently labelled lectins specific to mucin sugars (Table 3.1).

Lectin	Abbreviation	Sugar recognition
Wheat germ agglutinin	WGA	N-acetylglucosamine (GlcNAc) Neuraminic (sialic) acid
Succinylated wheat germ agglutinin	SWGA	N-acetylglucosamine (GlcNAc)
Sambucus nigra lectin	SNA	Neuraminic (sialic) acid
Ricinis communis agglutinin I	RCA	Galactose N-acetylglucosamine (GlcNAc) N-acetylgalactosamine (GalNAc)
Concanavalin A	Con A	Mannose
Lotus tetragonobolus lectin	LTL	Fucose
Ulex europaeus agglutinin	UEA	Fucose

Table 3.1 Specificity of lectins used in this study

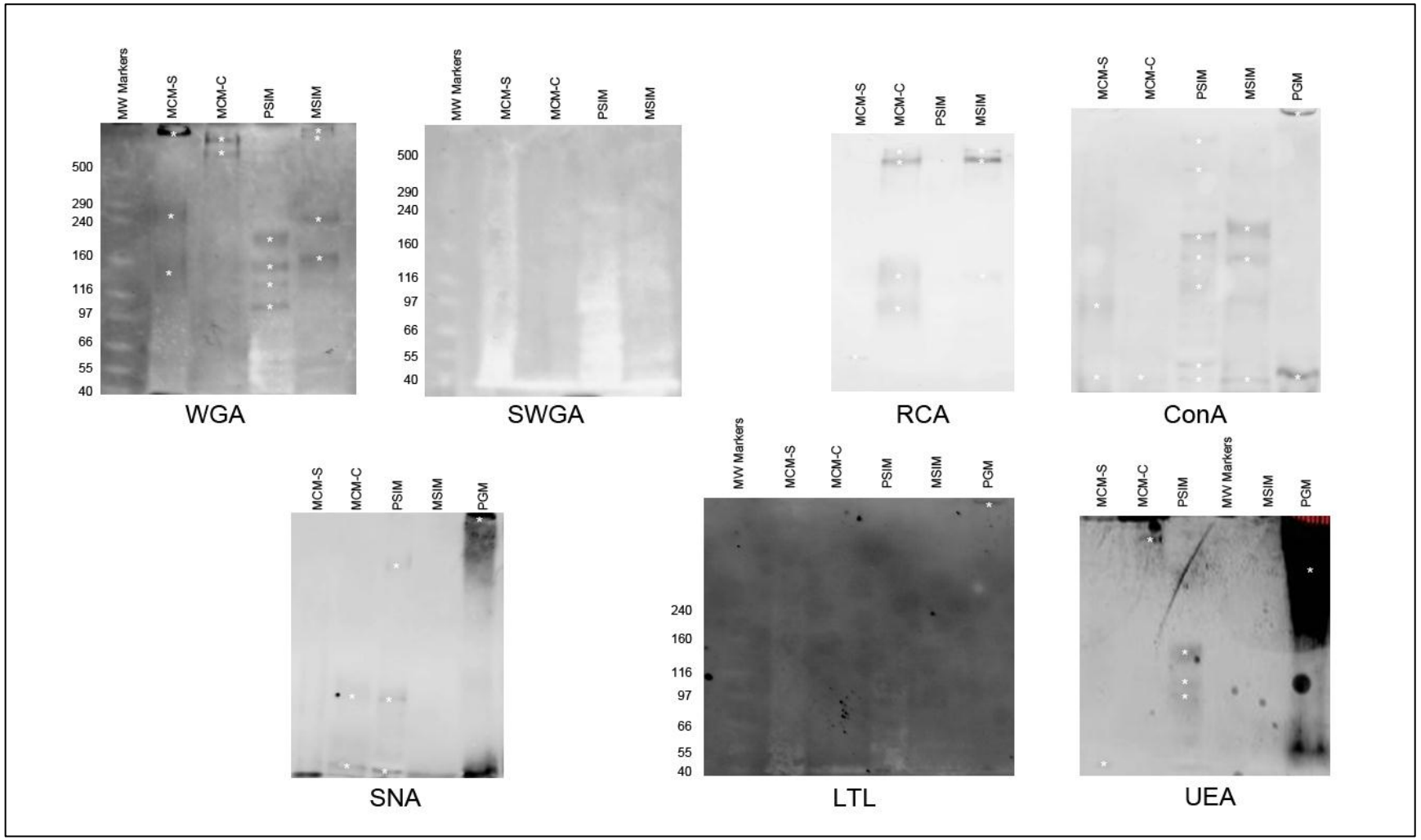


Figure 3.2 Western blot analysis of mucus samples separated by AgPAGE and probed with f-lectins (as indicated). Positive signals indicated with white stars.

Wheat germ agglutinin (WGA) recognises N-acetylglucosamine (GlcNAc) and has also been reported to interact with glycoproteins via sialic acid residues, whereas succinylated WGA (SWGA) does not recognise sialic acid (Table 3.1). All mucus samples tested were positive for WGA but negative for SWGA (Figure 3.2) indicating the presence of sialic acid in all samples. In order to further investigate the sialylated structure present in the mucus samples, the blots were probed with *Sambucus nigra* lectin (SNA), which binds preferentially to sialic acid attached to terminal galactose via an (α-2,6), and to a lesser degree, (α-2,3), linkage. There was a difference between the binding of WGA and SNA in all samples, indicating variation in the sialic acid forms present.

Ricinis communis agglutinin I (RCA) recognises galactose, GlcNAc and N-acetylgalactcosamine (GalNAc) (Table 3.1). All samples apart from PSIM were positive for RCA (Figure 3.2). Since there was no SWGA signal, which may indicate a lack of GlcNAc, the RCA recognition may be attributed to presence of galactose or GalNAc. Concanavalin A (ConA) recognises mannose and showed positive signals for all samples although with a weaker signal for MCM-C, indicating mannose content across the mucus samples, which is present in low amounts in mucin, and along with other contaminating sugars may come from shed epithelial cells present in the native mucus extracts.

Ulex europaeus agglutinin (UEA) recognises α-linked fucose, whereas *Lotus tetragonobulus* lectin (LTL) recognises fucose linked to the C-2 of the galactose of βDGal(1-4)DGlcNAc, but not on βDGal(1-3)DGlcNAc [257]. There was positive recognition by UEA of bands in PSIM, MCM-C and MCM-S samples (Figure 3.2), but there was no recognition in any mucus sample by LTL, which suggests that fucose in the mucus samples is α-linked or on C-2 of βDGal(1-3)DGlcNAc. The PGMIII positive control, showed positive bands for all lectins tested.

Taken together these results showed that although different profiles were observed between colonic mucus and small intestinal mucus extracts, all mucus samples displayed similar responses to lectin probing, indicating the presence of expected sugar residues.

3.2 **Development, optimisation and standardisation of the mucus adhesion assay**

An adhesion assay was developed for assessment of bacterial adhesion to mucus. Briefly, carboxyfluorescein (cF) labelled bacteria [258] were incubated on mucus coated wells, non-adhering bacteria washed off and the bound cells were lysed before the fluorescence signal was measured at 520 nm using a plate reader. The use of cell permeant cF diacetate (cFDA) to label the bacterial cells was chosen over a range of alternatives due to its mechanism of action. Upon entering the cells, cFDA undergoes hydrolysis by intracellular esterases, releasing cF which is retained in the cell by an intact membrane. The two advantages of this method are that, only viable cells are labelled and furthermore since cF is internalised in the cells, the surface of the bacteria and therefore the cell surface proteins potentially involved in adhesion remain unaffected by labelling. However in order to measure fluorescence the bacterial cells need to be lysed (see Materials and Methods section 2.3.3.1).

Immobilised BSA was used as a control, as it was found to give minimum signal compared to casein, β-lactoglobulin, ovalbumin and an equivalent signal to PBS (data not shown). The adhesion assay was optimised and standardised both in terms of mucus coating and preparation and bacterial cell numbers, viability and growth phase (see section 3.2.1-3.2.2 below).

3.2.1 Saturation of mucus coating and mucus preparation comparison

In order to ensure that the microtitre plate wells were saturated with immobilised mucus, to avoid non-specific binding of the bacteria to the wells, the binding of cF labelled *L. reuteri* strain ATCC 53608 was tested against wells coated with a serial dilution of MCM-S ranging from 2 mg ml^{-1} to 0.24 µg ml^{-1} (Figure 3.3). Binding is represented by the level of fluorescence detected after non-adhered bacteria were washed off and bound cells lysed to release the fluorescence. Immobilised BSA was included as a control.

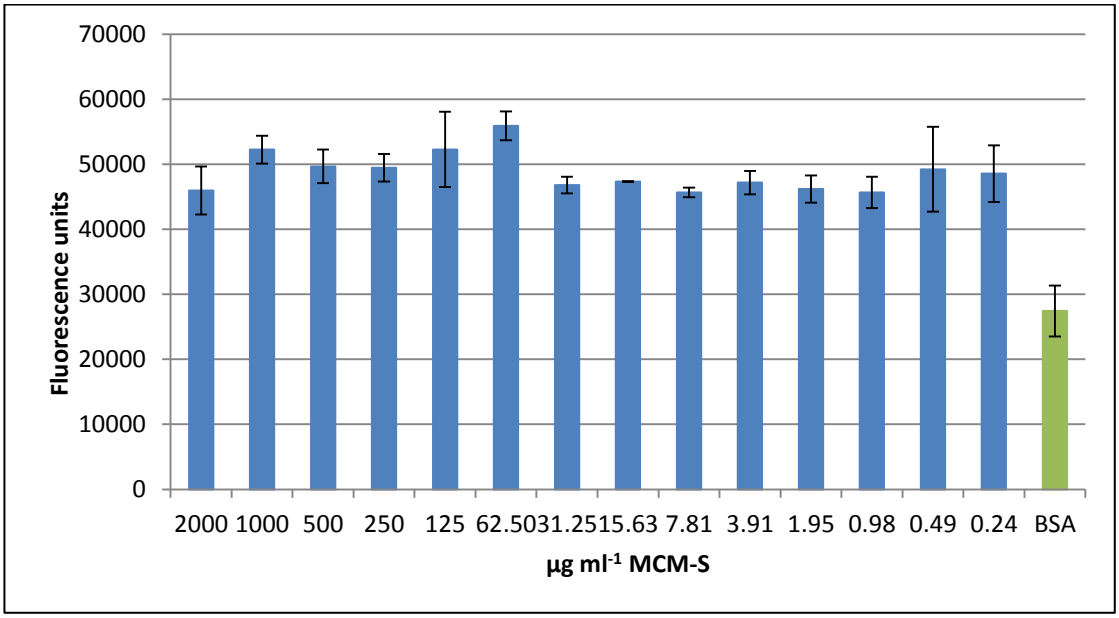


Figure 3.3 Binding of strain ATCC 53608 to varying concentrations of MCM-S (blue bars) immobilised on microtitre wells. Control BSA (green bar).

No significant variation was observed within the range of mucus tested, indicating that the concentrations used were above the saturation level. A value of 1 mg ml^{-1} was used for MCM-S and 0.1 mg ml^{-1} for further MSIM and PSIM adhesion studies.

Since two different methods were used for the MCM extraction, resulting in the samples MCM-C and MCM-S, the adhesion of strain ATCC 53608 was tested at two concentrations of both mucus preparations.

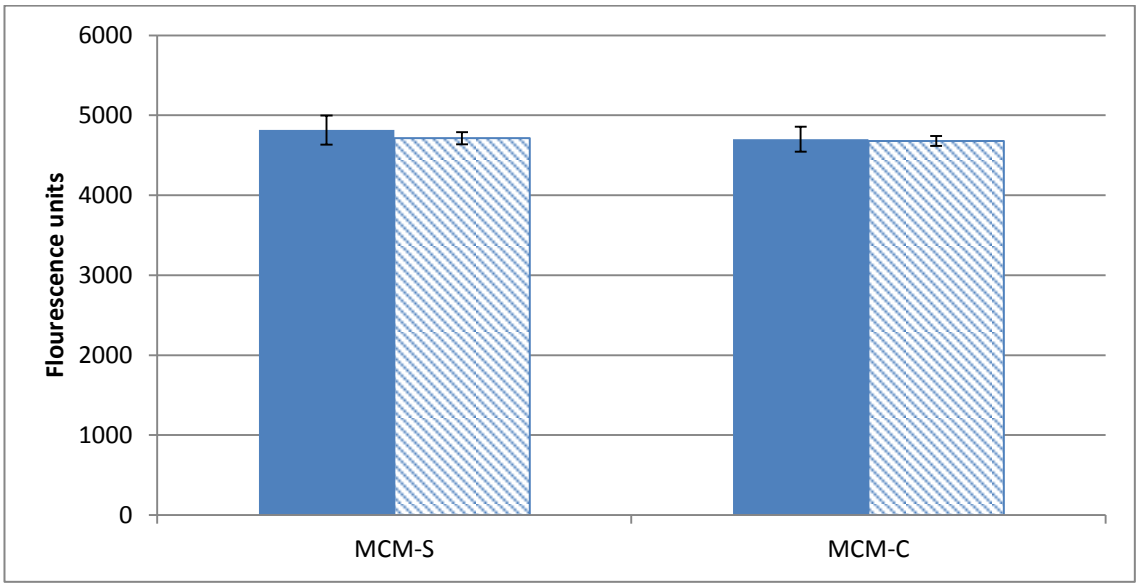


Figure 3.4 Effect of MCM preparation and concentration 1 mg ml^{-1} (blue bars), 0.1 mg ml^{-1} (blue and white bars) on binding ability of *L. reuteri* ATCC 53608.

There was no significant difference in the binding ability of strain ATCC 53608 to MCM prepared using the two different methods or at the two concentrations used (Figure 3.4), indicating that the method of extraction did not have an impact on the reproducibility of the assay.

3.2.2 Adhesion assay optimisation and standardisation; bacterial cell numbers, viability, labelling and growth phase

Adhesion assays generally rely on OD_{600} values of bacterial suspensions to normalise the number of cells added to each well. However, differences in the size and shape of the cells may impact on OD_{600} value, thus potentially affecting the dynamics of the adhesion assay by adding variable numbers of cells.

Flow cytometry (FCM) was used to count the cells and therefore determine whether the OD_{600} value corresponds to an equivalent number of cells across the *L. reuteri* strains tested. Briefly, a selection of strains were diluted to an OD_{600} of 0.5 and the number of events (corresponding to cells) was counted for 1 min at a constant flow rate using an FC500 cytometer (Table 3.2). The FC500 cytometer aspirates the cell suspension and the fluidics system passes each cell in front of a laser, the detectors measure the light scattered and the number of "events" passing through the laser corresponds to the number of cells.

Strain	Count*	No. cells added to microtitre well x 10^6
MM4-1a	282000	5.64
CF4-6g	284000	5.68
FJ1	283000	5.66
LMS11-3	283000	5.66
1063N	280000	5.6
ATCC 55739	284000	5.68
ATCC 53608	269000	5.38
DSM 17509	274000	5.48
DSM 20016	280000	5.6
ML1	284000	5.68
r13	277000	5.54
MF2-3	255000	5.1
sr11	275000	5.5
R2LC	281000	5.62
N2D	283000	5.66

Table 3.2 Determination of the number of *L. reuteri* cells by FCM across selected strains. * In cell suspension measuring OD_{600} 0.5.

The number of events counted was similar across the strains, with an average of 278267 +/- 7796, corresponding to ~ 5.6 x 10^6 cells per well in the binding assay.

To further optimise the adhesion assay, a range of bacterial cell concentrations was tested, the assay was performed with a 16 h culture of *L. reuteri* strain DSM 20016 at a range of OD_{600} values (Figure 3.5). Data are presented as percentage adhesion, comparing the fluorescent signal of bound bacteria to the fluorescent signal of the labelled bacterial aliquots originally added to the wells. The range of OD_{600} values chosen extends to above the theoretical maximum number of cells that can bind if 100 % of cells adhered. The theoretical maximum number of cells that can bind is 377 x 10^6, based on surface area of the well and size of cells, while an OD_{600} of 6.8 is equivalent to ~544 x 10^6 cells.

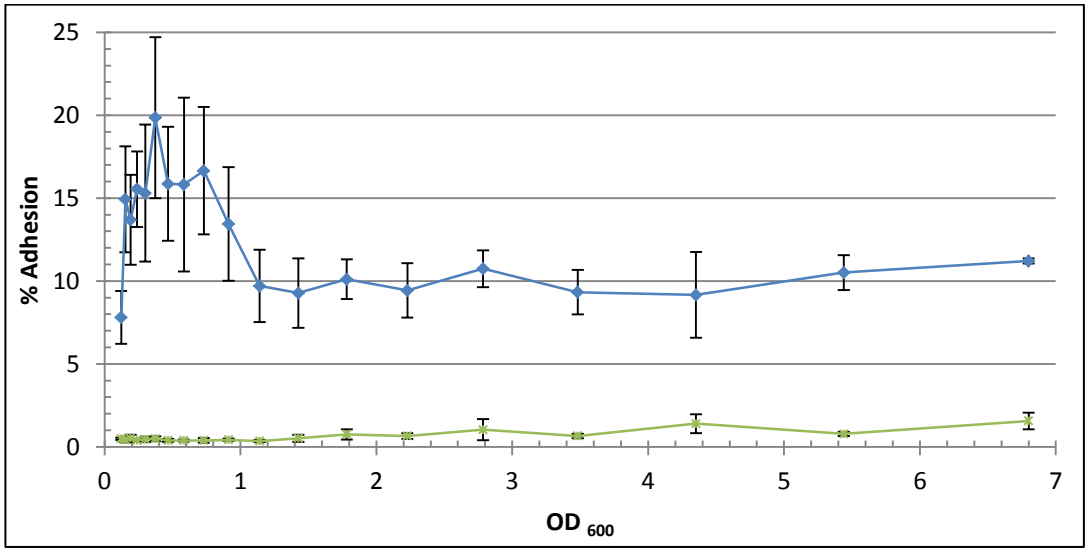


Figure 3.5 Effect of cell numbers on adhesion of strain DSM 20016 to MCM-S (blue diamonds) and immobilised BSA as a control (green crosses).

The measured maximum number of cells bound (11.2 % at OD_{600} 6.8) corresponds to ~61 x 10^6, indicating that the results are within the theoretical limits of the assay. A high percentage of adhesion of strain DSM 20016 to mucus was observed between OD_{600} 0.3 and 0.7 (Figure 3.5). Above an OD_{600} of 1, the adhesion stabilised at ~10 %, indicating that there is a proportional increase in the number of cells binding with the increase in cells added to the wells. A similar optimum adhesion range between OD_{600} 0.3 and 0.7 was observed for strain ATCC 53608 (data not shown), therefore an OD_{600} of 0.5 was chosen from the middle of this range in the rest of the study.

Only bound cF labelled bacteria are detected under the conditions of the binding assay. If unlabelled cells (corresponding to either dead cells or cells with leaky membranes) are present in the bacterial mix, they may interfere with the assay by binding to the immobilised mucus and thus competing with the cF labelled cells, leading to potential inaccuracies in the calculated binding percentage. Selected *L. reuteri* strains, prepared as described in section 2.2.2 for the adhesion assay, were characterised by FCM to determine the percentage of cells which are cF labelled (Figure 3.6).

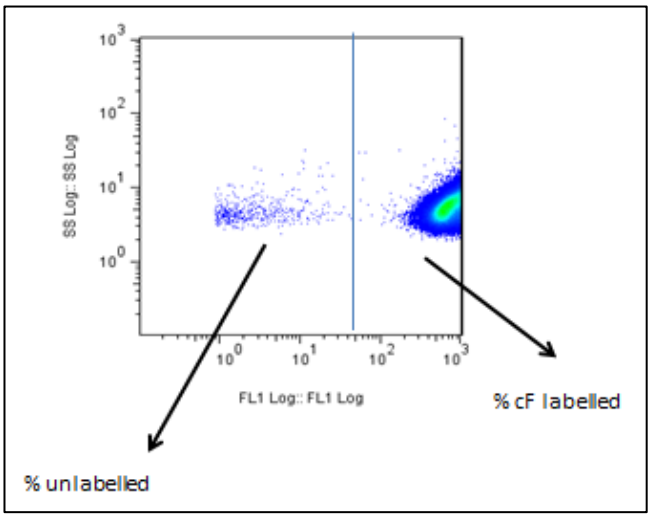


Figure 3.6 Example of a FCM plot used to distinguish cF labelled population of *L. reuteri* cells from unlabelled population of cells.

Cells are passed in single cell suspension through a laser and the fluorescent signal from the cells is detected. The SS signal gives information about the roughness and granularity of the cells, these two signals combined showed two distinct populations of cells, correspond to fluorescently labelled and unlabelled cells.

Strain	% cF labelled
MM4-1a	99.7
CF4-6g	99.8
FJ1	99.7
LMS11-3	99.7
sr11	96.6
1063N	99.8
ATCC 55739	99.8
ATCC 53608	99.8
DSM 20016	99.6
DSM 17509	99.5
ML1	99.3
r13	99.0
MF2-3	99.2
R2LC	98.3
N2D	99.7
Lr4020	99.3
MM4KO	99.7

Table 3.3 Percentage of cF-labelled bacteria, across *L. reuteri* strains as determined by FCM

For the majority of the strains tested, > 99 % of cells were cF-labelled (Table 3.3), indicating that any effect of unlabelled cells on the adhesion assay will be minimal. Two strains (sr11 and R2LC) showed higher numbers of unlabelled cells, sr11 (3.45% unlabelled) and R2LC (1.7% unlabelled), which may slightly affect the adhesion values of these two strains.

In order to investigate the influence of growth phase on bacterial adhesion to mucus, the binding ability of strain ATCC 53608 was tested in both mid-exponential and early stationary phase, diluted to an OD_{600} 0.5.

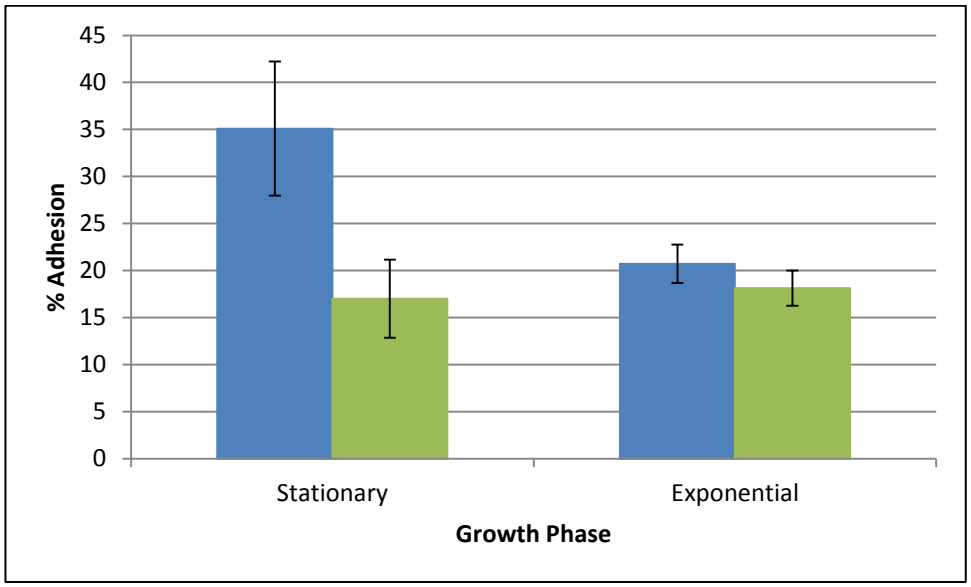


Figure 3.7 Impact of growth phase on adhesion of strain ATCC 53608 binding to MCM-S (blue bars) and BSA (green bars).

Significantly higher binding to mucus was observed when the cells were harvested in early stationary phase (16 h) compared to those in exponential phase (9.5 h) (Figure 3.7), probably due to differential expression of cell surface proteins. Early stationary phase was chosen for the rest of the study.

3.3 **Mucus adhesion ability of *L. reuteri* strains**

In order to investigate the mucus binding ability of *L. reuteri*, 26 strains (Table 3.4) were screened for adhesion ability to MCM-S, MSIM or PSIM (and BSA as non-specific binding control), using the microtitre plate binding assay. The identity of the *L. reuteri* strains in the collection was first confirmed by sequencing of a 194 bp fragment of 16S rRNA amplified by PCR from cells of the *L. reuteri* strains, showing 100 % identity with the sequence from type strain DSM 20016 (data not shown). The strains were originally isolated from five different vertebrate hosts, pig, human, chicken, mouse and rat (Table 3.4).

L. reuteri strain	Vertebrate host origin
ATCC 53608	Pig
1063N	Pig
Lp167-67	Pig
20-2	Pig
3c6	Pig
JW2015	Pig
DSM 20016	Human
MM4-1a	Human
MM4KO	Human
MF2-3	Human
CF4-6g	Human
FJ1	Human
MF14-C	Human
LMS11-3	Human
sr11	Human
LB54	Chicken
#20	Mouse
r13	Mouse
Lr4020	Mouse
ML1	Mouse
CR	Rat
One-One	Rat
DSM 17509	Rat
N2D	Rat
R2LC	Rat
ATCC 55739	Rat

Table 3.4 Collection of *L. reuteri* strains used in this study

In order to compare adhesion between the strains, percentage adhesion was normalised to the mucus binding of ATCC 53608 which was set to a value of 100 %.

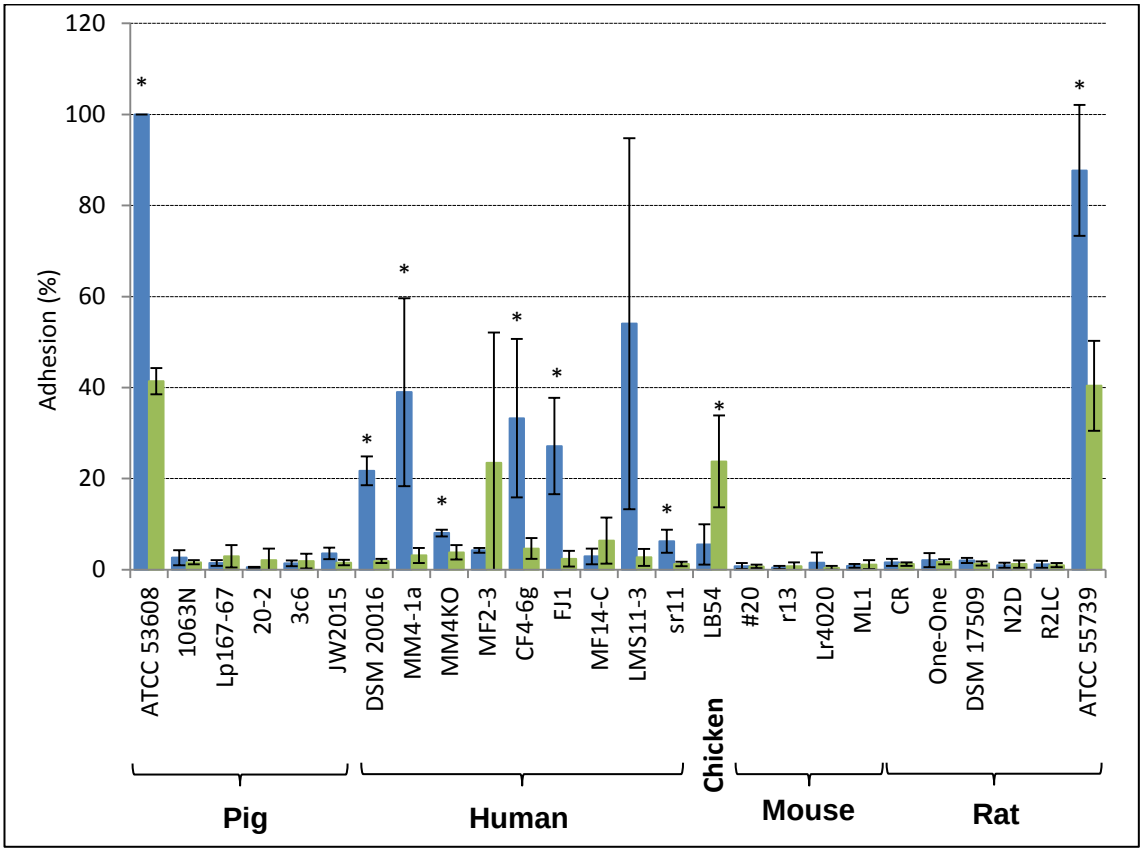


Figure 3.8 Adhesion of *L. reuteri* strains to MCM-S (blue bars) with BSA as a control (green bars). * P <0.05.

Adhesion to MCM-S was markedly varied for the different strains, with eight strains showing significantly higher binding to mucus compared to binding to BSA (Figure 3.8). Adhesion of *L. reuteri* strains to MCM-S was not correlated to the origin of the strain, i.e. there was no significant binding to mouse mucus by strains isolated from mice. Two strains (ATCC 53608 and ATCC 55739) showed significantly higher binding to mucus than to BSA but with a substantial binding to BSA. The six strains showing significant binding to mucus compared to BSA, but with very low levels of BSA binding (DSM 20016, MM4-1a, MM4KO, CF4-6g, FJ1 and sr11) were all human origin. Strain LB54 (chicken origin) showed significantly higher binding to BSA than to MCM-S.

In order to investigate whether mucus origin in the GI tract has an impact on *L. reuteri* ability to bind to mucus *in vitro*, the *L. reuteri* strains were tested against MSIM.

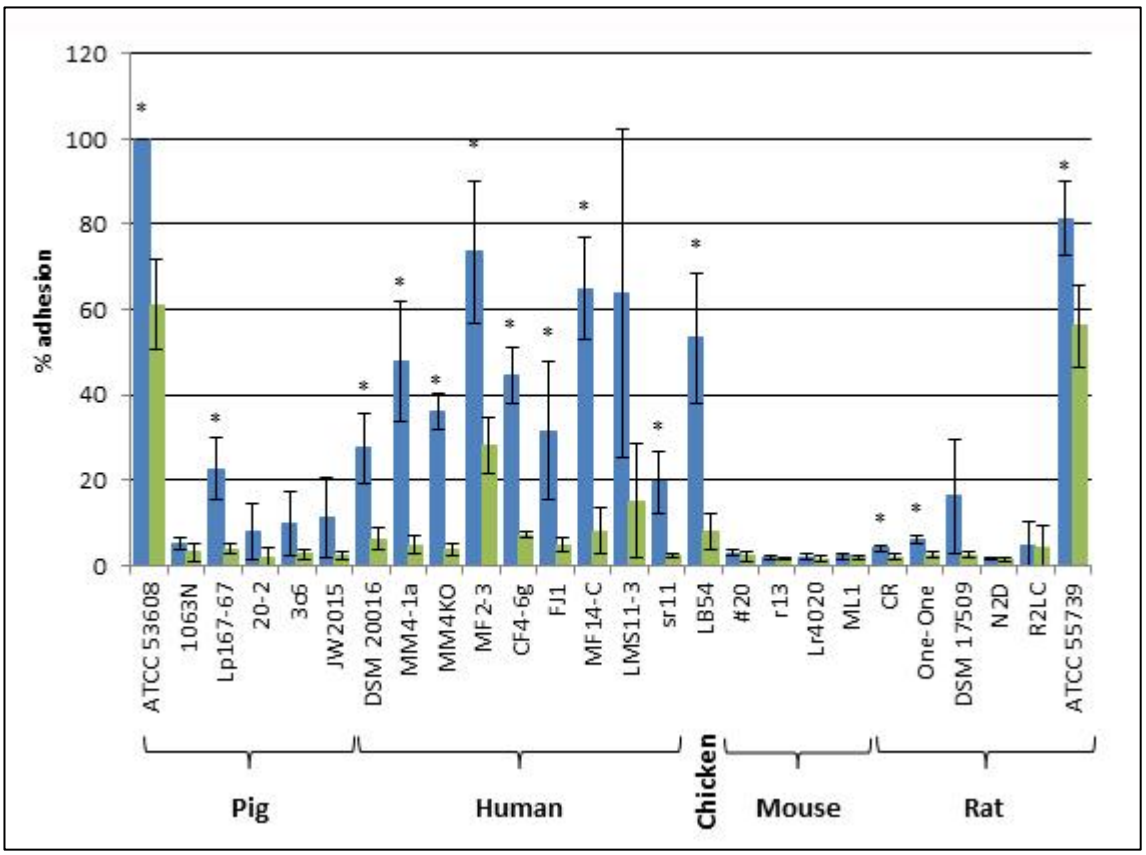


Figure 3.9 Adhesion of *L. reuteri* strains to MSIM (blue bars) with BSA as a control (green bars) *P <0.05

The eight strains showing significant binding to MCM-S all showed the same binding pattern to MSIM (Figure 3.9). Furthermore significant binding was observed for six other strains to MSIM, Lp167-67, MF2-3, MF14-C, CR, One-One and LB54, which indicates that the differences in mucus composition may affect the adhesion of specific strains and that adhesion is not specific to colonic mucus.

The adhesion assay was also carried out against PSIM to assess whether the host origin of the mucus could have an impact on the adhesion of *L. reuteri* strains to mucus (Figure 3.10).

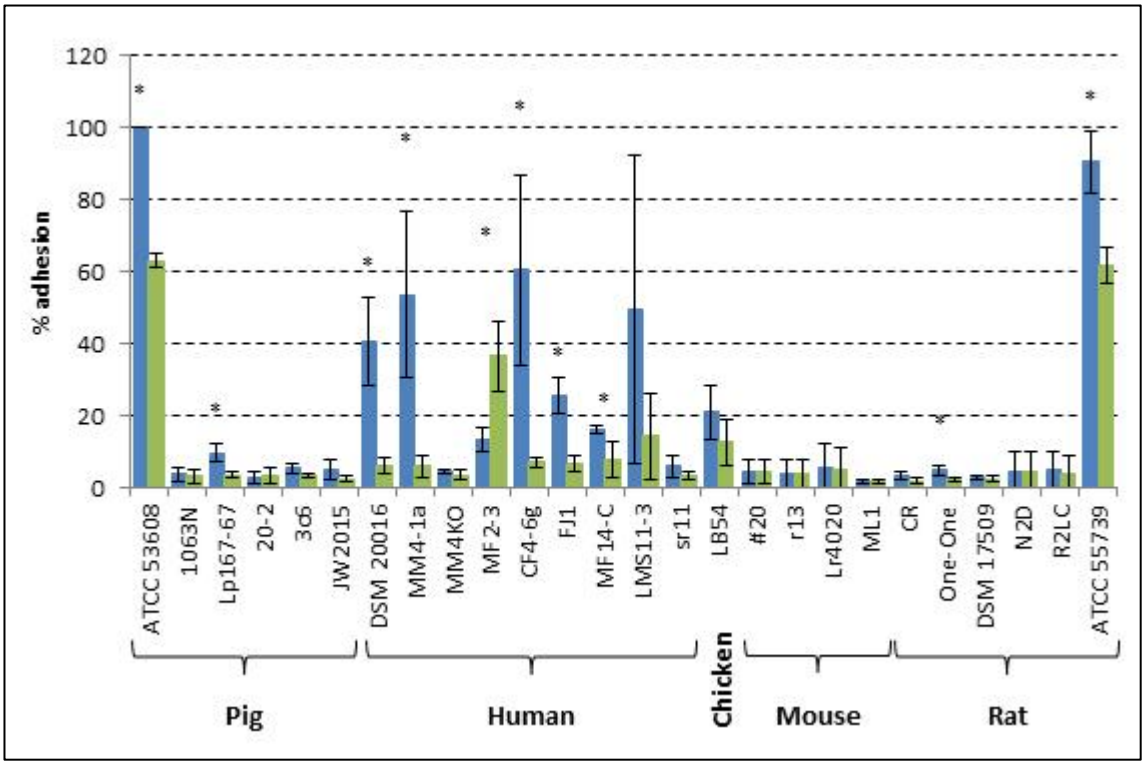


Figure 3.10 Adhesion of *L. reuteri* strains to PSIM (blue bars) with BSA as a negative control (green bars) *P <0.05

Comparable to the binding to MCM-S and MSIM, strains ATCC 53608 and ATCC 55739 showed very high binding to PSIM with high background binding to BSA, and human strains DSM 20016, MM4-1a, CF4-6g and FJ1 showed significant binding to PSIM. However, in contrast to the significant binding observed to MCM-S and MSIM, strains MM4KO and sr11 did not show any significant binding to PSIM. Strains Lp167-67, MF14-C and One-One showed significant binding to PSIM, whilst there was no significant binding observed to MCM-S and MSIM. Strain MF2-3 showed significant binding to BSA.

The specificity of the reported mucus binding ability can be inferred by comparing the binding to mucus to the binding to BSA. Binding ratios were calculated for each strain, where a higher ratio indicates a higher specificity for mucus (Table 3.5). Four strains (DSM 20016, MM4-1a, CF4-6g and FJ1) showed consistently high binding ratios along with significant binding to mucus. The ATCC 53608 and ATCC 55739 strains which displayed very high percentage binding to mucus, compared to the other *L. reuteri* strains tested in the present study, had a relatively low binding ratio (less than 2.5 for both strains), due to their high binding to BSA.

Strain	Ratio		
	MCM-S	MSIM	PSIM
ATCC 53608	2.42	1.63	1.59
1063N	1.62	1.61	1.19
Lp167-67	0.49	5.83	2.82
20-2	0.25	3.91	0.83
3c6	0.73	3.71	1.57
JW2015	2.34	4.48	2.09
DSM 20016	11.44	4.44	6.73
MM4-1a	12.53	9.88	8.88
MM4KO	2.11	9.69	1.23
MF2-3	0.18	2.62	0.37
CF4-6g	7.20	6.28	8.65
FJ1	11.36	6.51	3.83
MF14-C	0.45	7.95	2.10
LMS11-3	20.14	4.19	3.46
sr11	4.95	8.82	1.80
LB54	0.23	6.72	1.65
#20	1.12	1.43	1.04
r13	0.67	1.02	1.05
Lr4020	3.76	1.25	1.10
ML1	0.71	1.23	1.03
CR	1.31	2.01	1.70
One-One	1.22	2.43	2.11
DSM 17509	1.52	6.39	1.20
N2D	0.79	1.15	1.04
R2LC	1.21	1.17	1.25
ATCC 55739	2.17	1.45	1.46

Table 3.5 Binding ratios of *L. reuteri* strains (mucus: BSA).

Strains DSM 20016, MM4-1a, CF4-6g and FJ1 all isolated from human sources consistently showed significant binding to mucus. Eleven strains exhibited no binding under these conditions, porcine isolates 1063N, 20-2, 3c6 and JW2015, murine isolates #20, r13, Lr4020, ML1 and rat isolates DSM 17509, N2D and R2LC. The human isolate LMS11-3 displayed a highly variable binding ability, with a trend for higher binding to mucus than to BSA, but this was not statistically significant.

The pattern of mucus binding ability of *L. reuteri* strains remains similar across the mucus samples, with strains ATCC 53608 and ATCC 55739 consistently showing high binding to mucus.

3.4 **Role of autoaggregation in mucus binding**

Autoaggregation of *L. reuteri* has been reported [125], and this property may influence the mucus binding value reported in the above adhesion assay, since cell aggregates will release a higher fluorescent signal than individual cells.

In order to assess the potential role of autoaggregation in *L. reuteri* adhesion to mucus, the autoaggregation phenotype of all 26 *L. reuteri* strains was assessed by spectrophotometric assay. Bacterial cells were grown in MRS to early stationary phase, washed and diluted to an OD_{600} of 0.5 in PBS. The OD_{600} value was measured every 10 min for 5 h, at 37 °C closely mimicking the conditions of the binding assay. Strains which autoaggregate showed a rapid decrease in OD_{600} values due to the formation of aggregates settling out at the bottom of the cuvette, compared to non-autoaggregating strains.

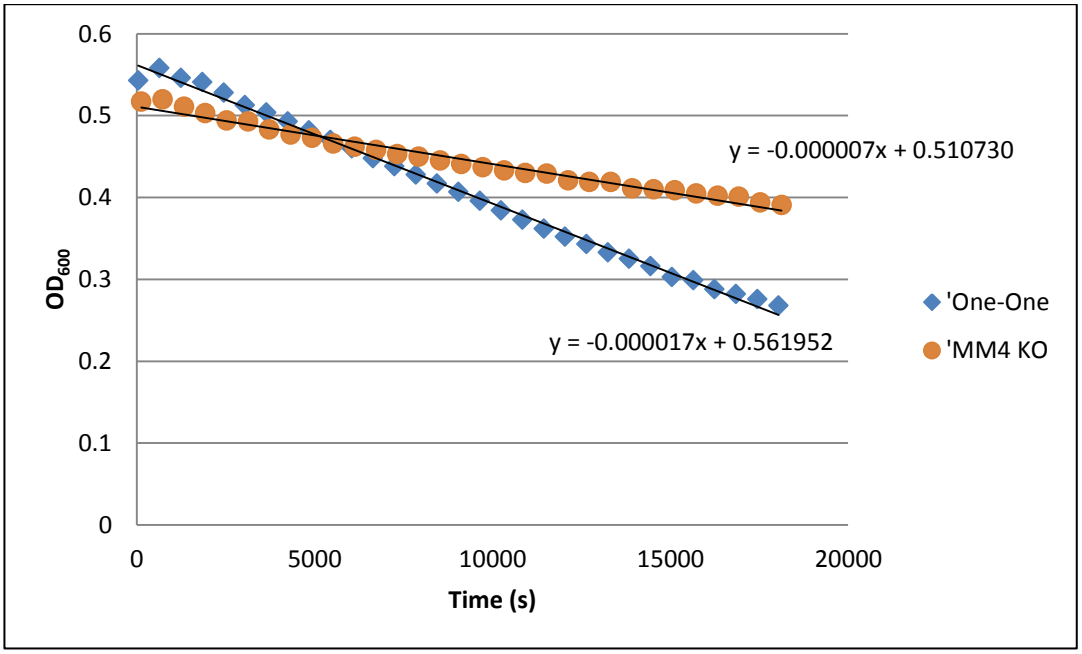


Figure 3.11.Measurement of OD_{600} over time of *L. reuteri* strains. The gradient of the line is a surrogate marker for autoaggregation ability.

The OD_{600} values were plotted on a graph against time (Figure 3.11) and a line of best fit applied to the data points for each strain, the gradient of the line (m in equation y = mx+c), was used as a measure of the degree of autoaggregation ability.

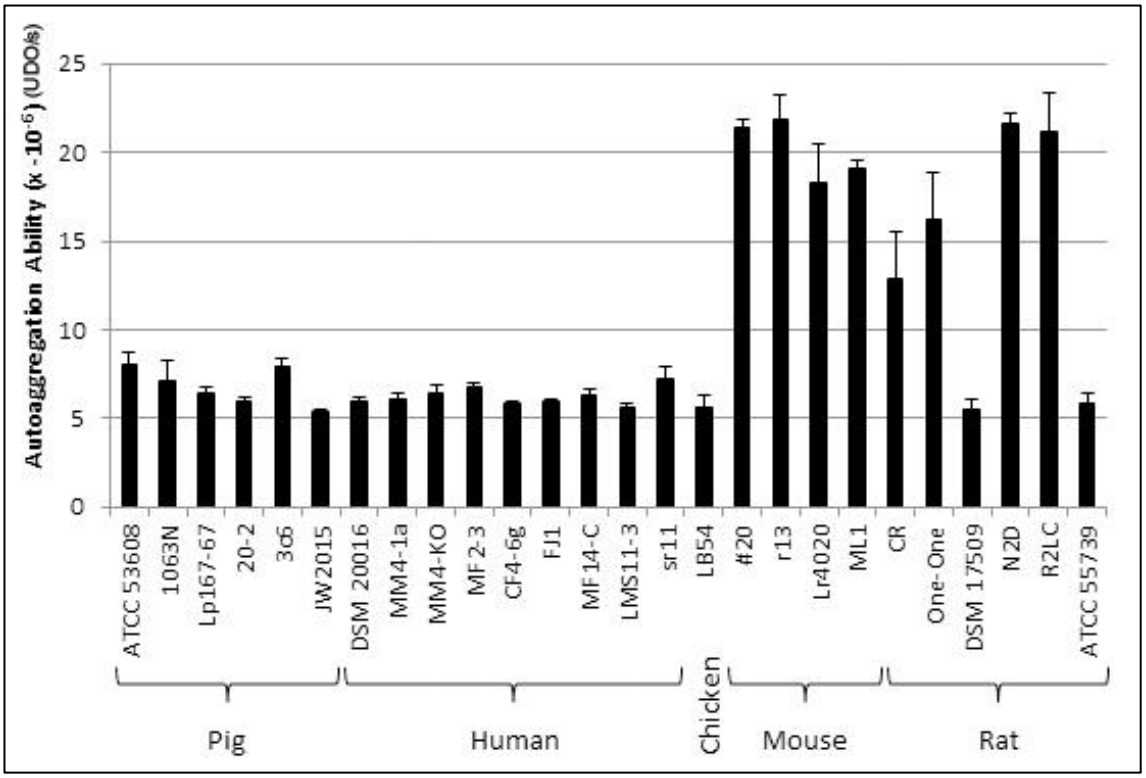


Figure 3.12 Measurement of autoaggregation of *L. reuteri* cells by spectrophotometry.

A clear autoaggregation phenotype was demonstrated for the majority of the rodent strains (#20, r13, Lr4020, ML1, CR, One-One, N2D, and R2LC) (Figure 3.12). Out of this group, only strains CR and One-One demonstrated mucus binding ability. Low levels of binding were exhibited by both strains, for CR 3.98 % to MSIM with a binding ratio of 2.01 while strain One-One showed binding to both MSIM and PSIM at 5.99 % and 2.33 %, with binding ratios of 2.43 and 2.11, respectively.

3.5 **Genome sequencing of *L. reuteri* ATCC 53608**

Since *L. reuteri* ATCC 53608 showed the highest binding ability across the mucus samples tested, this strain was selected for genome sequencing in order to obtain insights on the molecular determinants mediating *L. reuteri* binding to mucus.

Genomic DNA was extracted from early stationary phase culture of *L. reuteri* ATCC 53608 following a modified protocol described in [101] for Gram-positive bacteria. The genome was sequenced by 454 sequencing platform, with both shotgun and 3-kbp paired-end libraries in collaboration with The Genome Analysis Centre (TGAC, Norwich Research Park). This approach generated in excess of 365 Mbp of sequence, which were further assembled into a draft genome of 99 large contigs. [259]. The draft genome of *L. reuteri* ATCC 53608 is 1,969,869 bp in length with an average G+C content of 38.4 % which is slightly lower when compared to the other *L. reuteri* strains with genome sequences available (Table 3.6).

L. reuteri strain	RefSeq	Genome size (Mbp)	G+C content (%)	Genes	Proteins
ATCC 53608	CACS00000000.2	1.97	38.4	1910	1864
JCM 1112	NC_010609.1	2.04	38.9	1901	1820
DSM 20016	NC_009513.1	2.00	38.9	2027	1900
100-23	NZ_AAPZ00000000.2	2.31	38.7	2269	2181
ATCC 55730 (SD2112)	NC_015697.1	2.04	39.3	2370	2246
CF48-3A	NZ_ACHG00000000.1	2.03	38.7	2223	2164
MM2-3	NZ_ACLB00000000.1	1.94	38.7	2105	2045
MM4-1a	NZ_ACGX00000000.2	2.07	38.7	2226	2095

Table 3.6 Genomic characteristics of genome sequenced *L. reuteri* strains.

Automatic annotation of the genome sequence of ATCC 53608 using Glimmer3 and GeneMark software revealed 2024 protein coding sequences [259], with 1864 confirmed by RefSeq. Subsequently gaps in the genome were closed using PCR, automatic gene prediction was performed using Rapid Annotations using Subsystems Technology (RAST) [260] and prediction of secretion signals using SigP were performed in collaboration with Udo Wegman (IFR). Protein domain predictions were performed using the Interpro domain search tool from the European Bioinformatics Institute (EBI) website (www.ebi.ac.uk/interpro/).The characteristics of four large (> 750 aa) cell surface proteins are described in Table 3.7 and their schematic representation is shown in Figure 3.13.

Protein (AA)	Features (Interpro)	Similar proteins (GenBank)	Organisms (%identity/%coverage)
Lb_C_0736 (1166)	5 x MucBP LPxTG anchor	YP_005854578.1	*L. amylovorus* (36/56)
Lb_C_0892 (964)	No hits	ZP_03973805.1 ZP_03073444.1 YP_001270659.1	*L. reuteri* CF48-3A (98/100) *L. reuteri* 100-23 (96/100) *L. reuteri* DSM 20016 (91/100)
Lb_C_1034 (3269)	14 x MucBP Gram-pos anchor YSIRK signal	AAF25576.1 ZP_09814175.1	*L. reuteri* 1063 (100/100) *L. mucosae* LM1 (74/100)
Lb_C_1846 (787)	GH68 Gram-pos anchor KxYKxGKxW signal	ZP_03072364.1	*L. reuteri* 100-23 (66/100)

Table 3.7 Large surface proteins (> 750 aa) predicted from genome of *L. reuteri* ATCC 53608.

Two of the surface proteins are predicted to contain putative mucin-binding MucBP domains (Pfam PF06458), the largest of which is homologous to mucus binding protein MUB from strain 1063 (parent strain of *L. reuteri* ATCC 53608). Other large surface proteins included Lb_C_1846 with a glycoside hydrolase family 68 domain (Pfam PF02435) and Lb_C_0892 a protein of unknown function but with high similarity to proteins found in *L. reuteri* strains CF48-3A, 100-23 and DSM 20016.

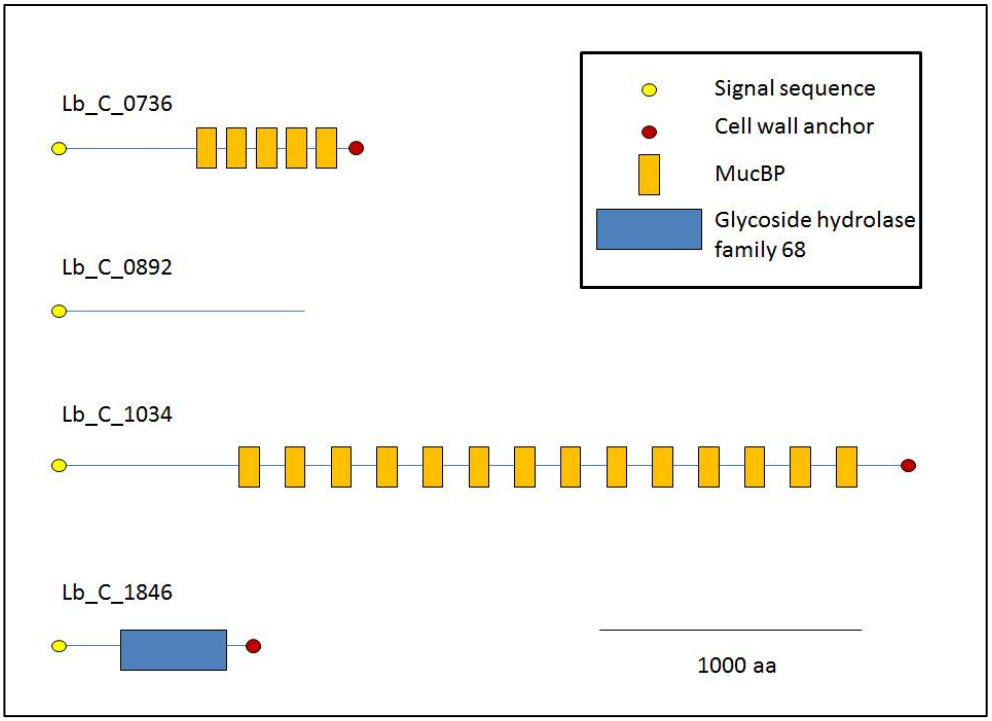


Figure 3.13 Large surface proteins (> 750 aa) predicted from genome sequence of *L. reuteri* ATCC 53608.

The genome sequence of ATCC 53608 along with those of strains MM4-1a, DSM 20016, JCM 1112, and 100-23 were interrogated for all mucus binding proteins identified so far in lactobacilli (Table 3.8). MUB (Lb_C_1034) was found to be a distinguishing feature of ATCC 53608, while genes encoding MapA and the cytoplasmic proteins EF-Tu, GroEL and GAPDH were present in all strains (GAPDH appears to be missing from strain DSM 20016). The genes encoding putative pili proteins were absent from all strains. The gene encoding Lar_0958 was found in *L. reuteri* strain JCM 1112 and a homologue was found in strain MM4-1a.

Mucus Binding Protein	Bacterial Strain	TBLASTN against genome of ATCC 53608		TBLASTN against genome of DSM 20016		TBLASTN against genome of JCM1112		TBLASTN against genome of MM4-1a		TBLASTN against genome of 100-23		Reference
		% Identity	% Coverage	% Identity	% Coverage	% Identity	% Coverage	% Identity	% Coverage	% Identity	% Coverage	
MUB (GenBank AF120104)	*L. reuteri* 1063	90	97	33	5	33	5	33	3	39	87	[124, 223]
Map A (Previously MAPP) (GenBank AJ293860)	*L. reuteri* 104R (previously named *L. fermentum* 104R)	95	100	94	100	94	100	94	100	95	100	[203, 222, 224-227]
EF-Tu (GenBank NP 964043)	*L. johnsonii* La1 NCC 533	84	100	84	100	84	100	84	100	84	100	[228]
GroEL (GenBank NP 964487)	*L. johnsonii* La1 NCC 533	70	98	70	98	70	98	70	98	70	98	[229]
GAPDH (GenBank AL935254)	*L. plantarum* LA 318	72	100	X	X	72	100	72	100	72	100	[230]
Pilin subunit SpaC (GenBank YP003170190)	*L. rhamnosus* GG	X	X	X	X	X	X	X	X	32	4	[173, 174]
Pilin subunit SpaF (GenBank YP003172118)	*L. rhamnosus* GG	X	X	X	X	X	X	X	X	X	X	[173, 174]
Pilin subunit SpaB (GenBank YP003170189)	*L. rhamnosus* GG	31	20	31	20	31	20	31	20	47	32	[173, 174]
Lar_0958 (GenBank YP001841954.1)	*L. reuteri* JCM1112	31	31	60	4	97	100	96	100	59	66	Personal Communication from Stefan Roos

Table 3.8 Predicted mucus binding proteins in genome sequenced *L. reuteri* strains

3.6 **Discussion**

In the lower GI tract the first point of contact between the bacteria and the host is the mucus covering the epithelial surfaces. Many studies have investigated direct interactions of the microbiota with epithelial cells and extracellular matrix (ECM) components of host [194]. These studies are more relevant for pathogens such as *Entamoeba histolytica* which can traverse the mucus layer and cause damage to the epithelium [261] or for bacterial-host interactions in the proximal regions of the GI tract where there is less mucus. Studies investigating adhesion interactions between gut microbes and mucus have focused on probiotic or pathogenic bacteria as mucus binding is generally thought to be a desirable trait for probiotic bacteria [188] and a virulence factor in pathogens [202], however the mechanisms underlying mucus binding is poorly understood for the resident microbiota.

The *Lactobacillus* species *reuteri* was used in this study as a model gut symbiont since it is found to inhabit the GI tract of many vertebrate species including, humans, pigs, horses, rodents, birds, and fish. Our study showed that the ability of *L. reuteri* to bind to mucus is strain specific, confirming previous findings using other lactobacilli species, for example *L. plantarum* [199]. In the present work, 17 out of the 26 strains tested showed consistent mucus binding phenotypes irrespective of the mucus origin, indicating a general absence of host specificity, since only two of the six pig isolates showed significant binding ability to the pig mucus, and three of the ten rodent strains showed mucus binding ability to mouse mucus. Such an absence of host specificity was previously reported in several other lactic acid bacteria species by Rinkinen *et al* 2003, including *L. rhamnosus* GG, *L. johnsonii* La1, and *L. pentosus* UK1A [193]. However, the human isolates in our study showed a trend for mucus binding ability, since eight out of the nine strains showed significant mucus binding ability to at least one type of mucus. This indicates that the mucus binding mechanism may be important in the colonisation of the particular niche specific to the human GI tract. Although *L. reuteri* is one of the most abundant species in GI tract of pigs rodents and chickens [113-116] and its prevalence is much lower in humans [110], lactobacilli are found in the human colon [71], where the mucosal associated microbial community is different to the lumen [262, 263] and microbial characteristics such as mucus binding are postulated to influence the colonisation of this microbial community [264]. This hypothesis is consistent with the genetic analysis of 126 *L. reuteri* strains showing host specific clades [101]. Following on from our work, the lack of mucus binding ability in mouse isolates may indicate that mucus binding is not a pre-requisite for *L. reuteri*

colonisation of this particular host environment. Instead the clear autoaggregating phenotype observed for the majority of rodent isolates indicates that this factor may be important for colonisation. Although inference of colonisation ability from *in vitro* mucus binding studies should be taken with caution, these data are in agreement with the colonisation of *L. reuteri* in rodents through biofilm-like association in the proximal regions (forestomach) [115, 265] of the GI tract where there is stratified squamous epithelium and reduced quantities of mucus compared to the colon. The idea that bacterial colonisation factors may vary depending on the target host is also indicated by the differential colonisation ability of *L. reuteri* strains Lr47 and Lr108. Lr47 was isolated from rat colon and found to have high colonisation ability in rats but not in humans while strain Lr108 which was isolated from human jejunum was found to have colonisation ability in humans, while no ability to colonise rats [266, 267].

The comparison of *L. reuteri* adhesion to mucus sourced from small or large intestine revealed that a small number of strains showed a specific preference (Lp167-67, MF14-C and One-One) for the small intestinal mucus. This observation of GI tract region specific preference is in line with the finding that a higher adhesion ability was exhibited by *L. acidophilus* I021 and *L. fermentum* I5007 to porcine ileal mucus compared to jejunal and duodenal mucus samples [111] and that mucosal conditions of the small intestine are different to those found in the colon in terms of amount of mucus [10], region-specific glycosylation of mucins [41], and surrounding pH [6]. This concept of region specific niches is also supported by the reported differences in the small intestinal microbiota compared to the colonic microbiota [71].

Mucus binding is regarded as a colonisation factor for many probiotics, for example the human probiotic *Lactobacillus rhamnosus* GG [268] has been shown to bind to mucus [269], and persists in human colonic mucosa more than a week after feeding [270]. However the term colonisation under these circumstances means persistence after consumption of the probiotic has ceased and is detected for a few days to weeks and it is unlikely that these probiotics would become permanent residents without continual input. Our study indicates that mucus binding may be a feature of autochthonous bacterial colonisation in the human GI tract for *L. reuteri* strains, however since 80 % of colonic mucosal associated bacteria remain uncultured [262] whether this is a general feature for all autochthonous bacteria remains to be determined.

Two very high mucus binders were highlighted by this study, strains ATCC 53608 (pig isolate) and ATCC 55739 (rat isolate), both of which also showed adhesion to BSA (Figure 3.8, Figure 3.9 and Figure 3.10). During the proteomic analysis of mouse colonic mucus, plasma proteins were identified including albumin [53], however it is not clear if this was due to a contamination during sample preparation or to plasma proteins secreted into the mucus, in which case albumin may be a potential adhesion target. The mucus binding and autoaggregation phenotype of strain ATCC 55739 was similar to the pig isolate ATCC 53608, which differs from the other rodent strains tested. Interestingly, the phylogenetic analysis performed by Oh *et al* showed that this strain clustered with the strains isolated from pigs (Figure 3.14) [101]. *L. reuteri* strains isolated from rodents generally show high colonisation ability in rodent models, such as strain 100-23 in a *Lactobacillus* free mouse model [102]. Additionally, rodent isolates R2LC and 6799jm-1 showed highest colonisation in a competition experiment with seven other *L. reuteri* strains from various origin performed in ex-germ-free mice [101]. In collaboration with Gerald Tannock, University of Otago, NZ, we tested the ability of strain ATCC 55739 to colonise a *Lactobacillus* free mouse model, unlike other rodent isolates, ATCC 55739 was unable to colonise, which taken together with previous results raises questions about the origin of this strain.

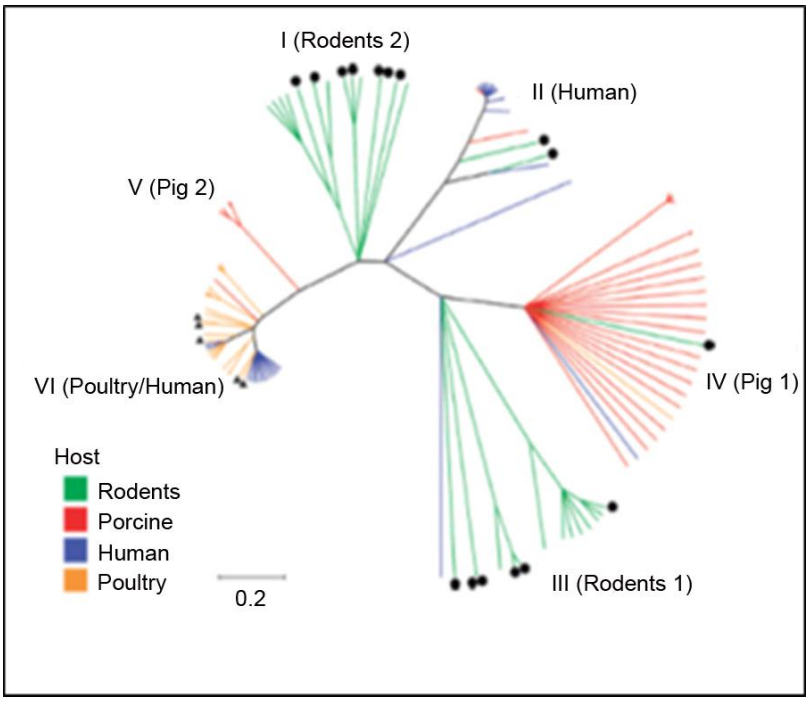


Figure 3.14 Genealogy of *L. reuteri* strains inferred by ClonalFrame [101], showing single green line of ATCC 55739 in Cluster IV (Pig 1).

Sequencing the genome of *L. reuteri* ATCC 53608 enabled the search for the presence or absence of known mucus binding proteins and a comparison with other *L. reuteri* strains. Initial bioinformatics approaches revealed four very large (> 750 aa) cell surface proteins, two of which contain the putative mucus binding MucBP domains. The larger of the two proteins with 14 domains was revealed to be a MUB homologue which has been well characterised as a mucus binding protein [124]. MUB contains 14 Mub repeats, each consisting of two domains (B1 and B2). The resolution of the crystal structure of one of the Mub repeats revealed that the B1 domain has structural homology to an immunoglobulin binding domain from *Peptostreptococcus magnus* and the B2 domain has structural homology to the MucBP domains [223]. The *in silico* search for Mub domains by Boekhorst *et al* 2006 [231], showed a wide distribution among the lactic acid bacteria and the recent *in silico* analysis of the exoproteomes of lactobacilli where a combination both MucBP domains and Mub domains to represent mucus binding proteins, a total of 47 were found in six *Lactobacillus* genomes and distributed over six separate Lactobacillales-specific clusters of orthologous protein-coding genes [232]. However no functional characterisation of MucBP domains has been reported in gut commensals.

Comparative genomics of the *L. reuteri* ATCC 53608 genome sequence with the other *L. reuteri* genomes available showed that MUB was specific to strain ATCC 53608, while Lar_0958 was specific to human isolates JCM 1112 and MM4-1a. The differences seen in mucus binding ability for these *L. reuteri* strains suggest that the specific expression of MUB and Lar_0958 may be important for mucus adhesion as the genes encoding these proteins are absent from the other *L. reuteri* genomes.

4 Role of MUB and Lar_0958 in *L. reuteri* adhesion to mucus

The *mub* gene has been identified in *L. reuteri* strain ATCC 53608, encoding a large (385 kDa) multimodular mucus binding protein with 14 Mub repeats, comprising six type 1 and eight type 2 repeats (Figure 4.1) (See Introduction, section 1.4.3 for more details).

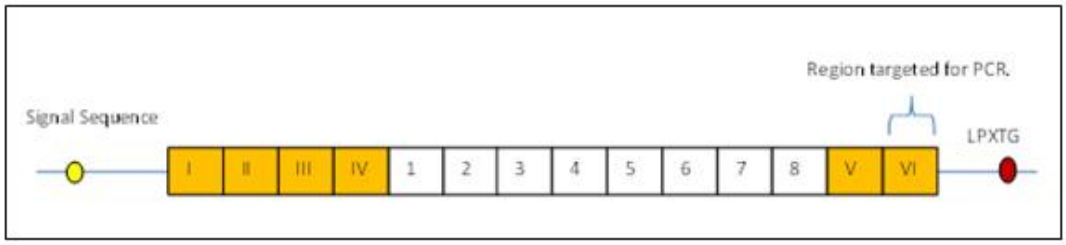


Figure 4.1 Schematic representation of mucus binding protein MUB, with six type 1 Mub repeats (orange) and eight type 2 Mub repeats (white). Blue bracket indicates region targeted by primers.

L. reuteri Lar_0958 is a smaller cell surface protein postulated to be involved in bacterial interaction with mucus and colonization of the digestive tract [271]. The 3690 bp *lar_0958* gene in *L. reuteri* JCM1112 encodes a protein of 1222 aa with a predicted molecular mass of 133 kDa. It is a modular protein containing six repeats domains, four of which are identical, the fifth has 99 % aa similarity and the sixth has only 46 % aa similarity (Figure 4.2).

The N terminal domain of both proteins contains an YSIRK signal sequence, [YF]SIRKxxxGxxS[VIA], which is commonly found in Gram-positive bacteria. Both proteins have an LPXTG anchor present at the C-terminal end for attachment to the cell wall PG.

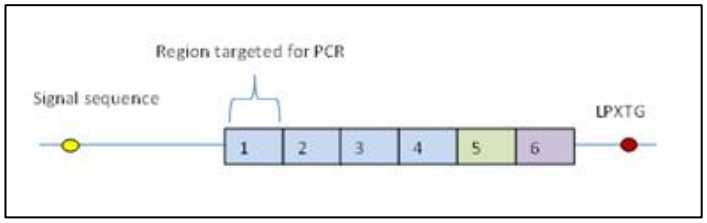


Figure 4.2 Schematic representation of Lar_0958 from *L. reuteri* JCM 1112, showing six Lar repeat domains, repeats 1-4 are identical (blue), repeat 5 has 99% aa similarity (green) and repeat 6 has 46 % aa similarity (purple).

In this chapter, the occurrence of the genes for these proteins across the collection of *L. reuteri* strains was tested, followed by an investigation into the role of these proteins in adhesion to mucus for selected strains.

4.1 **Occurrence of *mub* genes and proteins in *L. reuteri* strains**

In order to investigate the occurrence of *mub* genes across *L. reuteri* strains, PCRs were performed on cells of 26 strains, using primers designed to amplify a 624 bp fragment corresponding to Mub repeat RVI (between aa 3050 and 3236, QEAQAIF…….DMTINITYK) (Figure 4.1).

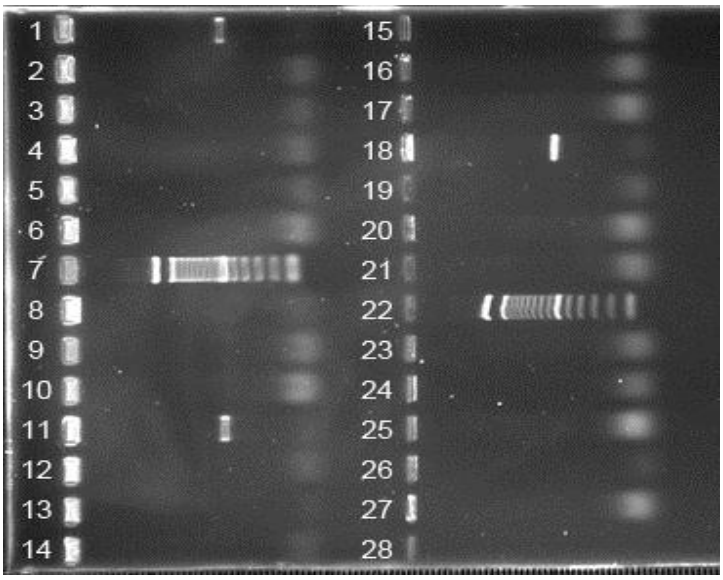


Figure 4.3 Agarose gel (1.5 %), used to separate *mub* PCR products amplified from *L. reuteri* strains. ATCC 53608 (lane1), MM4-1a (lane 2), MF2-3 (lane 3), CF4-6g (lane 4), FJ1 (lane 5), DSM 20016 (lane 6), MF14-C (lane 8), LMS11-3 (lane 9), sr11 (lane 10), 1063N (lane 11), JW2015 (lane 12), Lp167-67 (lane 13), 20-2 (lane 14), 3c6 (lane 15), CR (lane 16), One-One (lane 17), ATCC 55739 (lane 18), DSM 17509 (lane 19), N2D (lane 20), R2LC (lane 21), ML1 (lane 23), #20 (lane 24), r13 (lane 25), Lr4020 (lane 26), LB54 (lane 27), with DNA ladder (lanes 7 and 22) and negative control (lane 28).

A PCR product of the expected size was generated from three of the 26 strains, ATCC 53608, ATCC 55739 and 1063N (Figure 4.3). DNA sequencing of the PCR products confirmed 100 % identity to the predicted 624 bp fragment from *mub*.

In order to confirm MUB expression at the protein level, strains ATCC 53608, ATCC 55739, 1063N and *mub* negative DSM 20016 were grown overnight and the spent media (SM) extracts, soluble cytoplasmic extracts (SCE) and cell wall extracts (CWE) were analysed by electrophoresis. When required, proteins were transferred to PVDF membrane and probed with polyclonal anti-MUB antibody raised against the type 2

repeat MubR5 (anti-MubR5). For confirmation of their identity, protein samples were excised from SDS-PAGE and trypsinised before analysis by Matrix Assisted Laser Desorption Ionisation – Time of Flight (MALDI-ToF/ToF) mass spectrometry (MS) (in collaboration with Fran Mulholland at IFR).

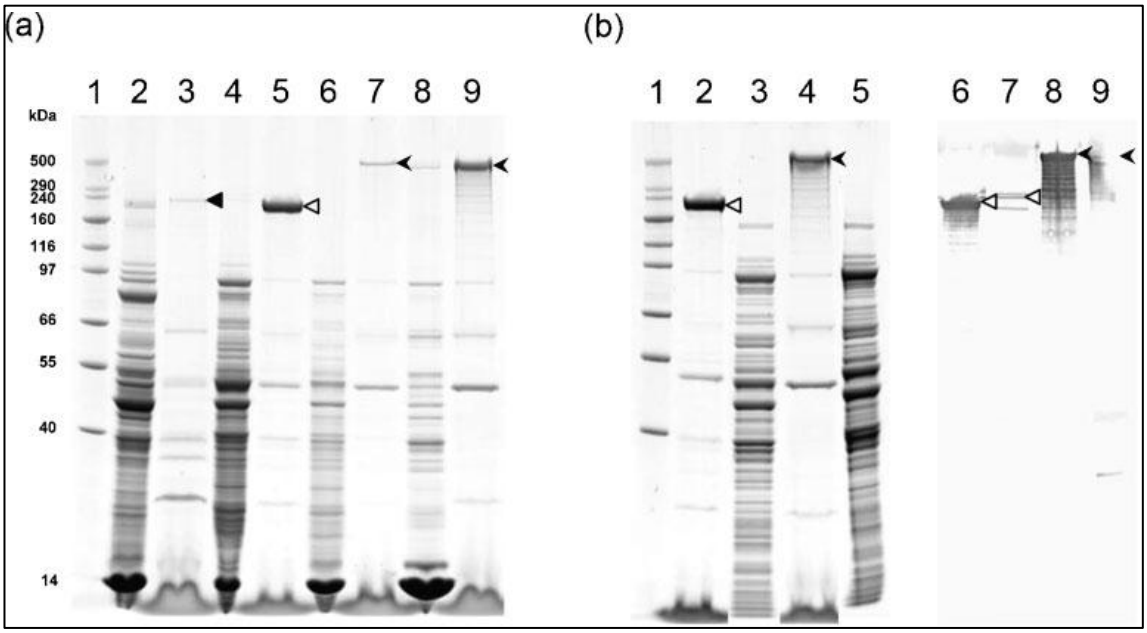


Figure 4.4 a) SDS-PAGE of cell wall extracts (CWE) and spent medium (SM) samples from *L. reuteri* strains. Lane 1, HiMark MW marker; lane 2, CWE from DSM 20016; lane 3, SM from DSM 20016; lane 4, CWE from 1063N; lane 5, SM from 1063N; lane 6, CWE from ATCC 55739; lane 7, SM from ATCC 55739; lane 8, CWE from ATCC 53608; lane 9, SM from ATCC 53608. MUBs are indicated by the arrowheads, the truncated MUB from 1063N by the open triangle and Lar_0958 from DSM20016 by the solid triangle. b) SDS-PAGE and anti-Mub Western blot of spent medium (SM) and soluble cytoplasmic extracts (SCE) from *L. reuteri* strains 1063N and ATCC 53608. Lane 1, HiMark MW marker; lanes 2 and 6 SM from 1063N; lanes 3 and 7 SCE from 1063N, lanes 4 and 8 SM from ATCC 53608, lanes 5 and 9 SCE from ATCC 53608. MUBs are indicated using the same symbols as in a). Taken from [271].

As indicated by SDS-PAGE and confirmed by MS, substantial amounts of MUB proteins were detected in the SM of strains ATCC 53608 and ATCC 55739 (Figure 4.4a, lanes 9 and 7 respectively), with an apparent size of 500 kDa, in accordance with earlier observations that large amounts of MUB are secreted into the media compared to the CWE [124]. For strain DSM 20016, no MUB was detected, however the high MW protein observed in the SM, was identified by MS as a Lar_0958 homologue which was not predicted from analysis of the genome sequence.

Strain 1063N produced a truncated MUB protein of approximately 200 kDa, substantial amounts of which were detected in the SM. Probing with anti-MubR5 confirmed the presence of the protein in both the SM and SCE (Figure 4.4b). MS data confirmed the identity of the protein and further analysis of 1063N DNA by PCR (in collaboration with

Donald MacKenzie from IFR) revealed the insertion of a duplicated 13 nt sequence in the *mub* gene causing a frameshift mutation leading to the secretion of a C-terminally truncated protein. The truncated MUB (MUB^{trunc}) has a predicted size of 1590 aa compared to full length MUB of 3269 aa, lacks the LPXTG cell wall anchor and consists of Mub repeats RI-RIV, R1 and the first part of Mub R2 (Figure 4.5) [271].

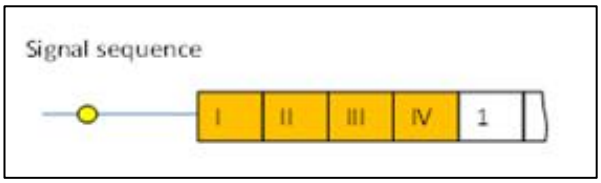


Figure 4.5 Schematic representation of the truncated mucus binding protein MUB^{trunc}, with type 1 Mub repeats (orange) and type 2 Mub repeats (white) from *L. reuteri* 1063N.

Expression of MUB on the bacterial cell surface was further assessed by FCM. *L. reuteri* strains ATCC 53608, ATCC 55739, 1063N and *mub* negative DSM 20016, grown to early stationary phase, incubated with primary antibody anti-MubR5, followed by a fluorescein labelled anti-rabbit IgG secondary antibody.

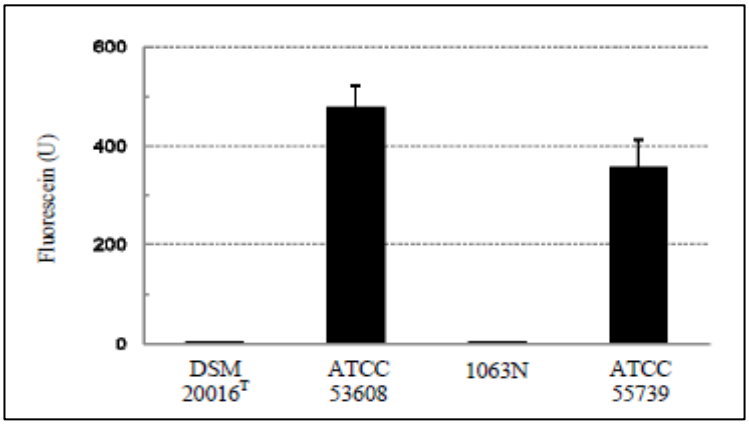


Figure 4.6 Quantification of cell surface MUB on *L. reuteri* strains detected by FCM. From MacKenzie *et al* 2010 [271].

MUB expression was confirmed for strains ATCC 53608 and ATCC 55739 with a 150-200 fold difference in MUB expression between ATCC 53608 and 1063N (Figure 4.6).
In order to further investigate the distribution of MUB at the cell surface of *L. reuteri* strains, we used immunogold labelling with polyclonal antibodies raised against the type 1 Mub repeat MubRI (anti-MubRI) and anti-MubR5, either separately or in combination.

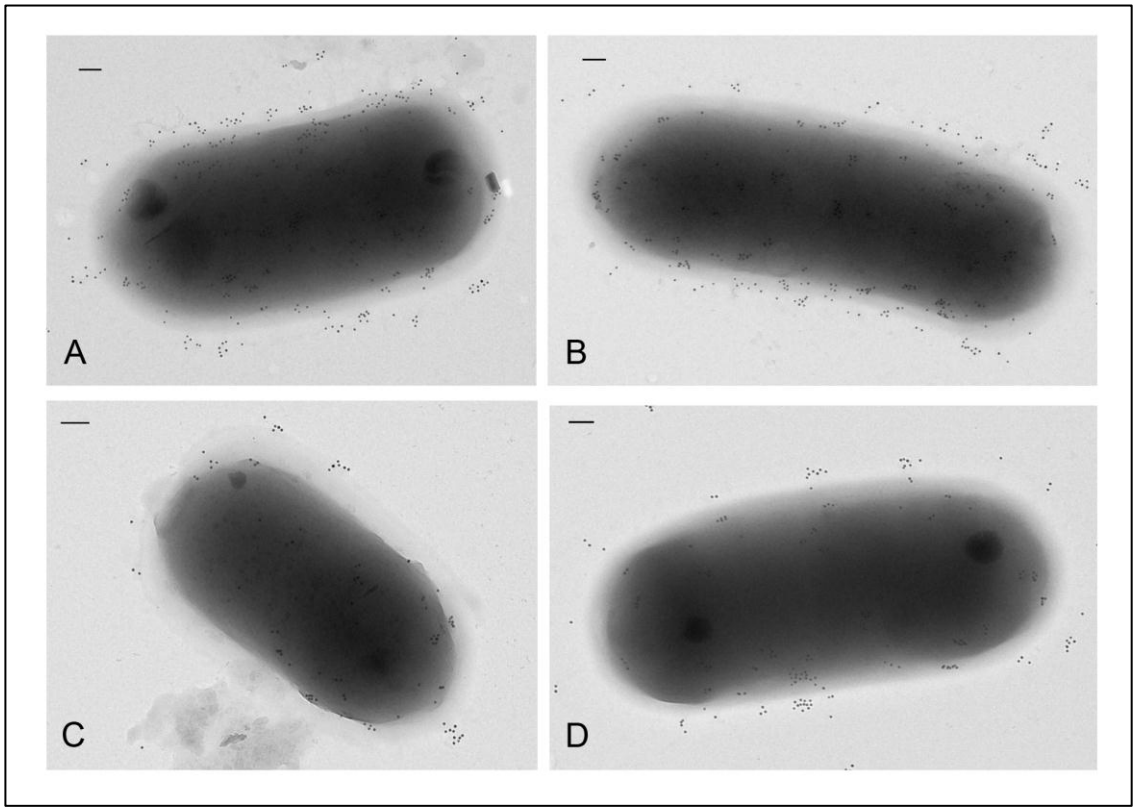


Figure 4.7 Immunogold labelling of MUB with combined anti-MubRI and anti-MubR5 antibodies on the surface of *L. reuteri* strains A) ATCC 55739, B) ATCC 53608 C) DSM 20016 and D) 1063N. Scale bar represents 100 nm.

As expected from the FCM data, strains ATCC 55739 and ATCC 53608 labelled positively with both anti-MubR5 and anti-MubRI antibodies (Figure 4.7). Labelling appears to be distributed over the cell surface and not localised to specific areas. MUB negative strain DSM 20016 showed low levels of non-specific background staining. Strain 1063N showed higher labelling than expected from the FCM data, which may indicate a difference in sensitivity or specificity between the antibodies, as confirmed in Figure 4.8 below, where only background binding was observed with anti-MubR5, while a higher labelling was observed with anti-MubRI.

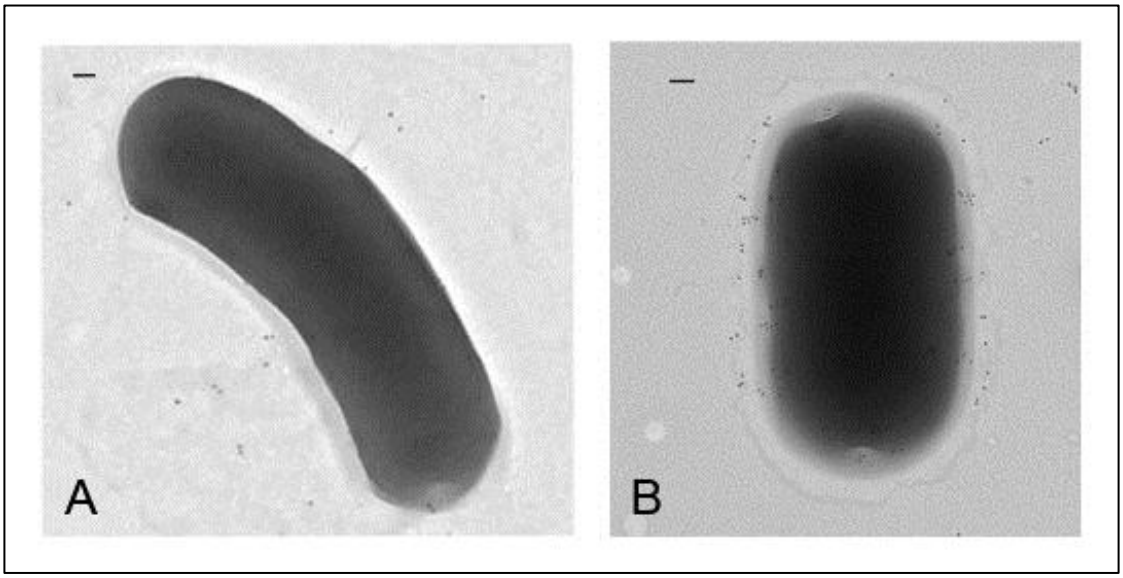


Figure 4.8 Immunogold labelling of MUB^{trunc} on surface of strain 1063N using A) anti-MubR5 antibody, or B) anti-MubRI antibody. Scale bar represents 100 nm.

The specificity of the two antibodies towards MUB was further assessed using 1063N and ATCC 53608 cell surface protein extracts, separated by electrophoresis, transferred to PVDF membrane and probed with anti-MubRI or anti-MubR5 (Figure 4.9).

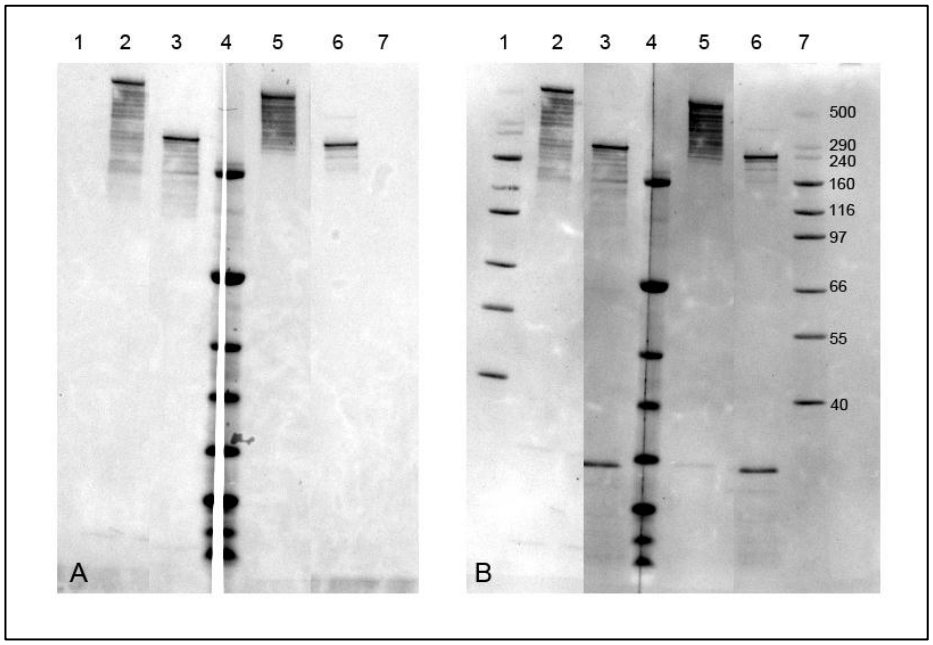
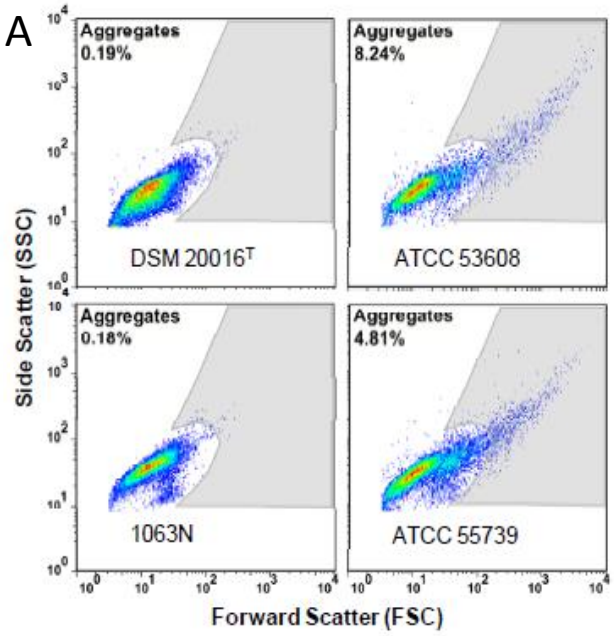


Figure 4.9 Cell surface protein extracts from *L. reuteri* strains 1063N and ATCC 53608 transferred to PVDF membrane after SDS-PAGE then A), probed with anti-MubRI (Lanes 1-3) or anti-MubR5 (Lanes 5-7) and B) followed by Gelcode blue stain of proteins. Lane 1 and 7, Hi MW markers; lane 2 and 5, extracts from ATCC 53608; lane 3 and 6, extracts from 1063N; lane 4, prestained markers.

Both polyclonal antibodies recognised full length MUB and MUB^{trunc} indicating that the anti-MubR5 cross reacts with Mub repeats, other than MubR5, which is not present in the truncated protein. Furthermore the specificity of the antibodies to MUB was confirmed since there was no recognition of other cell surface proteins. The immunogold labelling of 1063N by anti-MubRI may be due to higher sensitivity of the antibody to MUB^{trunc}, perhaps due to the number and type of Mub repeats present in the truncated form.

The autoaggregation phenotype of *L. reuteri* strain 1063, the parent strain of ATCC 53608, is well documented [125], however ATCC 53608 did not show a high autoaggregation ability using the spectrophotometry assay in section 3.4. Here FCM was used to quantify the autoaggregation phenotype of MUB expressing strains ATCC 53608 and ATCC 55739, MUB^{trunc} expressing strain 1063N and MUB negative strain DSM 20016. Briefly, bacterial cells were grown overnight, washed and diluted in PBS to an OD_{600} 0.5. Cells were aspirated and using the fluidics system passed through a laser, the light scatter recorded in the FS and SS detectors was used to assess the autoaggregation. Bacteria exhibiting an autoaggregating phenotype displayed a "tail" on the scatter plots, whereas non autoaggregating strains showed an oval shaped population. For analysis, strains showing a value of less than 90 % of events as single cells were considered as autoaggregating (Figure 4.10).

A



B

Strain	% of events which are single cells
ATCC 55739	85.7
ATCC 53608	86.9
1063N	99.3
DSM 20016	99.3

Figure 4.10 Autoaggregation of *L. reuteri* cells determined by FCM, A) quantified by determining the percentage of events with high FS and SS. From MacKenzie *et al* 2010 [271]. B) FCM analysis showing percentage of events which are single cells.

A clear autoaggregation phenotype was demonstrated for ATCC 53608 and ATCC 55739 compared to strains DSM 20016 and 1063N. The discrepancy between the apparent non-autoaggregating phenotype of ATCC 53608 and ATCC 5573 as detected by spectrophotometry and the aggregating phenotype as measured by FCM may be due to the high sensitivity of the flow cytometric assay. Furthermore the conditions used in both assays are not directly comparable, since for the FCM analysis the bacterial cells were analysed at RT within a fluidics system, whereas in the spectrophotometry assay the cells were maintained statically at 37 °C. Taken together, the FCM data showed a correlation between the level of MUB at the cell surface and the ability of strains ATCC 53608 and ATCC 55739 to autoaggregate, indicating a novel role for MUB in autoaggregation.

4.2 **Comparison of spontaneous mutant 1063N to wild type ATCC 53608**

In order to further characterise strain 1063N in comparison to ATCC 53608, Random Amplification of Polymorphic DNA (RAPD)-PCR analysis and cell surface proteomic surveys were conducted.

RAPD-PCR can be used to accurately differentiate between bacterial species [246], and strains [112]. To this end, random short primers are used to generate a profile of PCR products specific to the bacterial strain which is then used for comparison. Primers used previously for RAPD analysis on lactobacilli were selected from the literature [246, 272] and first tested on the genomic DNA extracted from strain ATCC 53608 (Figure 4.11).

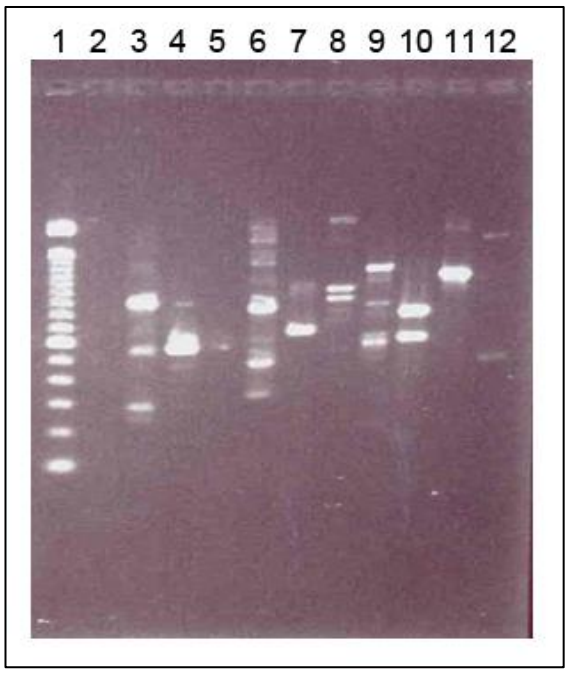


Figure 4.11 Agarose gel (1.5 %) was used to separate PCR products amplified from *L. reuteri* strain ATCC 53608, with primers M14 (lane 3), M13 (lane 4), XD8 (lane 6), XD9 (lane 7), OPL-04 (lane 8), Coc (lane 9), 1254 (lane 10), 1247 (lane 11) and BF2 (lane 12), with DNA ladder (lane 1) and negative control (lanes 2 and 5).

Four primers (M14, XD8, OPL-04 and Coc) produced a pattern of multiple PCR products and were subsequently used to obtain the RAPD profiles for strains 1063N and ATCC 53608.

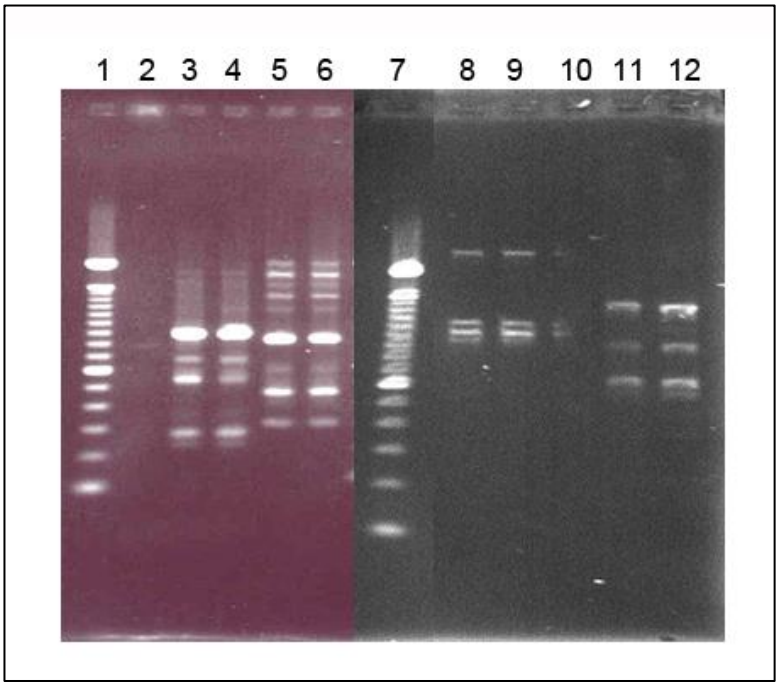


Figure 4.12 Agarose gel (1.5 %) was used to separate PCR products amplified from *L. reuteri* strains. ATCC 53608 (lanes 3, 5, 8 and 11) and 1063N (lanes 4, 6, 9 and 12) with primers M14 (lanes 3 and 4), XD8 (lanes 5 and 6), OPL-04 (lanes 8 and 9) and Coc lanes (11 and 12), with DNA ladder (lanes 1 and 7) and negative control (lanes 2 and 10).

RAPD profiles of strains ATCC 53608 and 1063N showed no apparent differences (Figure 4.12), suggesting that MUB is the major difference between the two strains although the genome of 1063N would need to be sequenced to confirm these findings.

In parallel, the two strains were compared in terms of their cell surface proteome (surfaceome). The extraction of the surfaceome using “shaving” methods has been successfully used to discover vaccine targets in pathogenic bacteria [273]. The use of proteases to digest the exposed peptides from the surface of intact bacterial cells can be problematic in terms of cytoplasmic protein contamination, which is why this technique is most suited to Gram-positive bacteria with their thick PG cell wall more resistant to spontaneous lysis.

Briefly, the surfaceomes of strains ATCC 53608 and 1063N were shaved using trypsin, three biological replicates were performed. These extracts were then further digested with an in-solution digest using one of two treatments, trypsin or chymotrypsin, before separation of peptides by liquid chromatography followed by analysis using MS on the LTQ-Orbitrap. After treatment, cell integrity was confirmed by SEM (
Figure 4.13) and FCM (data not shown).

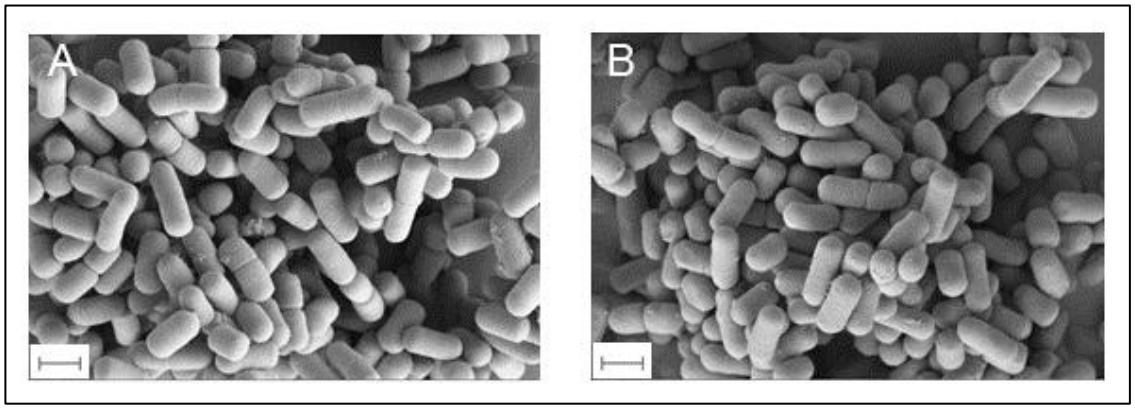


Figure 4.13 SEM images of intact *L. reuteri* ATCC 53608 bacterial cells. A) before trypsin shaving and B) after trypsin shaving. Scale bar represents 1 µm.

MS data were analysed by Mascott to identify proteins from the peptide samples using an in-house protein database based on the annotated genome sequence of *L. reuteri* ATCC 53608 (courtesy of TGAC), and the surfaceome comparison was carried out in Scaffold. Identified proteins were tested for cell localisation using a combination of bioinformatics tools including SignalP for signal peptides, PSORTb for subcellular localisation, LipoP for lipoproteins, TMHMM for transmembrane helices and SecretomeP for non-classical protein secretion.

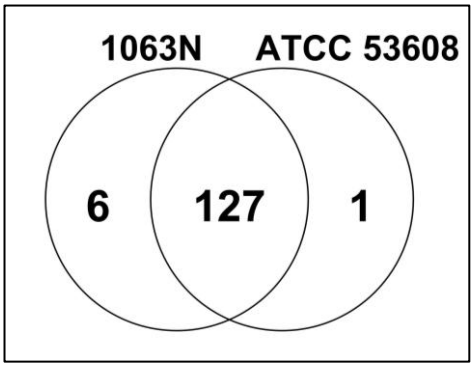


Figure 4.14 Numbers of proteins identified in the surfaceomes of *L. reuteri*, strains 1063N and ATCC 53608.

A total of 134 proteins were identified in the surfaceomes of ATCC 53608 and 1063N, 127 shared by both strains (Figure 4.14), with 51 predicted to be surface located and 83 cytoplasmic. The number of proteins predicted to be located in the cytoplasm should be taken with caution, as a number of them have been shown to have dual functions and localisation within cells, for example protein EF-Tu which is involved in protein synthesis via its guanosine nucleotide binding function when located in the cytoplasm and a role as a mucin binding protein when located on the cell surface of *L. johnsonii* NCC533 [228].

MUB was identified from both strains and a quantification based on the intensity of the peaks obtained by MS, using software Progenesis LC-MS, showed a MUB expression ratio of 29 : 1, for ATCC 53608 : 1063N, which is perhaps a more accurate representation than the difference provided by the FCM data as MS does not rely on antibody recognition. Proteins AggH and APF, known to be involved in autoaggregation in *Lactobacillus* strains [125, 274], were identified in the surfaceomes of strains ATCC 53608 and 1063N, with no differential expression found using Progenesis LC-MS. Of the strain specific proteins (1 for ATCC 53608 and 6 for 1063N), only NADH oxidase from 1063N was consistently found across the different biological and technical replicates. NADH oxidase is predicted to be a cytoplasmic protein produced during aerobic growth. This difference in expression by the two strains is perhaps due to a compensation for the loss of the autoaggregating phenotype for 1063N. During growth of ATCC 53608, the bacterial cells settle on the bottom of the culture flask where the oxygen concentration is at its lowest compared to the more dispersed growth of 1063N.

The proteomics survey supported by data from the RAPD-PCR analysis, indicate no major difference in protein expression at the cell surface, apart from MUB, suggesting that the differences in phenotype observed between strains ATCC 53608 and 1063N, with regard to mucus binding and autoaggregation are due to the absence of MUB on the 1063N cell surface.

4.3 **MUB contribution to bacterial cell adhesion to mucus**

As shown in section 3.3, the two strains expressing full length MUB on the surface of their cells (ATCC 53608 and ATCC 55739) showed highest mucus adhesion capacities, whereas MUB negative DSM 20016 showed lower percentage of adhesion to mucus and MUB^{trunc} strain 1063N showed no binding ability to MCM-S, MSIM, PSIM nor BSA.

In order to test further the contribution of MUB to bacterial adhesion to mucus, cF-labelled bacterial cells of strains ATCC 53608, ATCC 55739, DSM 20016 and 1063N were pre-incubated with anti-MubRI and anti-MubR5 antibodies, either individually (Figure 4.15) or in combination (Figure 4.16), or preimmume serum as a control, before adhesion to mucus was assessed.

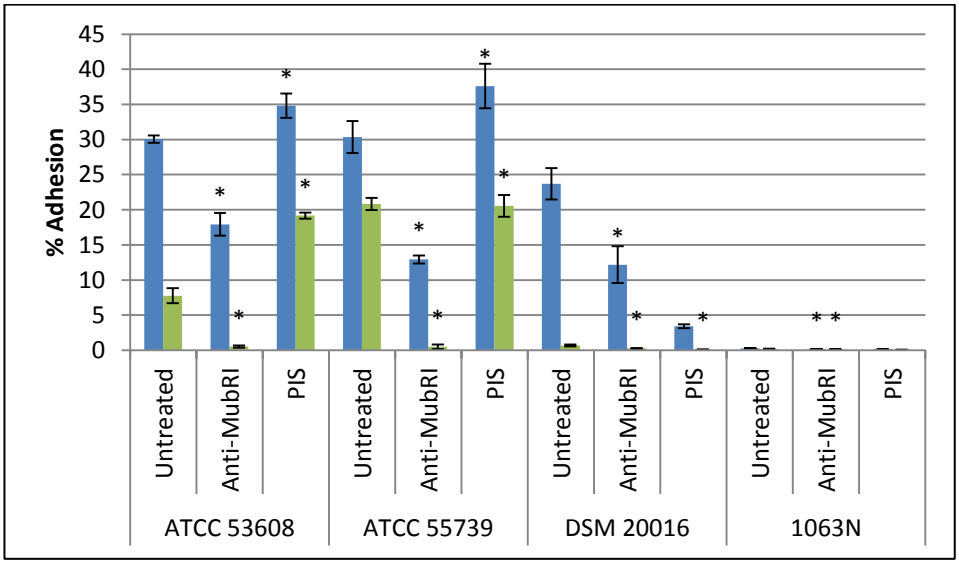


Figure 4.15 Effect of Anti-MubRI on *L. reuteri* ATCC 53608, ATCC 55739, DSM 20016 and 1063N adhesion to mouse colonic mucus (Blue bars) and BSA (Green bars). Pre-immune serum (PIS) was used as a control. * = $p < 0.05$.

Incubation of *L. reuteri* ATCC 53608 and strain ATCC 55739 with anti-MubRI (Figure 4.15) or anti-MubR5 (data not shown) significantly decreased the binding of the bacteria by ~2 fold and ~3 fold to MCM-S and BSA, respectively, providing indirect evidence of MUB involvement in bacterial adhesion. This difference was increased to ~4 fold and ~8 fold when the antibodies were used in combination (Figure 4.16), indicating the involvement of both types of Mub repeats in mucus binding. The non-binding phenotype of strain 1063N was unchanged by antibody incubation.

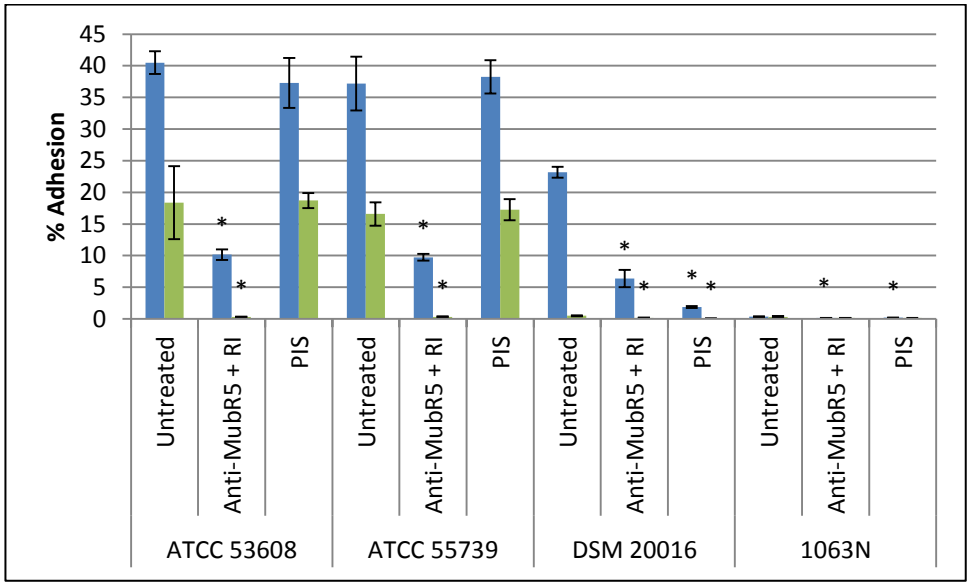


Figure 4.16 Effect of combined anti-MubR5 and anti-MubRI on *L. reuteri* ATCC 53608, ATCC 55739, DSM 20016 and 1063N adhesion to mouse colonic mucus (Blue bars) and BSA (Green bars). Pre-immune serum (PIS) was used as a control. * = p <0.05

L. reuteri DSM 20016, the MUB negative strain surprisingly showed a reduction in adhesion upon pre-incubation with anti-MubRI or anti-MubR5 (alone or in combination), or with the preimmune serum (Figure 4.15 and Figure 4.16), which indicates that *L. reuteri* strains may employ different molecular effectors for adhesion to mucus.

4.4 **Adhesion of MUB protein to mucus**

In order to further assess the role of MUB in binding of ATCC 53608 to mucus, native MUB was purified from SM of ATCC 53608 cultures. Briefly, *L. reuteri* ATCC 53608 cells were grown overnight in LDM II broth (Appendix 1) as used in [124]. Bacteria were centrifuged and the supernatant filtered through a 0.22 µm PVDF membrane to remove all bacterial cells. The vivaflow system was used on the filtered spent media to both remove low MW proteins (less than 50 kDa) and concentrate the extract. The extract was then dialysed extensively in PBS before further concentration using a 0.45 µm spin column.

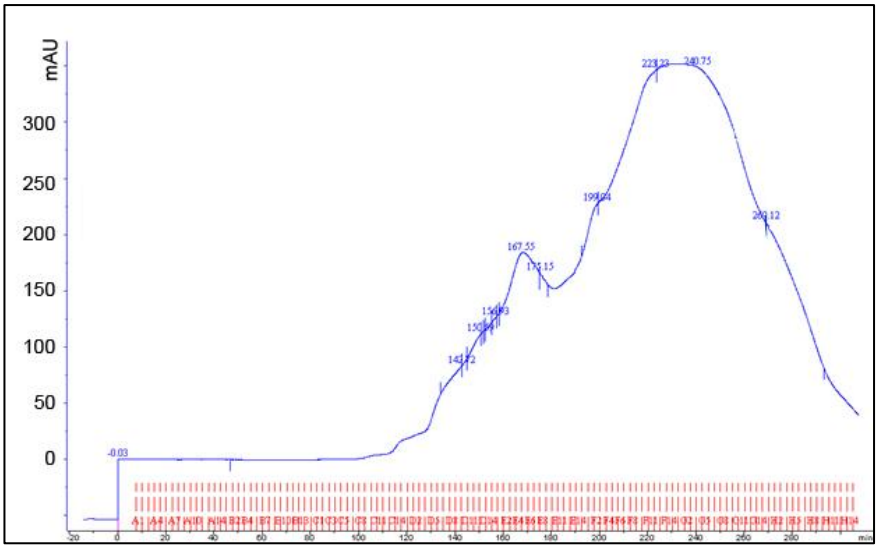


Figure 4.17 Elution profile (blue line) of MUB purification sample from FPLC with gel filtration column. (Fractions collected, indicated by red lines).

The proteins were then fractionated by FPLC using a Superose 6 column (Figure 4.17), using absorbance at 280 nm to follow elution profile. Fractions were collected and analysed by SDS-PAGE (Figure 4.18) and the identity of MUB was confirmed by MS. Fractions C15 - E2 were pooled, corresponding to the first peak observed on the gel filtration elution profile, which separates MUB from the contaminating proteins of the second peak as shown by SDS-PAGE in lanes 6-10 (Figure 4.18).

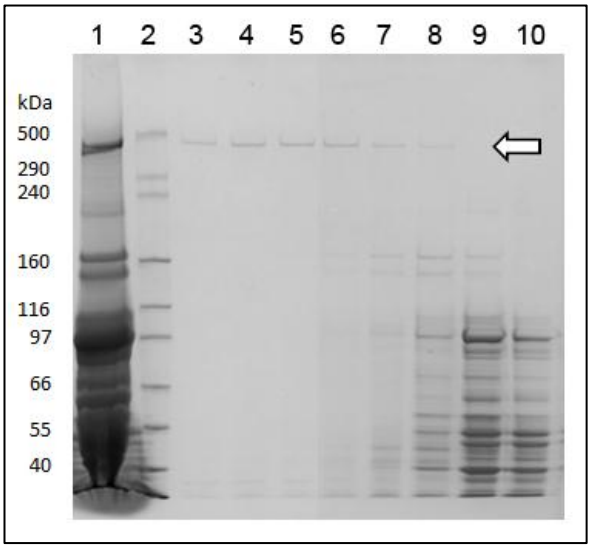


Figure 4.18 SDS-PAGE of selected fractions from gel filtration of MUB purification. Lane 1, SM pre-gel filtration column; lane 2, MW markers; lane 3, fraction D6; lane 4, fraction D10; lane 5, fraction D14; lane 6, fraction E4; lane 7, fraction E7; lane 8, fraction E13; lane 9, fraction F12; lane 10, fraction G7. MUB indicated by arrow.

The concentration of the purified MUB sample as measured by nanodrop (Abs_{280} 1 = 0.125 mg ml^{-1}) was 0.08 mg ml^{-1}. The MUB protein was used in mucus binding assays along with recombinant Mub repeat MubR5 and recombinant triple repeats MubRI-II-III (kindly provided by Donald MacKenzie and Sabrina Etzold, respectively).

The purified proteins were tested for mucus binding ability to immobilised PSIM, MSIM and MCM-C. Adhesion of full length MUB and MubR5 was detected using primary antibody anti-MubR5 while the adhesion of triple domain MubRI-II-III was detected using primary antibody anti-MubRI. Binding was revealed following incubation with anti-rabbit IgG-AP secondary antibody in combination with pNPP substrate.

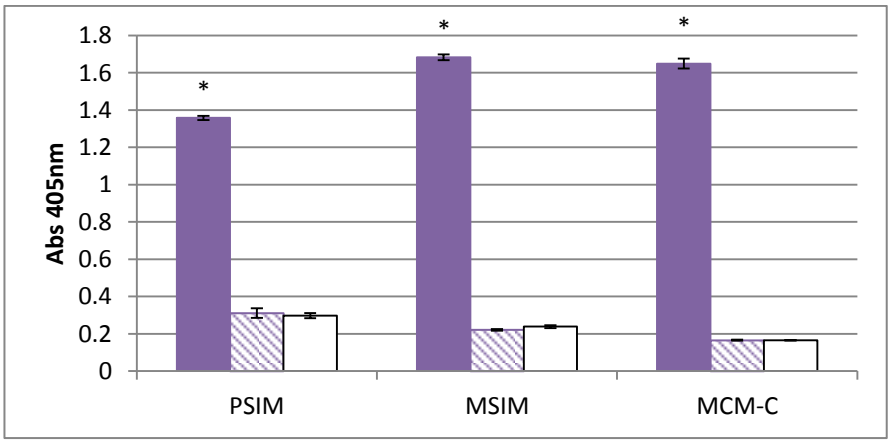


Figure 4.19 Full length MUB (purple bars) and recombinant MubR5 (purple and white bars) or PBS negative control (white bars) binding to mucus, PSIM, MSIM, and MCM-C. * = P>0.05.

The full length MUB was shown to bind to all three types of mucus tested (Figure 4.19), whereas the recombinant MubRI-II-III only showed significant binding to MCM-C (Figure 4.20), while the single repeat MubR5 did not show binding (Figure 4.19). These data indicate that the number and type of Mub repeats present in the protein may be significant for functional binding as well as for the specificity of the interaction.

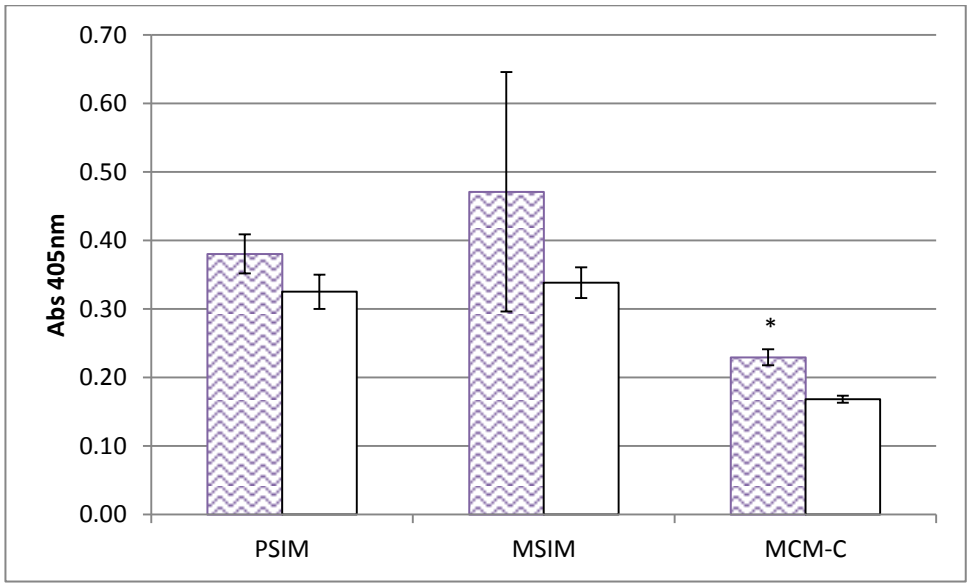


Figure 4.20 MubRI-II-III (purple and white bars) and PBS negative controls (white bars) binding to mucus, PSIM, MSIM, and MCM-C. * = P>0.05.

4.5 **Occurrence of *lar_0958* homologue genes and proteins in *L. reuteri* strains**

In order to assess the occurrence of *lar_0958* homologues in *L. reuteri*, PCRs were performed on bacterial cells from 26 *L. reuteri* strains using primers designed to amplify Lar repeat 1 (corresponding to aa 577-671, KVTY…….NPLGI), which is 100 % identical to repeat regions 2, 3, and 4 (Figure 4.2). PCR products of the expected size were generated in six of the 26 strains, DSM 20016, MM4-1a, CF4-6g, FJ1, LMS11-3 (Figure 4.21) and in strain MM4KO (data not shown).

Strain MM4KO (full name MM4-1a Lar_0958 K/O) is a Lar_0958 homologue mutant, generated by Rob Britton and co-workers (Michigan State University, USA). A ctg stop codon inserted after bp 41;
1 ATGCTATCAA GAAAAAATTA TAAGGAAACT ATACGAAAAC ActgACCTAC …..
resulted in a truncated translated product of 14 aa (MLSRKNYKETIRKH).

DNA sequencing of the PCR products showed 100 % identity to the predicted 285 bp fragment from *lar_0958* across the strains. Notably the six *lar_0958* positive strains were all human isolates.

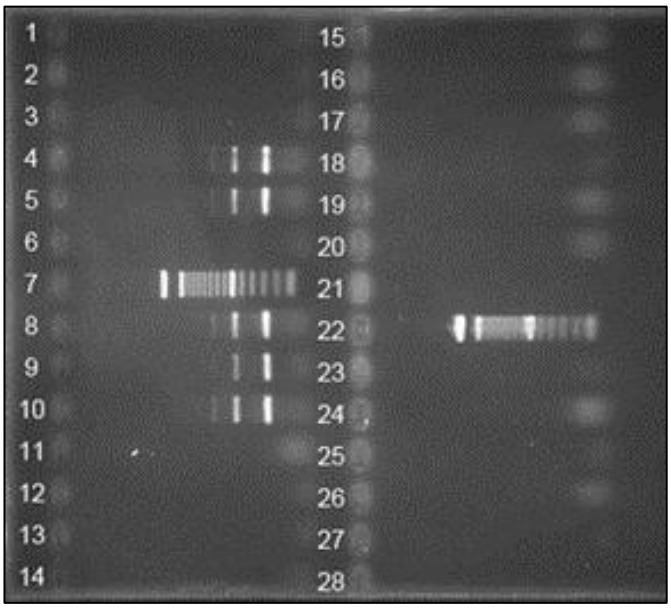


Figure 4.21 Agarose gel (1.5 %) separating PCR products of *lar_0958* amplified from *L. reuteri* strains. ATCC 53608 (lane 1), ATCC 55739 (lane 2), 1063N (lane 3), DSM 20016 (lane 4), MM4-1a (lane 5), MF2-3 (lane 6), CF4-6g (lane 8), FJ1 (lane 9), LMS11-3 (lane 10), sr11 (lane 11), JW2015 (lane 12), Lp167-67 (lane 13), 20-2 (lane 14), 3c6 (lane 15), CR (lane 16), One-One (lane 17), DSM 17509 (lane 18), N2D (lane 19), R2LC (lane 20), ML1 (lane 21), #20 (lane 23), r13 (lane 24), Lr4020 (lane 25), LB54 (lane 26), with DNA ladder (lanes 7 and 22) and negative control (lane 27). Lane 28 is blank.

Interestingly DSM 20016 gave a PCR product which was not predicted from the genome sequence. Strain JCM 1112 and DSM 20016 are both derived from the same human isolate (strain F275), however their genomes were sequenced in different laboratories and an alignment of the two sequences showed that strain JCM 1112 contains two large unique regions which results in a 40 kb difference between the two strains [275]. One of the “unique” regions in JCM 1112 contains the sequence of *lar_0958* which we have shown is present in DSM 20016. Originally the authors attributed the 40 kb difference between the two strains to the tendency of bacteria to undergo genomic changes during repeated laboratory culturing, however our work demonstrates the presence of at least part of one of the “unique” regions in the DSM 20016 genome.

Media extracts from overnight cultures were used to assess the expression of the Lar_0958 protein homologues by all PCR positive strains, DSM 20016, MM4-1a, MM4KO, CF4-6g, FJ1, LMS11-3 and negative control ATCC 53608. Protein extracts were analysed by electrophoresis followed by western blotting using the anti-Lar antibody directed against the 100 % identical repeat domain of Lar_0958. The blot was subsequently stained with gelcode blue for estimation of the apparent molecular mass by reference to the high MW marker.

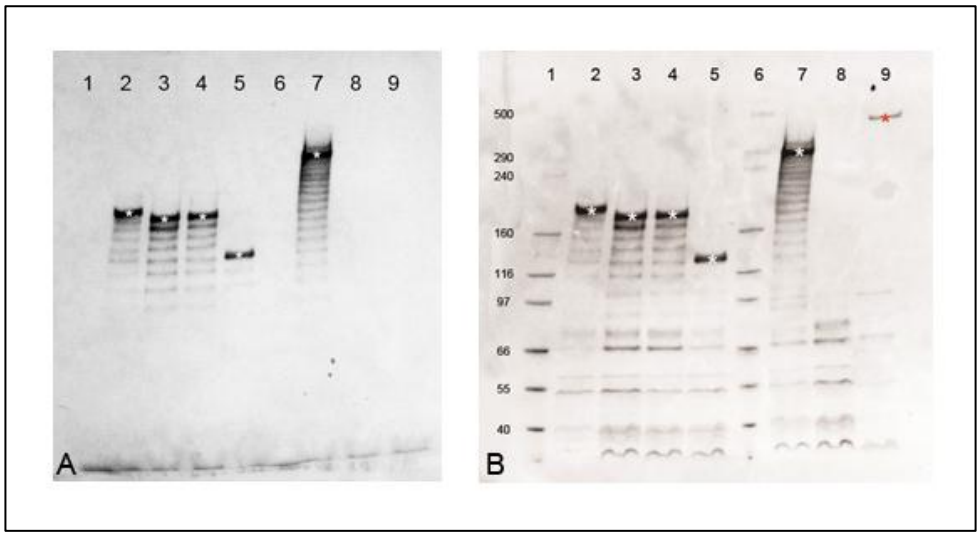


Figure 4.22 Western blot of Lar_0958 homologous proteins separated by electrophoresis from media extracts of *L. reuteri* strains, DSM 20016 (lane 2), MM4-1a (lane 3), CF4-6g (lane 4), FJ1 (lane 5), LMS11-3 (lane 7), MM4KO (lane 8), ATCC 53608 (lane 9), and HiMark MW markers (lanes 1 and 6). A) Blot probed with anti-Lar antibody. B) Blot subsequently stained with gelcode blue. Lar_0958 homologues indicated with white star. MUB indicated with red star.

Several protein bands of different molecular masses were recognised by the anti-Lar antibody in protein extracts from strains DSM 20016, MM4-1a, CF4-6g, FJ1 and

LMS11-3 (Figure 4.22). For each strain there was one strong band with a number of weaker bands evenly spaced at lower MW, this ladder effect may correspond to the degradation of the full length proteins or be a result of transcriptional processing due to the repeat nature of the DNA sequence. No Lar_0958 protein homologue was identified in protein extracts from strains MM4KO or ATCC 53608, as expected from the genome sequences. The apparent molecular mass of the Lar_0958 homologues was determined using TotalLab software showing that strains DSM 20016, MM4-1a, CF4-6g, FJ1 and LMS11-3 expressed proteins of apparent MW ~182 kDa, ~174 kDa, ~176 kDa, ~129 kDa and ~279 kDa, respectively. The difference in the size of Lar_0958 homologues between the different *L. reuteri* strains may be due to the presence of a different number of repeat regions.

FCM was used to determine whether the Lar_0958 homologues are expressed on the bacterial cell surface. Cells from overnight cultures of strains MM4-1a, MM4KO, DSM 20016 and ATCC 53608 were incubated with primary anti-Lar antibody or PIS, followed by the secondary fluorescein labelled anti-rabbit IgG antibody before analysis by FCM (Figure 4.23). SS signals along with the fluorescent signals detected in the FL1 channel were used to measure the Lar_0958 expression at the cell surface.

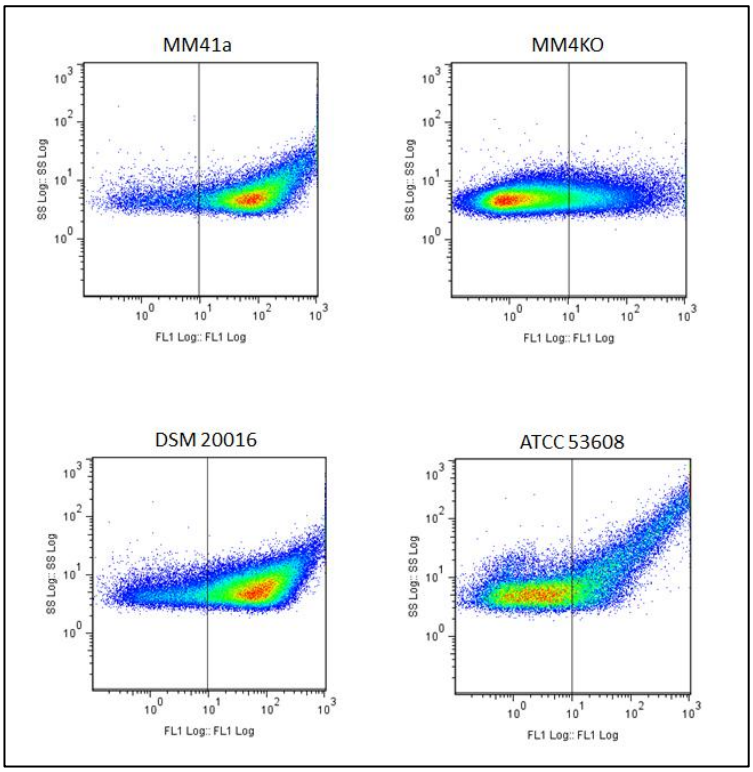


Figure 4.23 Quantification of Lar_0958 on cell surface of *L. reuteri* strains. Bacterial cells with high fluorescent signal were measured by FCM.

Lar_0958 homologues were clearly detected on the cell surface of strains MM4-1a and DSM 20016 (Figure 4.23), with a 22-33 fold higher expression of Lar_0958 compared to MM4KO and ATCC 53608 (Figure 4.24).

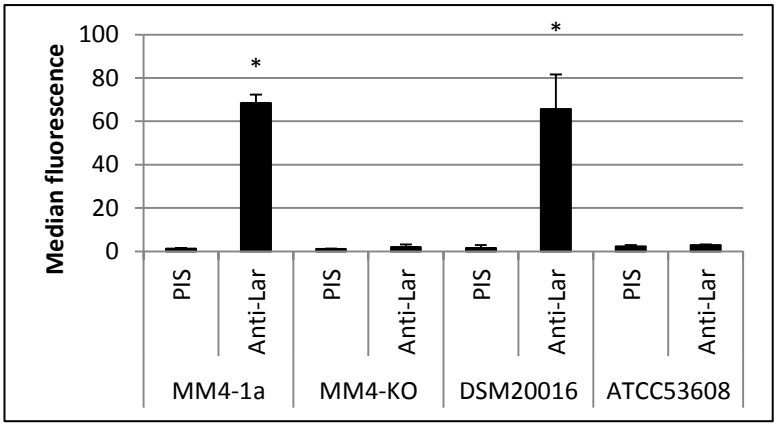


Figure 4.24 FCM quantification of Lar_0958 homologue expression by selected *L. reuteri* strains, with anti-Lar_0958 antibody compared to preimmune serum (PIS).

The localisation of Lar_0958 homologues on the cell surface of strains MM4-1a and DSM 20016 was further investigated by immunogold labelling using the anti-Lar antibody. Strain MM4KO was included as a negative control.

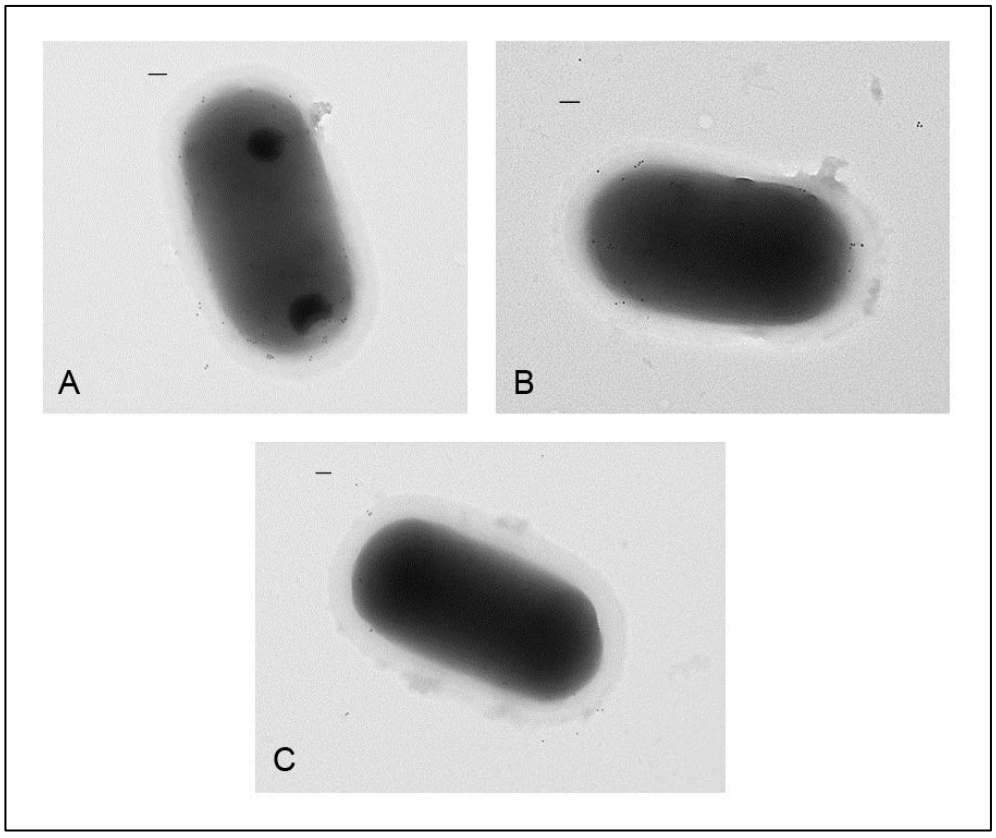


Figure 4.25 Immunogold labelling of Lar_0958 homologues on the surface of *L. reuteri* strains with anti-Lar0958 antibody. A) MM4-1a, B) DSM 20016, C) MM4KO. Scale bar represents 100 nm.

Lar_0958 homologues were detected on the cell surface of strains MM4-1a and DSM 20016 and absent from the cell surface of MM4KO (Figure 4.25), confirming the FCM data, although a low level of labelling was observed indicating that this technique is less sensitive than FCM, and prohibiting an accurate assessment of the protein distribution on the cell surface.

4.6 **Lar_0958 homologue contribution to bacterial cell adhesion to mucus**

The role of Lar_0958 homologues in the adhesion capacity of *L. reuteri* to mucus was investigated using two Lar_0958 expressing strains, MM4-1a and DSM 20016, and two non-expressing strains, MM4KO and ATCC 53608. A binding assay was performed where bacterial cells from overnight cultures were pre-incubated with either anti-Lar antibody, PIS, or left untreated, before testing their binding to MCM-S.

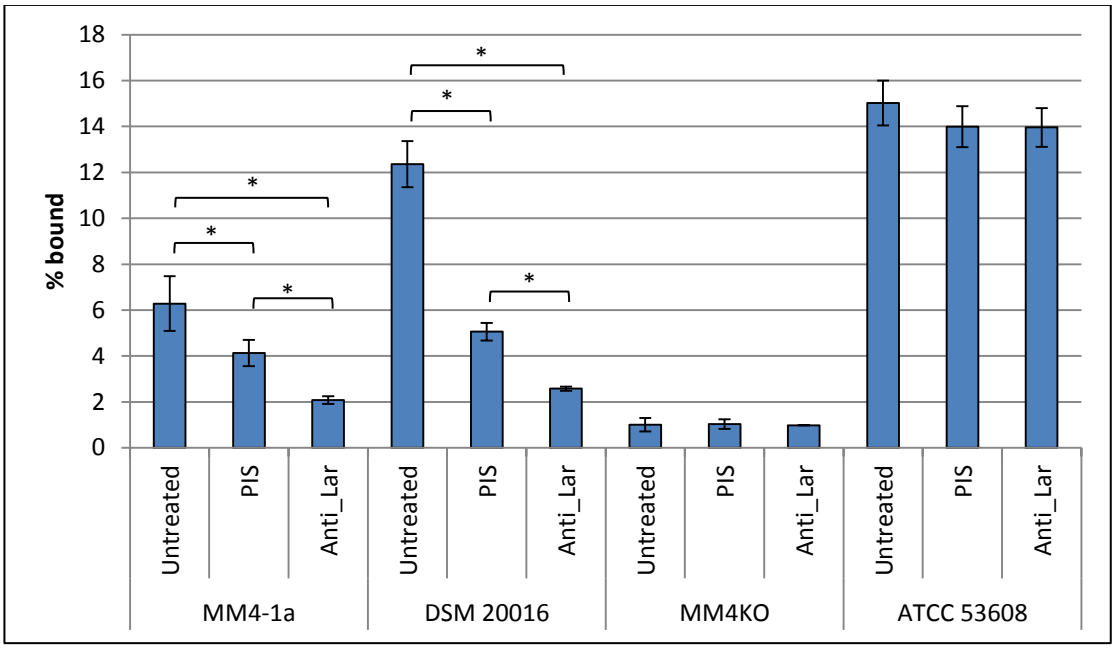


Figure 4.26 Adhesion of *L. reuteri* strains to MCM-S with pre-incubated bacterial cells, with anti-Lar_0958 antibody, PIS or left untreated. * P <0.05

Adhesion of untreated bacterial cells to MCM-S confirmed the binding results in section 3.3, with strain ATCC 53608 showing highest binding ability, MM4KO showing low levels of binding and strains MM4-1a and DSM20016 showing intermediate levels of binding. Pre-incubation of the bacterial cells with the anti-Lar antibody led to a significant decrease in the binding of strains MM4-1a and DSM 20016 to MCM-S, but had no effect on strains ATCC 53608 and MM4KO (Figure 4.26) which do not possess a Lar_0958 homologue. However the PIS also impacted significantly on the binding ability of Lar_0958 expressing strains MM4-1a and DSM 20016, and although the anti_Lar antibody has a more pronounced effect, this may indicate either an interaction of Lar_0958 with a component of the PIS or a non-specific interaction with other proteins.

In order to test if the PIS recognises Lar_0958 homologues or other proteins, strains MM4-1a, MM4KO, DSM 20016 and ATCC 53608 were grown in LDM II, the proteins in

the spent media were separated by electrophoresis, transferred to PVDF membrane and probed with PIS.

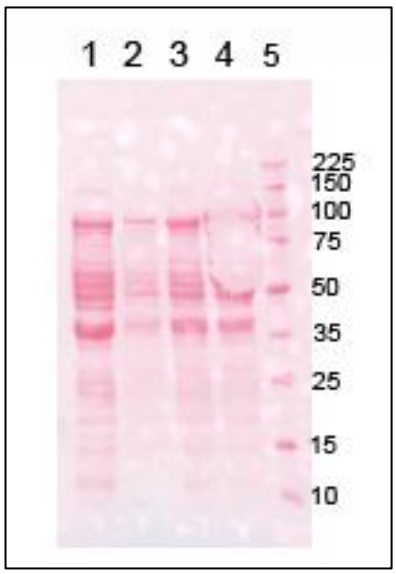
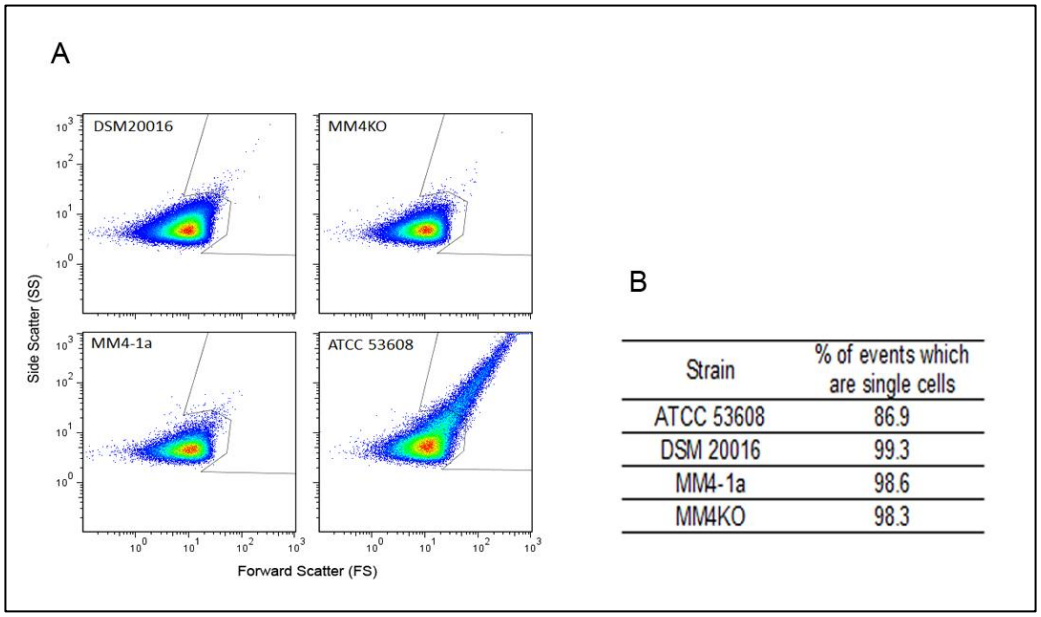


Figure 4.27 Western blot of SM proteins separated by electrophoresis from *L. reuteri* strains, ATCC 53608 (lane 1), DSM 20016 (lane 2), MM4KO (lane 3), MM4-1a (lane 4), with Broad range MW markers (lane 6), stained with ponceau red.

Although proteins from the SM extracts were shown to have been efficiently transferred to the PVDF membrane by reversible ponceau red staining (Figure 4.27), no signal was observed when PIS was used to probe the blot (data not shown), indicating that the reduction in binding observed in the competition assay was not due to interactions of PIS with *L. reuteri* proteins.

Since autoaggregation may also play a role in the ability of bacterial cells to bind to mucus, the autoaggregating phenotype of MM4-1a and MM4KO was investigated by FCM and compared to the non-aggregating DSM 20016 and the autoaggregating ATCC 53608 strains. FS and SS parameters were used to distinguish single cells from aggregates and the percentage of single cells was determined. Strains showing a single cell percentage value of less than 90 % were considered as autoaggregating (Figure 4.28).



Strain	% of events which are single cells
ATCC 53608	86.9
DSM 20016	99.3
MM4-1a	98.6
MM4KO	98.3

Figure 4.28 Autoaggregation of *L. reuteri* cells determined by FCM, A) quantified by determining the percentage of events with high FS and SS. B) FCM analysis showing percentage of events which are single cells.

The results indicate that strains MM4-1a, MM4KO and DSM 20016 are non-aggregating, therefore the observed binding to mucus in these strains is not an effect of autoaggregation.

4.7 **Adhesion of Lar_0958 to mucus**

In order to investigate the direct binding of Lar_0958 to mucus, a single Lar repeat, from aa 577 to 672 of DSM 20016 Lar_0958 homologue (Figure 4.29) was heterologously expressed in *E. coli* TUNER(DE3)pLacI cells using pETBlue-1 AccepTor (Novagen) vector and purified by ion exchange chromatography (in collaboration with Donald MacKenzie).

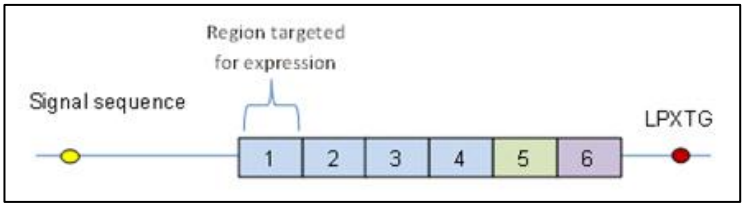


Figure 4.29 Schematic representation of Lar_0958 from *L. reuteri* JCM 1112, showing six Lar repeat domains, repeats 1-4 are identical (blue), repeat 5 has 99 % aa similarity (green) and repeat 6 is 46 % aa similarity (purple). Blue bracket indicating the region targeted for Lar repeat expression.

The recombinant Lar repeat was incubated with immobilised PSIM, MSIM, MCM-S, or BSA, unbound protein was washed off and the bound Lar repeat was detected using the anti-Lar antibody followed by secondary antibody, anti-rabbit IgG-AP and pNPP substrate. The single Lar repeat did not bind significantly to any of the mucus preparations, when compared to BSA (data not shown). The high background noise meant that once subtraction had occurred the levels of binding detected were negligible. The low level of binding may due to the nature of the recombinant Lar_0958 protein which contained only a single repeat.

Therefore the purification of native Lar_0958 homologue from spent media of bacterial cultures was undertaken. *L. reuteri* strains MM4-1a and DSM 20016 were first tested for their ability to secrete a Lar_0958 homologue into different media (MRS or LDM II). Lar_0958 homologues were produced in all conditions. No differences were observed between DSM 20016 and MM4-1a strains (Figure 4.30). Strain MM4-1a grown in LDM II was chosen for the larger scale purification of Lar_0958 homologue.

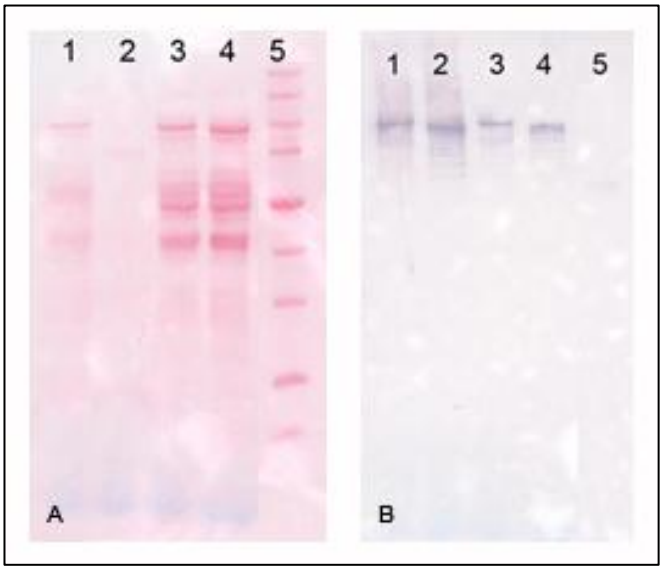


Figure 4.30 Western blot of *L. reuteri* SM samples separated by electrophoresis. Extracted from different *L. reuteri* strains, DSM 20016 (lanes 1 and 3), and MM4-1a (lanes 2 and 4), with Broad range MW markers (lane 5), when cells were grown in different media, MRS (lanes 1 and 2), LDM II (lanes 3 and 4). A) Reversible staining with Ponceau Red B) Recognition of Lar_0958 homologues with anti-Lar antibody.

L. reuteri MM4-1a cells were grown overnight in LDM II broth, bacteria were centrifuged and the supernatant was filtered through 0.22 µm PVDF membrane to remove all bacterial cells and concentrated using the vivaflow system. The sample was then dialysed extensively in PBS before further concentration of proteins using a 0.45 µm spin column. The proteins were then fractionated on a Superose 6 column, where the elution profile showed one large peak (Figure 4.31). Fractions were collected and analysed by SDS-PAGE (Figure 4.32).

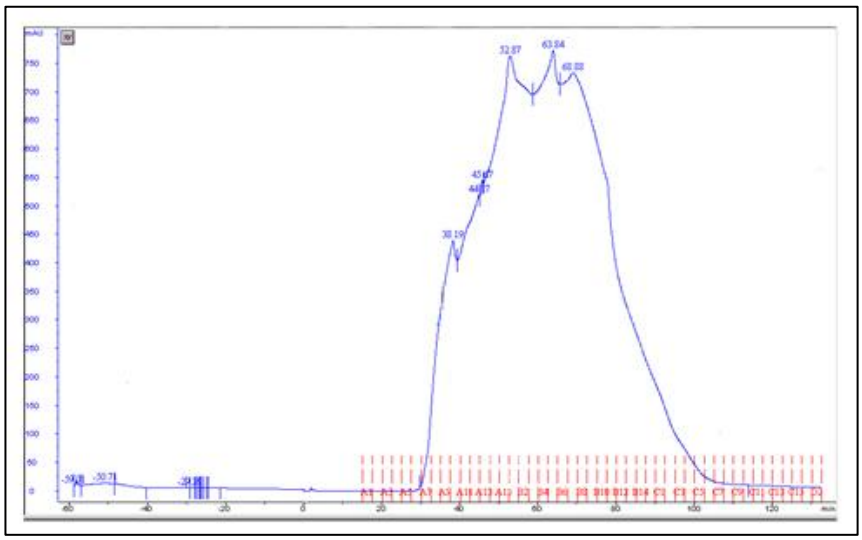


Figure 4.31 Elution profile (blue line) of Lar_0958 homologue purification sample from FPLC with gel filtration column. (Fractions collected indicated by red lines).

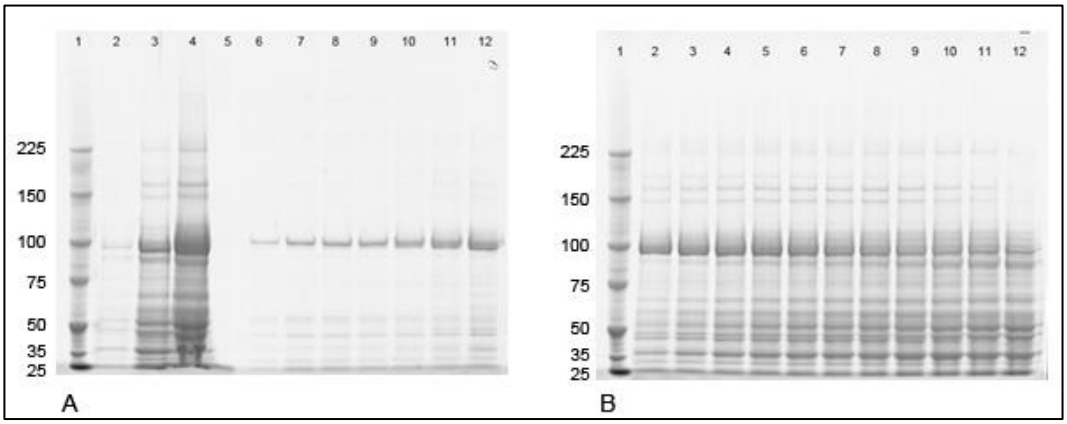


Figure 4.32 SDS-PAGE of Lar_0958 homologue purification stages. A) Lane 1, Broad range MW markers; lane 2, SM after filtration, lane 3, SM after vivaflow; lane 4, SM pre-gel filtration column; lane 5, fraction A6; lane 6, fraction A7; lane 7, fraction A8; lane 8, fraction A9; lane 9, fraction A10; lane 10, fraction A11; lane 11, fraction A12; lane 12, fraction A13. B) Lane 1, Broad range MW markers; lane 2, fraction A14, lane 3, fraction A15; lane 4, fraction B1; lane 5, fraction B2; lane 6, fraction B3; lane 7, fraction B4; lane 8, fraction B5; lane 9, fraction B6; lane 10, fraction B7; lane 11, fraction B8; lane 12, fraction B9.

The transfer of proteins to PVDF membrane and probing with anti-Lar antibody (Figure 4.33), in conjunction with excision from gel and MS analysis, confirmed the identity of the Lar_0958 homologue as a faint protein band at ~200 kDa, while the stronger protein band occurring at approximately 100 kDa did not react with the anti-Lar antibody (Figure 4.33), but was identified by MS to be an aldehyde-alcohol dehydrogenase with a MW of 97 kDa. The low recovery of Lar_0958 at this stage (10-20 fold less than MUB), prevented further pursuit of native Lar_0958 purification.

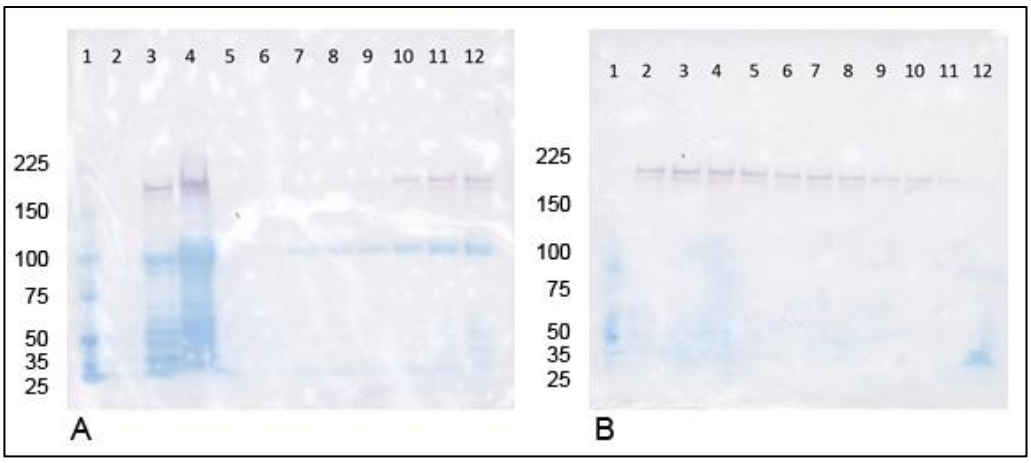


Figure 4.33 Western blot of SDS-PAGE of Lar_0958 homologue purification stages, probed with anti-Lar antibody (purple signal). A) Lane 1, Broad range MW markers; lane 2, SM after filtration, lane 3, SM after vivaflow; lane 4, SM pre-gel filtration column; lane 5, fraction A6; lane 6, fraction A7; lane 7, fraction A8; lane 8, fraction A9; lane 9, fraction A10; lane 10, A11; lane 11, fraction A12; lane 12, fraction A13. B) Lane 1, Broad range MW markers; lane 2, fraction A14, lane 3, fraction A15; lane 4, fraction B1; lane 5, fraction B2; lane 6, fraction B3; lane 7, fraction B4; lane 8, fraction B5; lane 9, fraction B6; lane 10, fraction B7; lane 11, fraction B8; lane 12, fraction B9.

4.8 **Discussion**

Cell surface proteins are thought to play a major role in mediating bacterial adhesion to mucus, however very few of the bacteria-mucus binding studies investigate the molecular mechanisms of the interactions. Only a few proteins have been functionally shown to bind to mucus and MUB, which was originally identified in *L. reuteri* 1063 (parent of ATCC 53608), was the first to have the crystal structure of one of the Mub repeats determined, providing structural insights into mucus binding proteins in Gram-positive bacteria [223]. Our work showed that both MUB and Lar_0958 homologues are mediators of mucus adhesion of *L. reuteri* strains. MUB homologues are found in many lactic acid bacteria [231], and specifically in the exoproteomes of lactobacilli isolated from the GI tract [232]. Currently there are 949 homologous proteins identified across all bacterial phylotypes in the NCBI non-redundant protein sequences database. Our study showed that the full length MUB was expressed by *L. reuteri* ATCC 53608 and ATCC 55739, whereas, Lar_0958 homologues were expressed by a larger number of *L. reuteri* strains (human isolates DSM 20016, MM4-1a, CF4-6g, FJ1, and LMS11-3). Lar_0958 is a novel mucus binding protein and currently 306 homologous proteins have been identified across all bacterial phylotypes in the NCBI non-redundant protein sequences database.

MUB and Lar_0958 share some structural and biochemical properties, they are both large, modular proteins with a cell surface anchor, they are much larger than the other functionally characterised mucus binding proteins in lactobacilli (Table 4.1) but share a similar acidic pI with the exceptions of MapA and SpaB.

Mucus Binding Protein	MW	Predicted pI
MUB	353	4.75
Lar_0958	133	5.6
Map A	26	9.7
EF-Tu	43	4.9
GroEL	57	4.7
GAPDH	36	5.4
Pili proteins SpaC	91	5
SpaF	104	5
SpaB	21	8

Table 4.1 Mucus binding proteins in lactobacilli.

Pili subunit SpaB is a small protein with a high pI (Table 4.1), interacts with mucus involving non-specific electrostatic interactions [174], due to the net difference in charge between mucus and SpaB. Although mucins remain net negatively charged at all values of pH, MUB and Lar_0958 with an acidic pI will have a net neutral to negative

charge in the GI tract, it is therefore likely that interactions between these mucus binding proteins and mucus involve specific interactions. The current hypothesis is that the target of mucus binding proteins are the O-glycan structures which decorate the mucin protein backbone, although this remains to be proved. Multivalency is the term used to describe the adhesion of multiple ligands with multiple receptors, a situation found in many biological interactions [276]. The repeat nature of both MUB and Lar_0958 homologues and the absence of mucus binding for single repeats may indicate the adaptation of these mucus binding proteins to the multivalent substrate. Due to the complexity of the mucin O-glycan structures, having an array of adhesive units with different sugar specificity would be an advantage for a mucus binding protein. It is also probable that the targets of adhesion involve the non-mucin mucus components as indicated by the binding of MubR5 to Igs [223] and the demonstrated role of MUB in autoaggregation.

There was a clear autoaggregation phenotype for MUB expressing strains ATCC 53608 and ATCC 55739. The difference in autoaggregation phenotype observed between ATCC 53608 and the non aggregating strain 1063N can be attributed to MUB. This dual role of adhesion and autoaggregation has also been shown for the extracellular protein, transaldolase in *Bifidobacterium bifidum* A8 [277]. Furthermore, aggregation factors glucosyltransferase and inulosucrase from *L. reuteri* TMW1.106 were shown to have a dual role with biofilm formation leading to a reduction in colonisation ability in an ex-*Lactobacillus*-free mouse model [128]. In contrast, aggregation was not implicated in the specific mucus binding ability of Lar_0958 expressing human isolates DSM 20016 and MM4-1a, suggesting that differences in the host environment require different mechanisms for host-bacterial interactions.

We showed that MUB and Lar_0958 proteins are located on the bacterial cell surface and are also released into the growth media, as reported previously for MUB in strain 1063 by Roos & Jonsson (2002) [124]. This feature is perhaps an indication of an alternative function for these adhesive molecules. The secretion of adhesins into the surrounding milieu is postulated to secure a competitive advantage, by acting to aggregate competing bacteria and facilitate clearance, as shown for example with GroEL from *L. johnsonii* La1 which mediates aggregation of *H. pylori* [229].

The competition assays showed that MUB and Lar_0958 homologues are not the sole mediators of mucus adhesion. There are a number of other possible effectors,

including non-proteinaceous cell surface structures such as EPS which has been implicated in the colonisation of *L. reuteri* strain 100-23 in a *Lactobacillus* free mouse model [278]. While proteins with assigned functions may have a dual role with a secondary function in mucus binding, as highlighted by the cytoplasmic proteins EF-Tu [228], GroEL [229] and GAPDH [230], the mechanisms of when and how they become associated with the cell surface remain unknown. This may be through an as yet unidentified secretion pathway or as cells lyse and release their contents the cytoplasmic proteins become associated with the surface of neighbouring cells. Another possible type of mediator may be other mucus binding proteins as yet unidentified. Recently there has been a growing interest in the surface exposed proteomes of bacteria, with advances in both proteomic [279, 280] and *in silico* [281, 282] approaches. However the functions of up to 60 % of predicted extracellular proteins in lactobacilli are unknown [232], indicating a need for future functional studies.

5 Investigating novel *L. reuteri* mucus binding proteins

As we showed in the previous chapters, *L. reuteri* adhesion to mucus is strain specific and involves large cell surface proteins, MUB for strains ATCC 53608 and ATCC 55739 and Lar_0958 homologues for strains MM4-1a and DSM 20016, both proteins being constitutively expressed *in vitro*. These proteins are not the sole mediators of binding and the aim of the work reported in this chapter was to identify novel *L. reuteri* mucus binding protein candidates. A higher mucus binding phenotype can be induced in specific *L. reuteri* strains by the presence of mucin in the growth media, and using this characteristic in combination with a quantitative proteomics approach we investigated differentially expressed cell surface proteins when *L. reuteri* was grown in the presence and absence of mucin.

5.1 **Induction of bacterial binding to mucus**

L. reuteri strains DSM 20016, 100-23, ATCC 53608 and MM4-1a, for which the genome sequence is available, and related strains DSM 17509, 1063N, and MM4KO were tested for their mucus binding ability when grown in the presence of mucin, along with strains 11284 and DSM 17938 whose responses to mucin supplementation were reported earlier [191]. The factors responsible for this induction are unknown although changes in gene expression are likely to occur.

Briefly, bacteria were grown overnight in MRS broth in the presence and absence of mucin supplementation and the adhesion of cells to immobilised PSIM was examined under an inverted microscope and quantified at 460 nm [191].

Strains 100-23, MM4KO and 11284 showed a significant increase in mucus binding when the bacteria were grown in medium supplemented with mucin (Figure 5.1). In contrast, strain DSM 17509 which is derived from the same parent strain as 100-23 showed no difference in PSIM binding when cells were grown in the presence or absence of mucin. The difference in behaviour observed between strains DSM 17509 and 100-23 may be due to the tendency of bacteria to undergo spontaneous changes during regular laboratory culturing. Strain ATCC 53608 showed a decrease in adhesion ability to PSIM when cultures were grown in the presence of mucin. In addition, the cells were observed by microscopy to form large aggregates which may affect binding to mucus. No increase in binding was observed with strains 1063N, MM4-1a, DSM 20016 or DSM 17938.

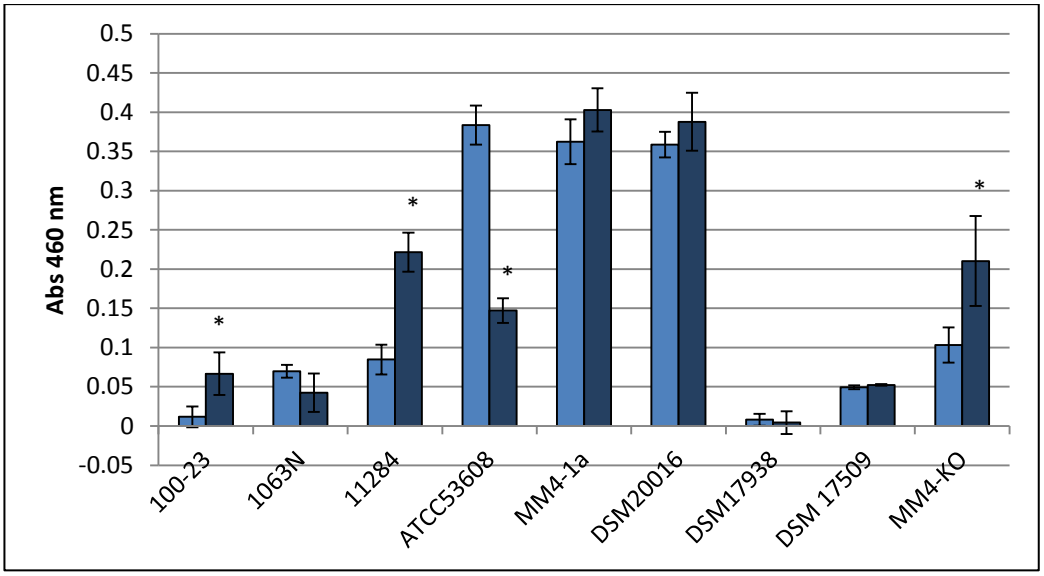


Figure 5.1 Binding of *L. reuteri* strains grown in MRS (pale blue bars) or MRS supplemented with mucin (dark blue bars) to PSIM * = p <0.05.

The binding of strain MM4KO to immobilised mucus, when cells were grown in the presence or absence of mucin, was further investigated with different mucus substrates, PSIM, MCM-C, and MSIM, with immobilised BSA as a control.

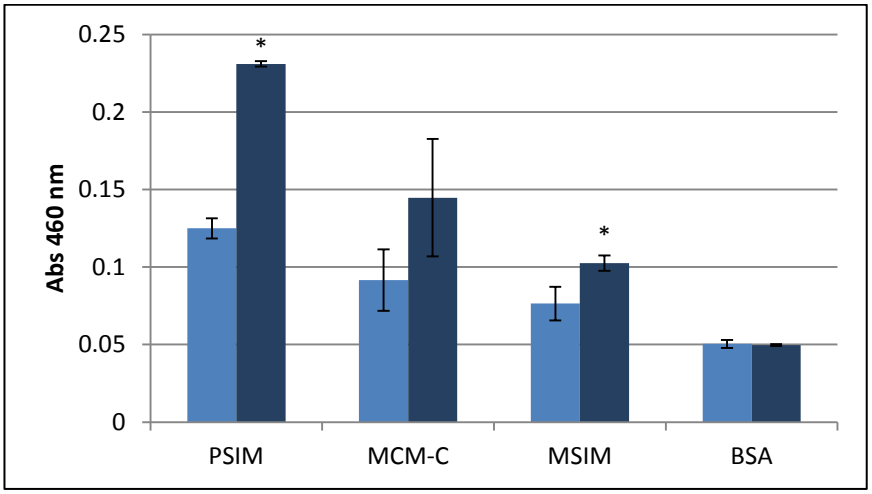


Figure 5.2 Binding of *L. reuteri* strain MM4KO grown in MRS (pale blue bars) or MRS supplemented with mucin (dark blue bars) to mucus * = p <0.05.

Induction of higher binding phenotype was observed for all mucus types tested, with binding to PSIM and MSIM showing a significant increase when cells were grown in the presence of mucin (Figure 5.2). The enhanced adhesion phenotype from non-induced to induced was most notable when cells were tested against PSIM with 1.8 fold change, compared to 1.3 fold change when binding was to MSIM. This may result from an adaption of the response to the porcine mucin used in the growth media.

In order to investigate the nature of the molecular mechanisms underlying the induction of a higher binding phenotype of strain MM4KO, the bacterial cells were pre-treated with trypsin before assessing binding to immobilised PSIM, non-induced cells were included as a control (Figure 5.3).

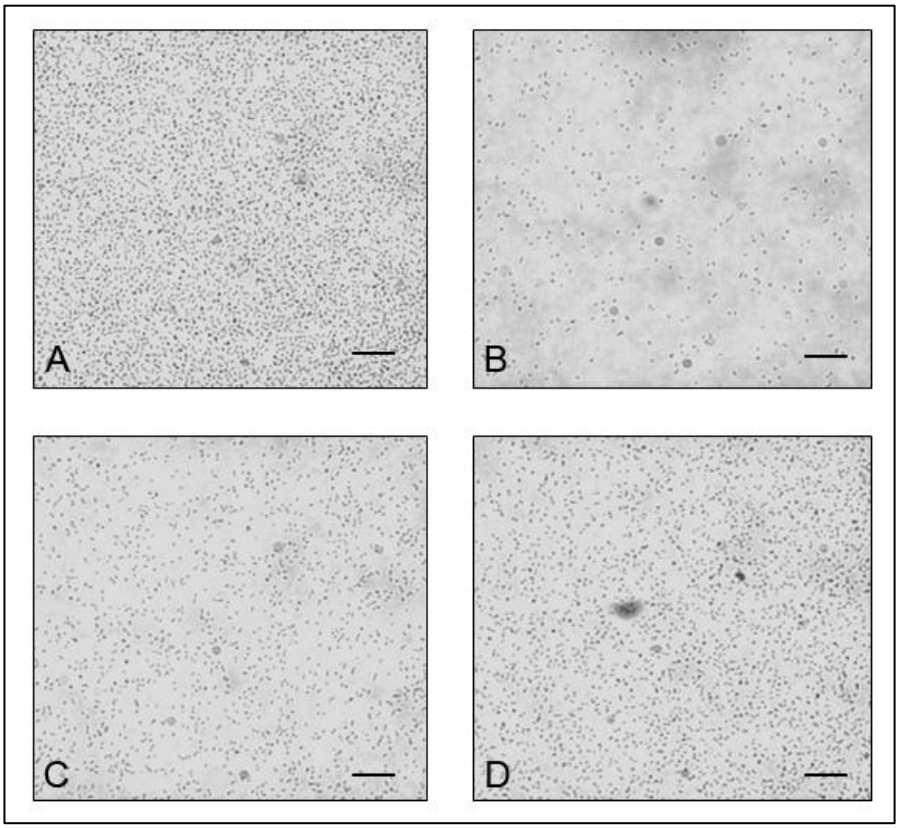


Figure 5.3 Adhesion of *L. reuteri* strain MM4KO to immobilised PSIM. Cell were grown in MRS, A) supplemented with mucin, B) supplemented with mucin then treated with trypsin, C) cells grown in the absence of mucin, D) grown in the absence of mucin then treated with trypsin. Scale bar represents 20 µm.

The protease treatment of the bacteria resulted in a reduction of mucus binding phenotype of strain MM4KO when grown in the presence of mucin (Figure 5.3 A and B), indicating the contribution of proteins expressed at the cell surface to the observed phenotype. No significant difference in adhesion was observed for cells grown in the absence of mucin followed by trypsin treatment (Figure 5.3 C and D), which may indicate that without induction, non-proteinaceous factors may contribute to the basal mucus binding level of strain MM4KO.

Since MUB and Lar_0958 are not expressed by strain MM4KO, the present findings suggest that a novel cell surface protein involved in mucus binding may be expressed by strain MM4KO when the bacteria are grown in media supplemented with mucin.

5.2 **Quantitative proteomic approach for differential expression of proteins.**

In order to quantitatively measure the differential expression of proteins when *L. reuteri* strain MM4KO is grown in the presence and absence of mucin, we used a proteomics approach called stable isotope labelling with amino acids in cell culture (SILAC). SILAC was originally developed within the context of human tissue culture however, the technique is perfectly suited for culturable bacteria due to their fast growth and relatively small genome sizes as shown by its successful use with *Bifidobacterium longum* [283], and *E. coli* [284].

By growing bacterial cells in media containing “heavy” aa (with stable isotopes of carbon ^{13}C and nitrogen ^{15}N) the cells incorporate the labelled aa into all cellular proteins. The common usage of trypsin in MS to digest proteins to peptides specifically cleaving after arginine or lysine residues has prompted the use of heavy labelled arginine and lysine in many SILAC experiments.

In a basic SILAC experiment, one cell population is labelled with heavy aa (population A), and the other one is labelled with light (unlabelled) aa (population B). The cells are mixed and their proteomes are extracted and measured by MS. MS spectra provide peptide mass and intensity information. Identification of peptides and therefore proteins is achieved through matching MS/MS spectra against a sequence database. In a SILAC experiment, peptide and protein ratios are obtained by direct comparison of the light and heavy isotopes as each peptide appears as a pair in the mass spectrometer, the peptide with the higher mass contains heavy aa and originates from population A, and the peptide with the lower mass contains the light aa and originates from population B. The difference in mass is dependent on the heavy aa used for example with heavy arginine ($^{15}N_4{}^{13}C_6$-arginine) the difference would be 10 Da. If the SILAC peptide pair has a one-to-one, heavy to light (H/L) ratio then there is no difference in the abundance of this protein between the proteomes.

The main advantage of SILAC over other quantitative techniques is the early pooling of samples which reduces variation due to sample processing [285, 286]. Another advantage is the sensitivity of SILAC compared to label free methods enabling the detection of smaller changes in protein expression [287].

In order to label proteins efficiently with heavy aa, the growth medium must be defined and lack the corresponding light aa. For that reason SILAC experiments require the

use of a synthetic chemically defined media such as LDM [288], which does not contain the peptone and meat extract present in MRS. LDM III was specifically adapted for this purpose from LDM II to control the aa composition, by replacing the vitamin-free casamino acids with individual aa. “Light” cultures used LDM III with unlabelled aa, while “heavy” cultures replace arginine with arginine $^{13}C_6{}^{15}N_4$ and lysine with lysine $^{13}C_6$ (Appendix 1).

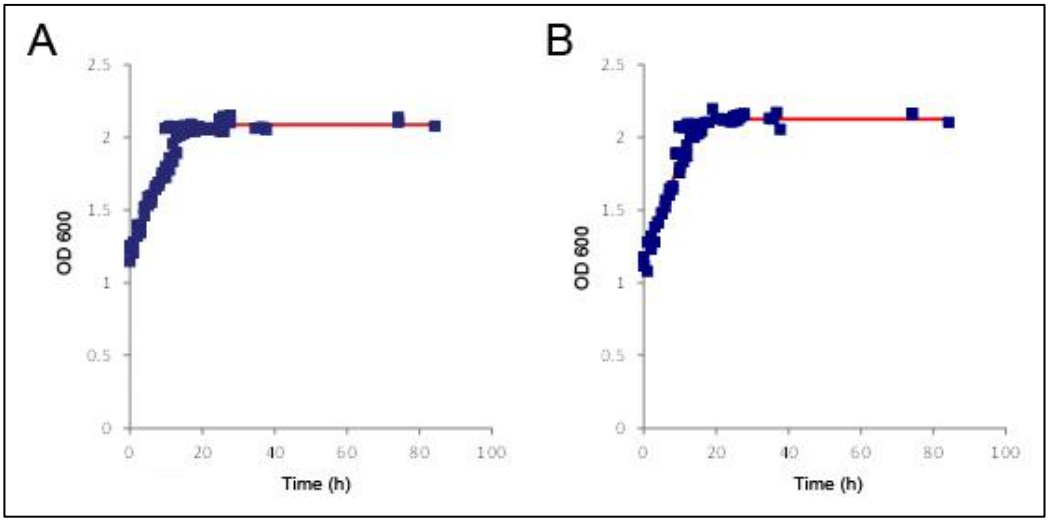


Figure 5.4 Growth curves of *L. reuteri* MM4KO in LDM III media A) in absence of mucin, B) in presence of mucin.

There was no difference between the growth curves when *L.* reuteri MM4KO was grown in LDM III in the presence or absence of mucin (Figure 5.4).The bacteria reached an OD_{600} ~2 in LDM III whereas a higher stationary phase OD_{600} was achieved when cells were grown in MRS (OD_{600} 6.3-6.8), reflecting the more stringent conditions of the synthetic medium. No growth was observed for *L. reuteri* MM4KO in mucin supplemented LDM III lacking both arginine and lysine (data not shown) indicating that MM4KO is auxotrophic for arginine and/or lysine.

The binding of *L. reuteri* strain MM4KO to PSIM was performed using cells grown in LDM III, in the presence or absence of mucin, showing that the induction of higher binding phenotype is not dependent on the media used for growth (Figure 5.5) and that the conditions are suitable for identifying novel potential protein mediators of the binding to mucus.

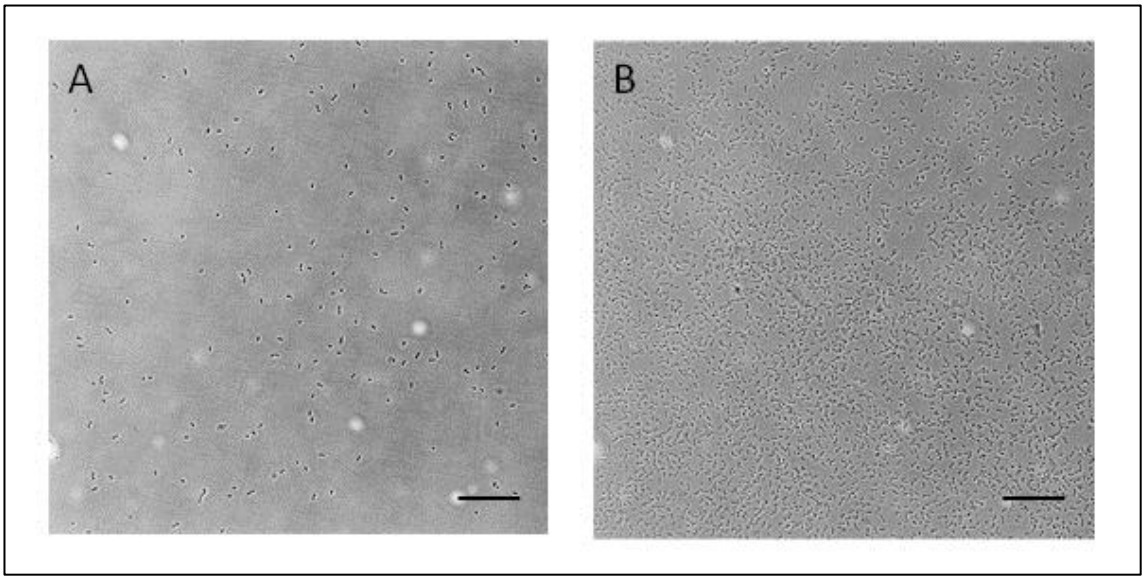


Figure 5.5 Binding of strain MM4KO to PSIM, when grown in LDM III medium, A) in absence of mucin, or B) presence of mucin. Scale bar represents 20 µm.

Since the ratio of heavy to light (H/L) labelled peptides is the measurement of change of protein expression under two experimental conditions (absence or presence of mucin), it was important to confirm that the heavy labelling of proteins attained a level > 95 % to ensure accurate ratio calculation.

We tested the incorporation of heavy arginine and heavy lysine into the proteins of *L. reuteri* strain MM4KO by growing cells in the presence of heavy aa, and performing a spent media protein extract before analysing the samples using LTQ-Orbitrap. Incomplete incorporation is indicated by identified peptides which exhibit a dual population of masses relating to both heavy and light labels.

Using the most abundant proteins to assess the incorporation of heavy arginine and heavy lysine into the proteome of *L. reuteri* MM4KO, for example Elongation factor Tu (Figure 5.6 and Table 5.1), we found that heavy arginine was incorporated to > 99 %. However heavy lysine incorporation was much lower (~81 %) therefore only heavy arginine labelling was selected for our experimental setup. Addition of mucin to the media did not affect the incorporation of heavy arginine (data not shown),

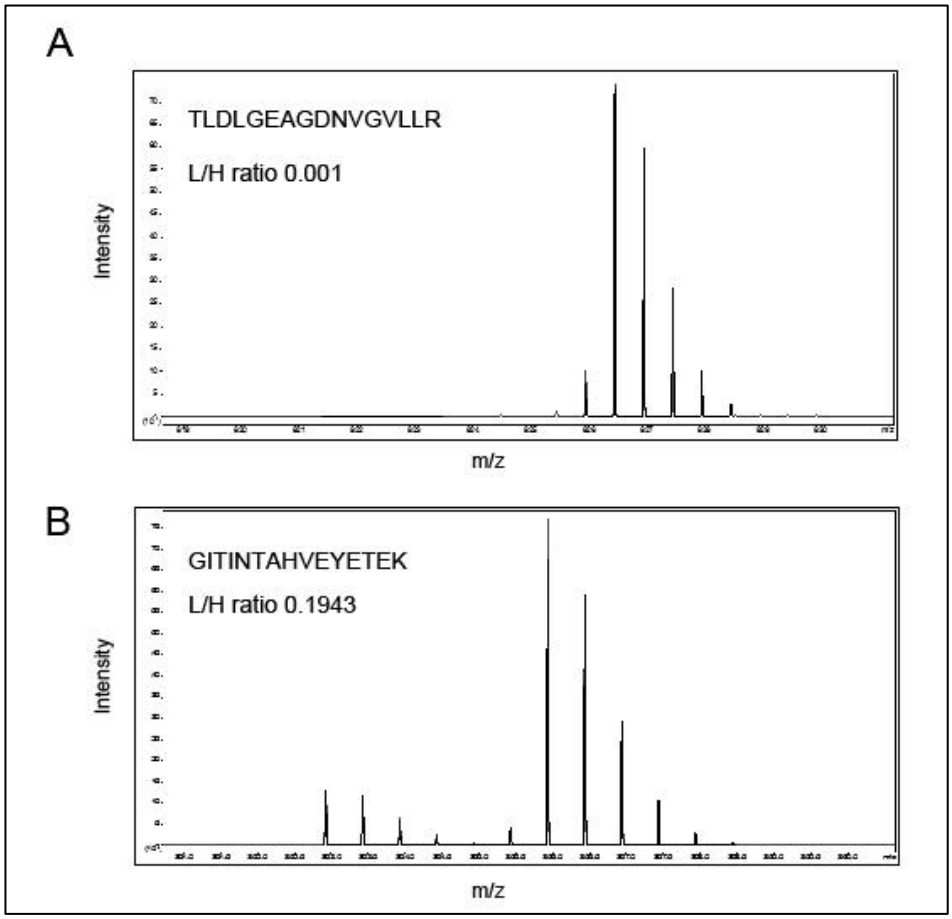


Figure 5.6 Example MS plots, two of the peptides used to identify protein Elongation factor Tu, from *L. reuteri* MM4KO grown in LDM III with heavy aa, showing incorporation of A) heavy arginine and B) heavy lysine.

Since bacterial conversion of arginine to proline, is a factor which may potentially affect the H/L ratios [289], we checked the proline containing peptides from the most abundant proteins of strain MM4KO for the occurrence of heavy proline. No evidence of arginine to proline conversion was found, indicating that the H/L ratios remained unaffected.

z	Sequence	Incl.	L/H	Std.Err.	Intensity	Modifications
3	GISHDQIQR	X	0.000924	2.1E-05	1.35E+07	
2	GISHDQIQR	X	0.001594	0.00083	8.37E+06	
2	ALEGDPEQEK		0.1831	0.1101	2.91E+05	
3	STVTGLEMFHK		0.2304	0.01785	2.89E+07	
3	STVTGLEMFHK		0.2193	0.02741	2.32E+06	(8) Oxidation (M)
2	STVTGLEMFHK		0.1924	0.01841	1.42E+06	(8) Oxidation (M)
3	GQVLAEPGSIQTHK		0.2279	0.01932	3.56E+07	
2	GQVLAEPGSIQTHK		0.2204	0.01872	2.02E+07	
2	QVGVQYIVVFLNK		0.2397	0.00882	1.06E+07	
2	AEDYADIDAAPEEK		0.2081	0.02582	2.36E+06	
2	TLDLGEAGDNVGVLLR	X	0.000952	8E-06	2.32E+07	
3	HYAHIDAPGHADYVK		0.1988	0.1608	1.19E+04	
4	HYAHIDAPGHADYVK		0.1711	0.0361	6.23E+04	
2	HYAHIDAPGHADYVK		0.1502	0.3469	1.91E+05	
3	GITINTAHVEYETEK		0.227	0.0307	2.51E+06	
2	GITINTAHVEYETEK		0.1943	0.04154	1.67E+06	
3	VGDEVEIVGLTEDVLK		0.2258	0.01237	7.34E+05	
2	VGDEVEIVGLTEDVLK		0.2434	0.00801	6.23E+06	
2	AEDYADIDAAPEEKER	X	0.002834	0.00014	7.21E+05	
2	GITINTAHVEYETEKR	X	0.00232	0.00016	3.47E+06	
4	GITINTAHVEYETEKR	X	0.007291	9.8E-05	1.45E+07	
2	DLLSEYDFPGDDVPVVR	X	0.000032	0	3.89E+06	
3	VILHLMDVIDDYIPTPK	X	0.2362	0.01807	3.27E+06	
2	VILHLMDVIDDYIPTPK	X	0.2075	0.01344	1.74E+06	
2	VILHLMDVIDDYIPTPK		0.1221	0.2767	3.31E+04	(6) Oxidation (M)
3	VILHLMDVIDDYIPTPK	X	0.2182	0.06384	1.16E+05	(6) Oxidation (M)
3	TDLVDDDELVDLVEMEVR	X	0.000028	0	1.84E+04	
2	TDLVDDDELVDLVEMEVR	X	0.00003	0	6.20E+04	
2	TDLVDDDELVDLVEMEVR	X	0.000035	0	5.32E+04	(15) Oxidation (M)
4	RPTDKPFMMPVEDVFTITGR	X	0.004892	8.8E-05	4.55E+06	
3	RPTDKPFMMPVEDVFTITGR	X	0.000348	3.4E-05	7.07E+06	
3	RPTDKPFMMPVEDVFTITGR	X	0.005092	0.00164	2.24E+05	(8) Oxidation (M)

Table 5.1 Complete list of peptides used for identification of protein Elongation factor Tu. The two highlighted in yellow are shown in more detail in Figure 5.6.

5.3 **SILAC experimental setup**

In order to identify differentially expressed proteins produced by strain MM4KO when grown in the presence or absence of mucin, we carried out a SILAC experiment using heavy arginine $^{13}C_6{}^{15}N_4$ (heavy vs. light arginine) with reciprocal labelling and three biological replicates.

One culture of *L. reuteri* MM4KO was labelled with heavy arginine (population A), and grown in the presence of mucin, the other culture was labelled with light arginine (population B); this configuration was prepared in triplicate. The reciprocal arrangement was also prepared i.e. the mucin present in the media of population B. Since one of the cultures failed to grow at the same rate, it was excluded from the experiment resulting in two biological replicates for the reciprocal labelling and a total of five experimental samples (Figure 5.7).

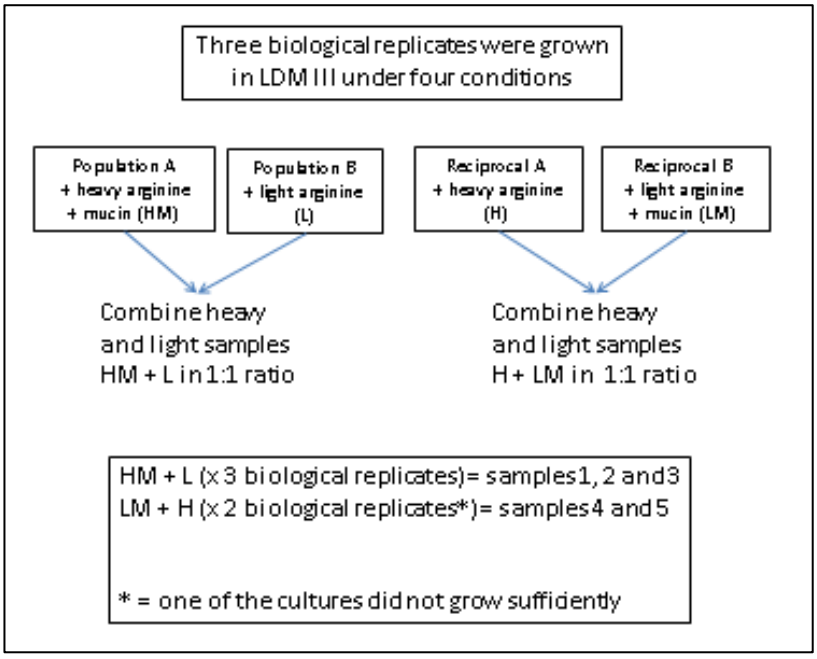


Figure 5.7 Growth conditions of *L. reuteri* MM4KO cultures for SILAC experiment

In order to extract the secreted proteome (SP), the five SILAC samples were centrifuged and the supernatant collected and concentrated. The five SILAC cell pellets were “shaved” with trypsin in order to extract the cell surface proteome (CSP) as described previously in section 4.2. The cell wall proteome (CWP) was obtained after the five shaved SILAC cell pellets were lysed, any contaminating soluble cytoplasmic proteins were washed off and the cell wall proteins were extracted with lysozyme and mutanolysin. The proteins present in the SP and CWP samples were separated by

SDS-PAGE gel, and each lane was cut into five slices in order to reduce the complexity of the peptide mixture injected onto the MS (Figure 5.8).

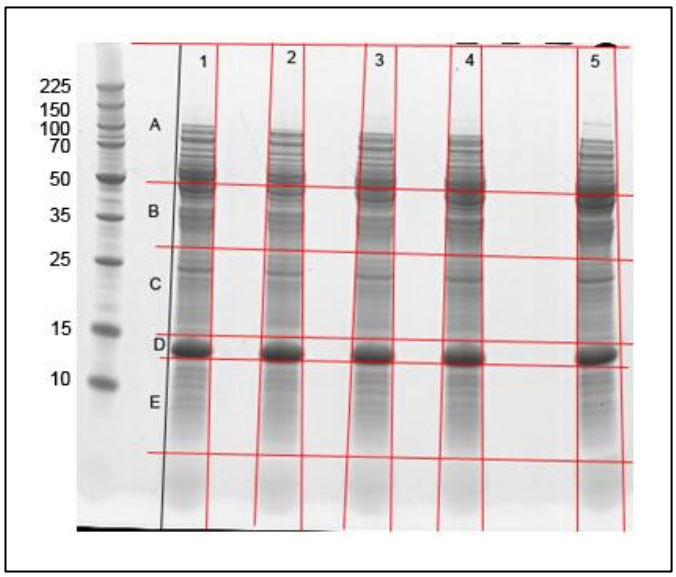


Figure 5.8 Cell wall protein extracts separated by electrophoresis, samples 1-5, were cut into 5 slices A-E as shown by the red lines.

The proteins in all samples were digested to peptides with trypsin; the SP and CWP extracts were processed with an in-gel digest protocol, while the CSP extracts were processed with an in-solution digest protocol. All samples were analysed by LTQ-Orbitrap, with duplicate injections to ensure technical reproducibility (Figure 5.9).

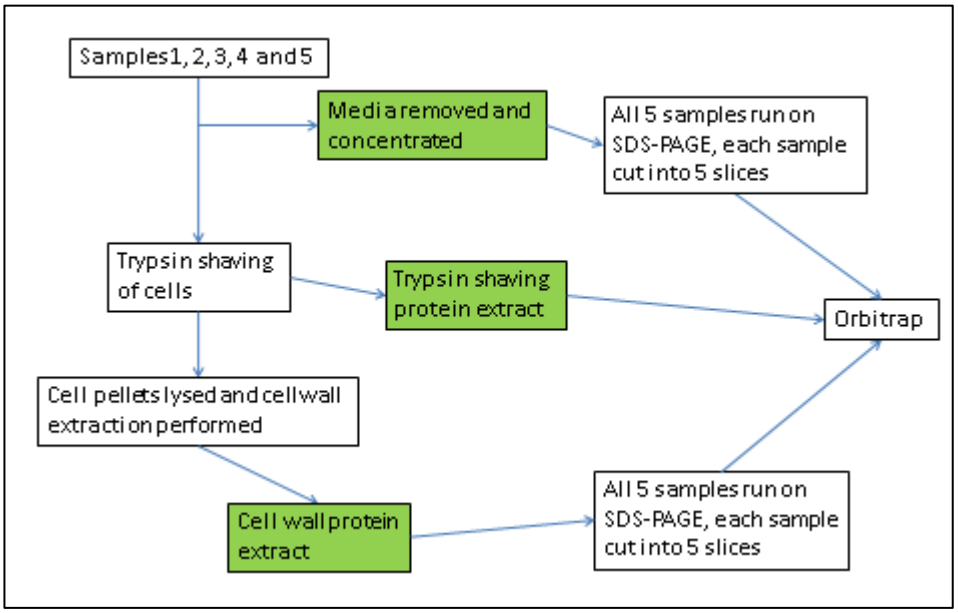


Figure 5.9 Sample processing pipeline for SILAC experiment

5.4 **SILAC data processing and protein identification**

Data were analysed using the MaxQuant software suite containing the Andromeda search engine, which, along with the statistical programme Perseus, have been specifically designed to achieve the highest possible quantitative accuracy in conjunction with SILAC. For validation purposes the data were also analysed with Mascot Distiller combined with Scaffold for statistical analysis.

MaxQuant uses a probabilistic approach for the identification of peptides that was originally pioneered by Mascot, i.e. calculating the probability that the observed number of matches between the calculated and measured fragment masses could have occurred by chance. As a commercial program, the exact algorithms used by Mascot Distiller are unknown. MaxQuant employs algorithms that determine the mass precision and accuracy of peptides individually combined with a "second peptide identification" algorithm used to resolve the frequent occurrence in complex mixtures of two peptides co-fragmenting. The first stage of the data analysis uses MS/MS spectra to give information on peptide sequences, to compare to known protein sequences in the *L. reuteri* database.

Using MaxQuant linked to search engine Andromeda, a total of 690, 1046 and 532 proteins were identified in CWP, SP and CSP respectively with high confidence across the biological and technical replicates (Appendix 2). Protein identifications were only accepted if the following quality parameters were met; a false discovery rate (FDR) of < 0.1 %, a minimum number of 2 unique peptides and a posterior error probability of < 0.05.

Using Mascot Distiller, a total of 429, 642 and 298 proteins were identified in CWP, SP and CSP respectively. The quality parameters used for acceptable protein identification were; a FDR of < 0.1 %, a minimum number of 2 unique peptides and a protein identification probability > 95 %. Considerably fewer identifications were made using the Mascot search engine compared to Andromeda. The sensitivity and specificity of both search engines was previously compared and found to be equivalent [249]. The differences can occur during the process of peak picking, the highest scoring peptide for each MS/MS spectrum is taken, which is not necessarily the same for Mascot and Andromeda scoring.

Among the potential mucus binding proteins previously identified in *Lactobacillus* species (Table 1.5), no peptides corresponding to MUB or pili (SpaC, SpaF, SpaB) were identified as expected from the genome sequence of parent strain MM4-1a. Lar_0958 was also absent as expected from the mutant strain. EF-Tu, GroEL, and GAPDH were identified in all three proteome extracts in agreement with their reported cell surface expression. However the lack of MapA identification was somewhat surprising since the gene encoding the protein is present in parent strain *L. reuteri* MM4-1a, and MapA is implicated in the adhesion of *L. reuteri* 104R to mucus [224] and *L. reuteri* DSM 20016 to Caco2 cells [225], consistent with a cell surface localisation.

5.5 **SILAC ratio calculation and statistical analysis**

During the second stage of the analysis, the H/L ratios determined for the identified proteins in each of the five SILAC samples were normalised across all data sets, since for the majority of proteins, no change in protein expression was expected under the two different experimental conditions. Not all identified proteins will possess a H/L ratio, only peptides containing arginine can be used for quantitation. In order to be considered in the statistical analysis a minimum of 3 H/L ratios must be present for each protein. Theoretically there may be proteins expressed only in one experimental condition, which would result in a H/L ratio of infinity. By searching the data for signal intensity values it is possible to identify proteins with a light only or heavy only label signal, it was confirmed that none of the proteins identified in these samples were expressed only in one experimental condition.

Data were exported from Mascot Distiller to Scaffold, where Log_2 fold change of 2 was used to indicate differential expression. The data exported from MaxQuant to Perseus, was tested for significant changes using the Significance B test with Benjamini Hochberg FDR threshold of 0.05. Differentially expressed proteins were then subjected to bioinformatics analysis for cell localisation using SigP, SecretomeP, LipoP, TMHMM and PSORTB.

SP	CSP	CWP
↓ Zinc/iron ABC super family ATP binding cassette transporter (MP)	↑ Cystathionine gamma-lyase (MP)	↑ mannosyl-glycoprotein endo-β-N-acetylglucosamidase (MP & MS)
↑ Phage replication protein (MP)	↓ SSU ribosomal protein S14P (MP)	↓ ATPase AAA 2 domain protein (MS)
↑ SSU ribosomal protein S3P (MP)	↑ Translation initiation factor IF-2 (MS)	
↑ 50S ribosomal protein L19 (MS)	↑ 1,3 propanediol dehydrogenase (MS)	
↑ 30S ribosomal protein S12 (MS)	↑ FAD dependent pyridine nucleotide disulphide oxidoreductase (MS)	
↓ Peptidyl-tRNA hydrolase (MS)	↑ Cold shock DNA binding protein (MS)	
	↑ UDP-N-acetyl enolpyruvoylglucosamine reductase (MS)	
	↑ Putative cold shock protein (MS)	

Table 5.2 Proteins differentially expressed in the different proteome extracts from *L. reuteri* MM4KO grown in the presence of mucin. Arrows indicate up-or down expression. Data analysis performed in MaxQuant and Perseus (MP) or Mascot Distiller and Scaffold (MS).

In total 16 proteins were found to be differentially expressed across the different proteome extracts (Table 5.2). Identified in all samples, mannosyl-glycoprotein endo-β-N-acetylglucosamidase was found to be differentially expressed in the CWP samples and was the sole protein identified by both computational analysis approaches. Mannosyl-glycoprotein endo-β-N-acetylglucosamidase was also the only differentially expressed protein predicted by SigP, SecretomeP, LipoP, TMHMM and PSORTB to be cell surface located. All other proteins identified as differentially expressed were predicted to be cytoplasmic, indicating that their involvement in cell surface adhesion mechanisms is unlikely, although these predictions should be taken with caution as several proteins, predicted to be cytoplasmic have been shown to be present on the cell surface and involved in mucus adhesion [228-230]. Mucus binding proteins EF-Tu, GroEL, and GAPDH were not found to be differentially expressed in any of the proteome extracts indicating that they are not playing a role in induced mucus binding for *L. reuteri* MM4KO grown in presence of mucin. The protease treatment of cells indicated non-proteinaceous mechanisms for the basal level of mucus binding, indicating that these proteins are not playing any role in mucus binding for strain MM4KO.

The overall expression of mannosyl-glycoprotein endo-β-N-acetylglucosamidase showed two-fold Log_2 change when *L. reuteri* MM4KO cells were grown in LDM III in the presence of mucin. In addition, an increased expression was consistently detected across the different biological and technical replicates (Figure 5.10).

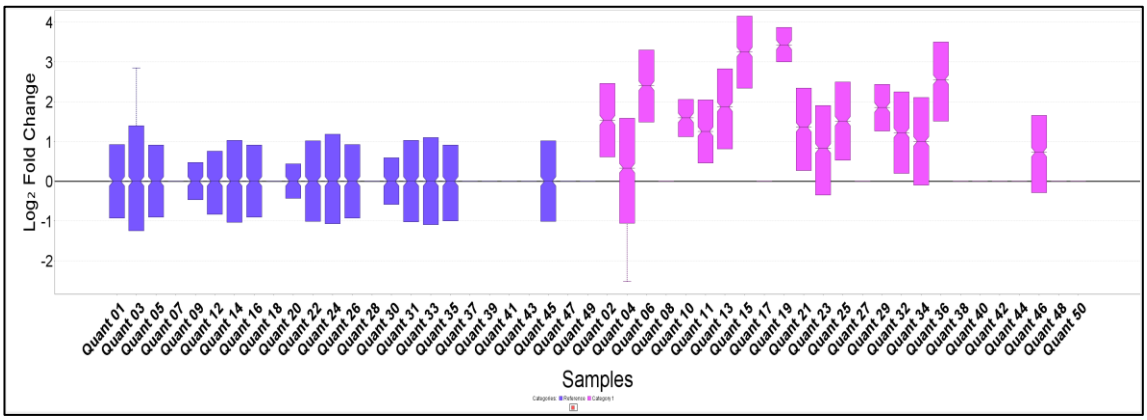


Figure 5.10 H/L ratios of mannosyl-glycoprotein endo-β-N-acetylglucosamidase peptides in Scaffold, identified in CWP samples from bacterial cells grown in LDM III (blue bars) or LDM III supplemented with mucin (pink bars). Samples on x axis refer to the biological and technical replicates.

The peptides detected from MM4KO gave a 37 % coverage (209/568 aa) of mannosyl endo-β-N-acetylglucosamidase (ABQ84091) from *L. reuteri* DSM 20016. The protein was predicted to be 56 kDa in size with a pI between 9.3-9.7. The deduced aa sequence of mannosyl endo-β-N-acetylglucosamidase contains three types of domain, KxYKxGKxW, FlgJ and LysM (Figure 5.11) (www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). The KxYKxGKxW domain is an N-terminal signal peptide to target the protein to the cell surface in Gram-positive bacteria.

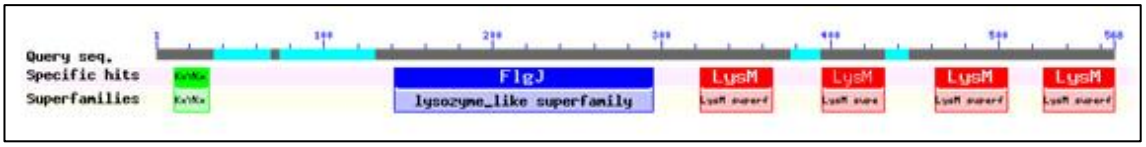


Figure 5.11 Domain structural homology of mannosyl endo-β-N-acetylglucosamidase

The FlgJ domain refers to the muramidase catalytic domain of the FlgJ protein, one of approximately ten proteins required to construct the basal rod portion of flagella and originally described in *Salmonella typhimurium* SJW1437. [290-293]. The muramidase domain belongs to the CAZy glycoside hydrolase (GH) family 73 (www.cazy.org), which contains enzymes which cleave the β-1,4-glycosidic linkage between N-acetylglucosaminyl (GlcNAc) and N-acetylmuramyl (MurNAc) moieties in the carbohydrate backbone of bacterial PGs. Enzymes from the GH73 family are mostly cell surface located and as for *L. reuteri* MM4KO mannosyl endo-β-N-acetylglucosamidase, exhibit repeat sequences involved in bacterial cell wall binding, in the form of LysM domains.

Four LysM domains were identified at the C-terminal end of *L. reuteri* mannosyl endo-β-N-acetylglucosamidase. LysMs are small (44-65 aa) globular domains, commonly found to non-covalently attach proteins to bacterial PG layer and also found to be involved in chitin binding in eukaryotes [294, 295]. The binding of LysM domains to PG is mediated through GlcNac, but whether this is the sole moiety recognised is yet to be determined [294]. The combination of a muramidase catalytic domain with multiple LysM domains is typical for cell wall hydrolases for example AcmA from *Lactococcus lactis* [296]. Cell wall hydrolases enzymatically degrade the PG layer of bacteria and are cell surface or secreted proteins, often classified into five groups in terms of their specificity, N-acetylmuramidases (lysozymes), N-acetylglucosaminidases, N-acetylmuramyl-L-alanine amidases, endopeptidases and transglycosylases [296]. Functions of cell wall hydrolases include cell separation, autolysis, cell wall turnover,

cell division, cell competence for DNA transformation, sporulation, bacterial motility, signalling and adhesion to surfaces.

No change in growth was observed when *L. reuteri* MM4KO was grown in the presence of mucin (Figure 5.4) and no evidence of autolysis was detected after 80 h growth, indicating that a role of mannosyl endo-β-N-acetylglucosamidase in mucin degradation/ utilisation or autolysis is unlikely in *L. reuteri* MM4KO. Hence the differential expression of mannosyl endo-β-N-acetylglucosamidase when *L. reuteri* MM4KO was grown in the presence of mucin, may indicate a role of this enzyme in mucus binding (possibly via LysM domains) as supported by the increased mucus binding phenotype of this strain reported under the same conditions.

5.6 **Discussion**

In this chapter we showed that the binding to mucus of *L. reuteri* strains can be induced by differential expression of proteins in response to mucin.

5.6.1 Stimulation of mucus binding

The environment in the GI tract influences the colonisation ability of the microbiota, in the same way, the outcome of *in vitro* adhesion assays can be influenced by the environment in which bacteria are grown, for example bile salts stimulate *E. coli* binding to HeLa cells [297], glucose stimulates *Staphylococcus epidermidis* binding to polymer surfaces [298], anaerobic growth induces mucus binding in *Salmonella typhimurium* [299] and mucin stimulates *L. reuteri* (strains T1, 11284, SD2112 and #71) adhesion to mucus [191]. In this study we showed that *L. reuteri* MM4KO and 100-23 can be induced to a higher mucus binding phenotype by the supplementation of the growth medium with mucin. The induced phenotype was observed across all mucus samples and was abolished when cells were treated with trypsin, suggesting that the nature of the factor responsible for the increased mucus binding was proteinaceous.

5.6.2 Methodology for quantitatively measuring cell surface proteins

In Gram-positive bacteria proteins can be sorted into four different destinations, the cytoplasm, the cytoplasmic membrane, the peptidoglycan cell wall and the extracellular environment [300]. It is the extracellular proteins and those protruding from the cell wall and cytoplasmic membrane which form the cell surface proteome, interacting with the host and other bacteria. Cell surface proteomes have been studied in both pathogenic bacteria for example *Streptococcus* in an attempt to identify targets for vaccines [273] and probiotic bacteria such as *Bifidobacteria* to explore mechanisms of bacterial-host interactions [283]. When extracting cell surface proteomes there is a well-documented risk of cytoplasmic protein contamination through cell lysis [301]. Through method development we limited the risk of cytoplasmic contamination. It should be noted that cytoplasmic proteins are commonly found in bacterial cell surface extracts, however their localisation on the cell surface should be confirmed by techniques such as immunogold labelling or FCM analysis [228, 301, 302].

Here SILAC was chosen as a sensitive quantitative proteomic approach. SILAC has been successfully applied to monitoring the changes in proteomes of bacteria in different conditions such as, i) stationary phase adaptation of *Bacillus subtilis* [303], ii)

response to nutritional challenges in *B. subtilis* [304], iii) comparison between pathogenic and non-pathogenic *E. coli* [284], and iv) the response to bile salts [283], and mucus [305] of *Bifidobacterium longum*. To date there has been no SILAC studies on *L. reuteri*. Here we used this method to both map the secreted, cell surface and cell wall proteomes, and also to determine the influence of mucin on protein expression level in these proteomes.

A requirement of SILAC is the complete labelling of proteins with the heavy aa in order to achieve accurate quantitation. Many bacteria possess metabolic pathways for aa synthesis and degradation, which may interfere with the heavy labelling of peptides [304]. *L. reuteri* strain JCM 1112 was extensively studied for metabolic pathways, lysine is non-essential for growth as JCM 1112 has a full complement of biosynthetic pathways, whereas arginine is an essential aa [306]. Our work indicated that strain MM4KO was similarly auxotrophic for arginine allowing > 99 % labelling with heavy arginine. The biological and technical replicates along with the reciprocal labelling led to a robust experimental design, however it was shown here and documented in previous SILAC experiments that some of the differentially expressed surface proteins were not found in all samples (proteomes and replicates) [284], indicating the importance of biological and technical replicates to decrease the number of false positives. The full list of proteins identified in the different proteomes can be found in Appendix 2.

5.6.3 Mannosyl glycoprotein endo-β-N-acetylglucosamidase is a candidate mucus binding protein

This study showed that expression of mannosyl glycoprotein endo-β-N-acetylglucosamidase was upregulated when *L. reuteri* strain MM4KO was grown in presence of mucin, which may contribute to the induction of higher mucus binding phenotype of this strain. N-acetylglucosamidases are cell wall hydrolases which may have multiple functions, for example AcmA from *Lactococcus lactis* ssp. *cremoris* MG1363 is involved in autolysis and cell separation [296, 307] and EndoD from *Streptococcus pneumoniae* has both hydrolase and transglycosylation activity [308]. Additionally the N-acetylglucosamidase from *B. longum* NCIMB8809 hydrolyses the glycans of host glycoproteins, thereby liberating nutrients to support growth [305]. Since data from the growth curves showed no evidence of autolysis or increased cell numbers in *L. reuteri* MM4KO when grown in the presence of mucin, this suggests that mannosyl endo-β-N-acetylglucosamidase does not play a role in autolysis and mucin

degradation/utilisation *in vitro*. Instead, this may indicate a role of the enzyme in mucus binding.

The LysM domains of muramidases are often found in multiple copies, the number of which is crucial to the function of the enzyme. For example, in AcmA from *L. lactis*, three LysMs are required for optimum enzyme activity and the removal or addition of LysM domains led to a reduction of autolysis and cell separation [309]. The enzyme activity of N-acetylglucosaminidase AltA from *Enterococcus faecalis* was found to be dependent on the presence of six LysM domains with a 580-fold decrease in activity when the domains were removed [310]. The mechanism by which LysM domains affect enzyme activity has not been elucidated but it may be due to proper positioning of the catalytic domain towards the substrate or to promote the correct folding of the catalytic domain itself. LysM domains have been implicated in directing the subcellular localisation of their proteins, as demonstrated for the cell wall hydrolases Sle1 and LytN from *S. aureus* Newman [311].

LysM containing proteins are also implicated in host-microbial interactions for example autolysin Aaa from *S. aureus* 4074 was shown to have fibrinogen and fibronectin binding activity [312] and the *in silico* analysis of 126 *L. reuteri* ATCC 55730 extracellular proteins found seven proteins with LysM domains, including four with putative hydrolase functions and three of unknown function, but conserved across bacteria and similar to each other [185]. Mutation of LysM containing protein Lr_70152 in *L. reuteri* 100-23 was shown to drastically decrease the colonisation of the mutant strain to the forestomach of germ free mice compared to the wild type strain, indicating that LysM domains may play an important role in bacteria-host interactions (Personal communication, Steve Frese and Jens Walter), although the direct involvement of LysM in mucus adhesion remains to be demonstrated. Taken together, these studies suggest that the increased binding phenotype of *L. reuteri* MM4KO when grown in the presence of mucin may be due to the induction of mannosyl glycoprotein endo-β-N-acetylglucosamidase mediated mucus binding via LysM domains.

6 Conclusions and Future Work

One of the main findings of this work is that the mucus binding ability and the autoaggregation phenotype of *L. reuteri* is strain-specific, highlighting a potential adaptation of the strains to specific hosts or regions of the GI tract via different mechanisms. Indeed the rodent strains displayed a strong autoaggregation phenotype but low mucus binding ability, whereas the human isolates showed consistent mucus binding but no autoaggregation ability. The adaptation potential of *L. reuteri* strains was demonstrated by comparative genomic analysis of 126 different *L. reuteri* strains suggesting fundamentally different trends of genome evolution in rodent and human isolates, with the former possessing a large and adaptable pan-genome while the latter being subjected to a process of reductive evolution [101].

In order to gain further insights into the mechanisms of adhesion of the pig isolate *L. reuteri* ATCC 53608, the genome was sequenced, assembled and automatically annotated. Genome analysis highlighted the presence of a number of genes encoding cell surface proteins which have been implicated in mucus binding, in particular MUB and MucBPs. A comprehensive genome exploitation for strain ATCC 53608 will take place once full manual annotation has been achieved. This will include a comparative genomic analysis with other pig isolates, pg_3b, Lp167-67, 20-2 and 3c6 (currently being sequenced at TGAC), in order to evaluate the number and types of mucus binding proteins in the pig pangenome.

Here we functionally characterised strain specific mediators of mucus binding i.e. the MUB protein from pig isolate ATCC 53608 and the Lar_0958 homologue found in human isolates MM4-1a, DSM 20016, CF4-6g, LMS11-3 and FJ1. We showed that their contribution to bacterial adhesion involved specific interactions with mucus components although these are not the sole mediators of the interaction. MUB and Lar_0958 are constitutively expressed *in vitro* and attached to the bacterial cell surface in accordance with their function. The MUB and Lar_0958 non-expressing strains (1063N and MM4KO respectively) showed a dramatic decrease in binding to mucus, although a basal level of binding was observed for MM4KO to MCM-S and MSIM. We also showed that MUB contributed to the autoaggregation phenotype of *L. reuteri* ATCC 53608. The interactions between MUB and mucus may be direct recognition of mucus components or an indirect mechanism by promoting autoaggregation of the bacterial cells and perhaps inducing biofilm formation. A dual role for mucus binding

and autoaggregation has been reported earlier for transaldolase from *B. bifidum* [277]. This dual functionality could act as an important colonisation factor favouring bacteria establishment in the gut. These cell surface proteins may thus represent a molecular target for rational selection of probiotic *L. reuteri* strains in the future.

Our SILAC experiment identified a novel *L. reuteri* potential mucus binding protein candidate, mannosyl-glycoprotein endo-β-N-acetylglucosamidase. Expression of this protein was upregulated when strain MM4KO was grown in the presence of mucin, thus closer to the conditions of the gut environment. Future mutagenesis studies will be required to abolish the expression of the enzyme in bacteria and assess binding activity of the *L. reuteri* K/O strains to mucus. Furthermore, heterologous expression of individual LysM and catalytic domains will enable discrimination of which of the domains are responsible for binding to mucus and help confirm the role of mannosyl glycoprotein endo-β-N-acetylglucosamidase in *L. reuteri* adhesion to mucus.

MUB and Lar_0958 are modular proteins, characterised by a variable number of repeats. The crystal structure of MubR5 revealed two domains, B1 with structural homology to an immunoglobulin binding protein and B2 with structural homology to MucBP domain [223]. Mucus adhesins with their modular nature may have adapted to recognise multiple ligands within mucus. The exact nature of the receptors is still a matter of debate. Potentially hundreds of heterogeneous glycans structures attached to mucins are present but it is difficult to identify the biologically accessible epitopes and focusing on individual oligosaccharide components does not reflect the native environment of the mucin glycans in mucus. Future work will exploit the recent construction of mucin glycan arrays which display mucins from GI tracts of different vertebrates [313, 314]. These will be used to reveal the molecular targets recognised by the different mucus binding proteins and protein modules we identified and characterised in this study and to further assess their host and tissue specificity to GI mucins.

The contribution of specific interactions and non-specific interactions of MUB and Lar_0958 to mucus adhesion needs further investigation. Methodologies such as AFM, FCM and SPR have been successfully used with whole bacteria to measure kinetic association constants and to obtain information on the differential contributions of non-specific and specific forces [196, 207, 315]. Taking advantage of the *L. reuteri* mutant strains 1063N and MM4KO and their corresponding adhesins MUB and Lar_0958

respectively, these techniques could be used in competition experiments to decipher the specific contribution of these cell surface proteins in the mucus and mucin binding ability of bacteria and to determine whether the adhesion is mediated by lectin-carbohydrate type interactions.

Despite a few functionally characterised mucus binding proteins, mainly in *Lactobacillus* species, there is a paucity of information regarding the nature and structure of the bacterial adhesins mediating binding to mucus and mucins in commensal bacteria. This is despite the critical role played by the mucus layer in the maintenance of a homeostatic relationship with the microbiota. Lactobacilli are dominant gut microbes in many domesticated animals species important in our food chain such as pigs, although they only contribute 0.01 % to the bacterial count in human faecal samples [110]. Work on these lactobacilli strains isolated from humans is therefore more relevant for the selection of probiotics with increased persistence in the gut. More functional and structural work is needed in order to exploit the data coming from the large genome and metagenome sequencing projects. Combining these approaches will help us to understand how to keep the beneficial relationship with our gut bacteria, which may lead to novel strategies to readjust microbial communities or prevent dysbiosis.

Appendix 1

Composition of LDM II and LDM III

LDM II broth:

Ingredient	Amount per L
K_2HPO_4 (anhydrous)	1.5 g
KH_2PO_4 (anhydrous)	1.5 g
Vitamin-free casamino acids	10 g
Sodium acetate	15 g
Sodium citrate	0.22 g
Tryptophan	50 mg
Asparagine	0.2 g
Cysteine-HCl	0.2 g
Thiamine-HCl	0.2 mg
para-Aminobenzoic acid	0.04 mg
Calcium pantothenic acid	0.4 mg
Niacin	1.0 mg
Pyridoxine-HCl	0.5 mg
Biotin	0.05 mg
Folic acid	0.1 mg
Riboflavin	0.4 mg
Adenine sulphate	10 mg
Uracil	20 mg
Guanine-HCl	10 mg
Cytidine (acid)	50 mg
Thymidine	1.6 µg
Tween-80	1.0 ml
$MgSO_4.7H_2O$	0.163 g
$MnSO_4.H_2O$	23.4 mg
$FeSO_4.7H_2O$	13 mg
Sucrose	20 g

LDM III broth:

Ingredient	Amount per L
K_2HPO_4 (anhydrous)	1.5 g
KH_2PO_4 (anhydrous)	1.5 g
Sodium acetate	15 g
Sodium citrate	0.22 g
Tryptophan	50 mg
Asparagine	50 mg
Cysteine	50 mg
Glycine	50 mg
Serine	50 mg
Alanine	50 mg
Phenylalanine	50 mg
Histidine	50 mg
Isoleucine	50 mg
Methionine	50 mg
Proline	50 mg
Threonine	50 mg
Valine	50 mg
Tyrosine	50 mg
Leucine	50 mg
Glutamine	50 mg
Aspartic acid	50 mg
Glutamic acid	50 mg
Thiamine-HCl	0.2 mg
para-Aminobenzoic acid	0.04 mg
Calcium pantothenic acid	0.4 mg
Niacin	1.0 mg
Pyridoxine-HCl	0.5 mg
Biotin	0.05 mg
Folic acid	0.1 mg
Riboflavin	0.4 mg
Adenine sulphate	10 mg
Uracil	20 mg
Guanine-HCl	10 mg
Cytidine (acid)	50 mg
Thymidine	1.6 µg
Tween-80	1.0 ml
$MgSO_4.7H_2O$	0.163 g
$MnSO_4.H_2O$	23.4 mg
$FeSO_4.7H_2O$	13 mg
Sucrose	20 g
Optional amino acids (arginine and lysine)	50 mg (each)

Appendix 2

Secreted proteome of *L. reuteri* MM4KO

Appendix 2 - SP Proteins Identified	Peptides	Peptides Sample 1	Peptides Sample 2	Peptides Sample 3	Peptides Sample 4	Peptides Sample 5	Sequence Coverage [%]	Mol. Weight [kDa]	Sequence Length
>gi|148531499|gb|ABQ83498.1| bacterial translation initiation factor 3 (bIF-3) [Lactobacillus reuteri DSM 20016]	6	4	5	3	5	4	40	19.487	170
>gi|148531703|gb|ABQ83702.1| LSU ribosomal protein L17P [Lactobacillus reuteri DSM 20016]	12	10	11	7	11	9	68.5	14.201	127
>gi|133930435|gb|ABO43789.1| DNA-binding protein [Lactobacillus reuteri];>	14	13	13	11	14	13	85.7	9.5238	91
>gi|183226688|dbj|BAG27204.1| pyruvate kinase [Lactobacillus fermentum IFO 3956];	15	10	11	12	9	12	29.4	51.641	473
>gi|337728600|emb|CCC03706.1| putative muramidase [Lactobacillus reuteri ATCC 53608]	15	13	10	11	14	11	39.1	60.348	565
>gi|194453977|gb|EDX42874.1| ribosomal protein L5 [Lactobacillus reuteri 100-23]	16	16	15	14	14	14	69.4	20.172	180
>gi|148531718|gb|ABQ83717.1| LSU ribosomal protein L5P [Lactobacillus reuteri DSM 20016]	16	16	16	15	15	15	69.4	20.171	180
>gi|148531711|gb|ABQ83710.1| LSU ribosomal protein L15P [Lactobacillus reuteri DSM 20016]	17	15	16	12	14	13	75.7	15.447	144
>gi|148532099|gb|ABQ84098.1| Alcohol dehydrogenase GroES domain protein [Lactobacillus reuteri DSM 20016]	18	13	16	15	17	14	68.5	35.918	336
>gi|183225377|dbj|BAG25894.1| 50S ribosomal protein L18 [Lactobacillus reuteri JCM 1112]	18	17	17	13	16	18	77.7	13.342	121
>gi|148530829|gb|ABQ82828.1| acetate kinase [Lactobacillus reuteri DSM 20016]ref	19	11	14	10	15	11	57.5	43.595	398
>gi|148532080|gb|ABQ84079.1| glycerol 2-dehydrogenase (NAD+) [Lactobacillus reuteri DSM 20016]	19	11	14	18	16	15	57.6	40.698	373
>gi|148531716|gb|ABQ83715.1| SSU ribosomal protein S8P [Lactobacillus reuteri DSM 20016]	19	18	17	13	18	18	87.9	14.536	132
>gi|148530404|gb|ABQ82403.1| Formate-tetrahydrofolate ligase [Lactobacillus reuteri DSM 20016]	20	14	15	17	18	12	42.3	60.251	553
>gi|148530792|gb|ABQ82791.1| RecA protein [Lactobacillus reuteri DSM 20016]	20	15	12	11	17	17	57.5	39.063	362
>gi|183226438|dbj|BAG26954.1| elongation factor Tu [Lactobacillus fermentum IFO 3956]	20	16	17	17	17	15	57.8	43.474	396
>gi|183224383|dbj|BAG24900.1| enolase [Lactobacillus reuteri JCM 1112]	20	18	19	19	20	18	57.1	49.951	457
>gi|148532119|gb|ABQ84118.1| Bleomycin hydrolase [Lactobacillus reuteri DSM 20016]	21	12	14	17	10	12	51.4	50.851	440
>gi|148531290|gb|ABQ83289.1| LPXTG-motif cell wall anchor domain [Lactobacillus reuteri DSM 20016]	21	13	17	18	17	16	33.1	80.889	752
>gi|148530989|gb|ABQ82988.1| aspartyl-tRNA synthetase [Lactobacillus reuteri DSM 20016]	21	15	16	17	14	12	38.5	68.43	600
>gi|148531743|gb|ABQ83742.1| Alcohol dehydrogenase GroES domain protein [Lactobacillus reuteri DSM 20016]	22	19	19	18	19	18	59.6	36.124	342
>gi|227186481|gb|EEI66552.1| D-alanine--D-alanine ligase [Lactobacillus reuteri CF48-3A]	23	18	19	18	17	16	68.8	42.569	378

>gi|183224464|dbj|BAG24981.1| D-alanine--D-alanine ligase [Lactobacillus reuteri JCM 1112]	23	18	20	18	18	17	69.9	43.001	382
>gi|337727906|emb|CCC02995.1| L-lactate dehydrogenase [Lactobacillus reuteri ATCC 53608]	23	21	16	17	19	19	50.8	34.069	319
>gi|148531688|gb|ABQ83687.1| aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit A [Lactobacillus reuteri DSM 20016]	23	21	17	14	18	14	59.4	53.009	490
>gi|148532092|gb|ABQ84091.1| Mannosyl-glycoprotein endo-beta-N-acetylglucosamidase [Lactobacillus reuteri DSM 20016	24	21	20	21	23	23	52.6	60.446	568
>gi|227070122|gb|EEI08498.1| malate dehydrogenase (NAD) [Lactobacillus reuteri MM2-3]	24	22	17	18	20	20	50.3	34.901	326
>gi|148530447|gb|ABQ82446.1| pyridine nucleotide-disulfide oxidoreductase dimerization region [Lactobacillus reuteri DSM 20016]	28	23	25	23	24	22	63.9	49.944	451
>gi|227071557|gb|EEI09855.1| glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) [Lactobacillus reuteri MM2-3	28	24	24	22	23	22	72.5	37.062	345
>gi|112943172|gb|ABI26299.1| glutamine synthetase [Lactobacillus reuteri	28	24	26	24	24	21	65.3	50.777	447
>gi|148531006|gb|ABQ83005.1| glycyl-tRNA synthetase beta chain [Lactobacillus reuteri DSM 20016]	28	25	24	23	23	22	44.6	78.536	691
>gi|148531459|gb|ABQ83458.1| glutamyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];	29	23	25	23	24	24	47.9	58.349	501
>gi|148530917|gb|ABQ82916.1| translation elongation factor 1A (EF-1A/EF-Tu) [Lactobacillus reuteri DSM 20016]	29	26	27	26	27	25	77.8	43.432	396
>gi|143798699|gb|ABF06644.2| sucrose phosphorylase [Lactobacillus reuteri]	30	26	21	26	23	26	49.5	55.969	485
>gi|148530345|gb|ABQ82344.1| Adenylosuccinate synthetase [Lactobacillus reuteri DSM 20016]	31	25	25	28	26	22	69.7	47.725	432
>gi|227186263|gb|EEI66334.1| maltose phosphorylase [Lactobacillus reuteri CF48-3A]	32	25	22	26	28	26	34.9	87.449	757
>gi|148531528|gb|ABQ83527.1| arginyl-tRNA synthetase [Lactobacillus reuteri DSM 20016]	32	27	28	27	26	26	53.9	63.878	562
>gi|148530903|gb|ABQ82902.1| GTP-binding protein TypA [Lactobacillus reuteri DSM 20016]	32	29	26	26	28	25	52.3	68.827	614
>gi|183225744|dbj|BAG26261.1| threonyl-tRNA synthase [Lactobacillus reuteri JCM 1112]	33	25	23	24	26	23	47.8	71.776	627
>gi|194454660|gb|EDX43557.1| methionyl-tRNA synthetase [Lactobacillus reuteri 100-23]	33	29	23	22	29	21	53.6	76.583	675
>gi|337729319|emb|CCC04448.1| ribonucleoside-diphosphate reductase alpha subunit [Lactobacillus reuteri ATCC 53608]	34	23	22	21	27	20	56.8	82.667	723
>gi|148532121|gb|ABQ84120.1| lysyl aminopeptidase, Metallo peptidase, MEROPS family M01 [Lactobacillus reuteri DSM 20016	34	27	28	27	31	27	42.3	95.323	843
>gi|227071019|gb|EEI09341.1| bifunctional GMP synthase/glutamine amidotransferase protein [Lactobacillus reuteri MM2-3	34	29	24	27	23	25	56.1	59.912	538
>gi|337729108|emb|CCC04231.1| alanyl-tRNA synthase [Lactobacillus reuteri ATCC 53608]	35	27	30	30	28	26	44.8	98.679	884
>gi|337728287|emb|CCC03382.1| translation elongation factor G [Lactobacillus reuteri ATCC 53608]	35	29	28	25	28	21	57.4	76.758	695
>gi|148530972|gb|ABQ82971.1| chaperone protein DnaK [Lactobacillus reuteri DSM 20016]	37	19	11	19	22	16	60.4	67.211	621
>gi|148530481|gb|ABQ82480.1| methionyl-tRNA synthetase [Lactobacillus reuteri DSM 20016]	37	31	28	26	33	24	60.7	76.638	675
>gi|148530594|gb|ABQ82593.1| ribonucleoside-diphosphate reductase class Ib alpha subunit	38	26	29	26	33	26	59.2	82.738	723

[Lactobacillus reuteri DSM 20016]									
>gi|227184598|gb|EEI64669.1| phosphoketolase [Lactobacillus reuteri CF48-3A]	38	33	29	31	37	32	43.2	91.435	803
>gi|148530417|gb|ABQ82416.1| IMP cyclohydrolase [Lactobacillus reuteri DSM 20016]	39	26	28	26	32	20	70.5	57.032	512
>gi|148530592|gb|ABQ82591.1| alcohol dehydrogenase AdhE / acetaldehyde dehydrogenase [Lactobacillus reuteri DSM 20016]	39	33	30	34	35	32	43.8	97.187	878
>gi|337729321|emb|CCC04450.1| aldehyde-alcohol dehydrogenase [Lactobacillus reuteri ATCC 53608]	39	34	25	29	32	28	42	97.181	878
>gi|183224518|dbj|BAG25035.1| alanyl-tRNA synthase [Lactobacillus reuteri JCM 1112]	40	30	35	34	31	30	49.4	98.648	885
>gi|145202295|gb|ABF06651.2| pyruvate kinase [Lactobacillus reuteri]	40	32	34	33	33	27	76.7	51.853	473
>gi|337728521|emb|CCC03625.1| glucose-6-phosphate 1-dehydrogenase [Lactobacillus reuteri ATCC 53608]	41	36	36	32	37	30	75.5	56.355	493
>gi|194452990|gb|EDX41888.1| chaperonin GroEL [Lactobacillus reuteri 100-23]	43	32	25	33	38	29	79	57.092	542
>gi|337728005|emb|CCC03094.1| isoleucyl-tRNA synthase [Lactobacillus reuteri ATCC 53608]	43	33	28	35	37	27	36.8	106.67	931
>gi|183224381|dbj|BAG24898.1| phosphoglycerate kinase [Lactobacillus reuteri JCM 1112]	43	34	33	36	36	32	85	42.96	401
>gi|337727862|emb|CCC02950.1| 30S ribosomal protein S1 [Lactobacillus reuteri ATCC 53608]	43	36	32	33	36	33	76.4	45.982	416
>gi|148531028|gb|ABQ83027.1| SSU ribosomal protein S1P [Lactobacillus reuteri DSM 20016]	43	36	34	35	36	34	76.4	45.995	416
>gi|148531733|gb|ABQ83732.1| translation elongation factor 2 (EF-2/EF-G) [Lactobacillus reuteri DSM 20016]	43	36	36	34	37	29	67.9	76.77	695
>gi|227070837|gb|EEI09162.1| glucose-6-phosphate 1-dehydrogenase [Lactobacillus reuteri MM2-3]	43	38	39	35	40	33	78.8	56.713	496
>gi|148530624|gb|ABQ82623.1| chaperonin GroEL [Lactobacillus reuteri DSM 20016]	44	33	26	34	39	30	78.2	57.123	542
>gi|148531571|gb|ABQ83570.1| hypothetical protein Lreu_1313 [Lactobacillus reuteri DSM 20016]	46	30	31	30	33	30	71.7	64.749	566
>gi|148530866|gb|ABQ82865.1| Isoleucyl-tRNA synthetase [Lactobacillus reuteri DSM 20016]	47	36	32	39	41	31	39.2	109.95	960
>gi|148530773|gb|ABQ82772.1| valyl-tRNA synthetase [Lactobacillus reuteri DSM 20016]	47	40	41	38	43	38	50.3	102.29	884
>gi|337728522|emb|CCC03626.1| 6-phosphogluconate dehydrogenase [Lactobacillus reuteri ATCC 53608]	50	43	37	37	44	37	92.7	53.444	478
>gi|183225653|dbj|BAG26170.1| 6-phosphogluconate dehydrogenase [Lactobacillus reuteri JCM 1112]	51	44	39	38	45	38	92.7	53.417	478
>gi|337728440|emb|CCC03541.1| xylulose 5-phosphate phosphoketolase [Lactobacillus reuteri ATCC 53608]	54	48	43	42	52	43	62.1	91.372	803
>gi|194454221|gb|EDX43118.1| Phosphoketolase [Lactobacillus reuteri 100-23]	54	48	44	44	52	45	60.1	91.374	803
>gi|148531926|gb|ABQ83925.1| Phosphoketolase [Lactobacillus reuteri DSM 20016]	54	49	45	44	52	44	62.1	91.403	803
>gi|183225401|dbj|BAG25918.1| DNA-directed RNA polymerase beta subunit [Lactobacillus reuteri JCM 1112]	55	46	43	43	49	41	48.5	135.36	1205
>gi|227185605|gb|EEI65676.1| DNA-directed RNA polymerase [Lactobacillus reuteri CF48-3A];	65	45	46	45	54	50	46.1	135.39	1211

>gi|148531738|gb|ABQ83737.1| DNA-directed RNA polymerase subunit beta [Lactobacillus reuteri DSM 20016]	66	45	48	47	55	51	46.7	135.42	1211
>gi|148530322|gb|ABQ82321.1| ATPase AAA-2 domain protein [Lactobacillus reuteri DSM 20016]	71	61	56	58	68	56	79.2	82.305	745
>gi|337728095|emb|CCC03185.1| methionyl-tRNA synthase [Lactobacillus reuteri ATCC 53608]	32	28	22	21	28	19	55	76.572	675
>gi|148530410|gb|ABQ82409.1| phosphoribosylaminoimidazole-succinocarboxamide synthase [Lactobacillus reuteri DSM 20016]	23	17	21	15	16	6	73.6	27.308	239
>gi|148530492|gb|ABQ82491.1| ribose-phosphate pyrophosphokinase [Lactobacillus reuteri DSM 20016]	15	10	12	13	12	12	40.7	36.16	329
>gi|148531725|gb|ABQ83724.1| LSU ribosomal protein L22P [Lactobacillus reuteri DSM 20016]	9	9	9	6	9	9	61.7	12.394	115
>gi|148530650|gb|ABQ82649.1| alpha-phosphoglucomutase [Lactobacillus reuteri DSM 20016]	26	20	23	20	23	18	47	63.706	574
>gi|194453644|gb|EDX42541.1| glutamine synthetase, type I [Lactobacillus reuteri 100-23]	25	21	22	20	21	19	57.5	50.84	447
>gi|148531706|gb|ABQ83705.1| SSU ribosomal protein S13P [Lactobacillus reuteri DSM 20016]	16	14	15	13	14	15	60.3	13.687	121
>gi|148531009|gb|ABQ83008.1| aminotransferase [Lactobacillus reuteri DSM 20016]	20	13	16	16	17	17	49	43.107	394
>gi|148531526|gb|ABQ83525.1| protein of unknown function DUF964 [Lactobacillus reuteri DSM 20016]	15	12	14	10	14	12	91.9	14.096	123
>gi|148530555|gb|ABQ82554.1| putative nicotinate phosphoribosyltransferase [Lactobacillus reuteri DSM 20016]	21	17	15	14	15	17	47.3	55.609	488
>gi|148530580|gb|ABQ82579.1| LSU ribosomal protein L1P [Lactobacillus reuteri DSM 20016]	17	12	10	8	12	13	45.8	27.362	253
>gi|227070018|gb|EEI08398.1| conserved hypothetical protein [Lactobacillus reuteri MM2-3]	12	10	11	9	10	11	70.9	15.313	134
>gi|133930433|gb|ABO43788.1| ribosomal protein L15 [Lactobacillus reuteri]	14	12	13	9	12	10	69.4	15.516	144
>gi|227186353|gb|EEI66424.1| membrane alanyl aminopeptidase [Lactobacillus reuteri CF48-3A]	26	21	21	21	22	22	31.6	95.388	843
>gi|148530679|gb|ABQ82678.1| phosphoglucosamine mutase [Lactobacillus reuteri DSM 20016]	18	14	11	12	11	9	47.2	48.902	451
>gi|148531932|gb|ABQ83931.1| (R)-2-hydroxyisocaproate dehydrogenase [Lactobacillus reuteri DSM 20016]	22	15	17	17	16	15	69.8	37.358	334
>gi|269930518|gb|ACZ53529.1| phosphoketolase [Lactobacillus reuteri]	15	13	12	11	15	11	87.3	21.654	189
>gi|269930626|gb|ACZ53583.1| phosphoketolase [Lactobacillus reuteri]	15	13	12	10	15	10	92.1	21.656	189
>gi|183225128|dbj|BAG25645.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112]	17	12	12	13	13	14	49.2	35.441	297
>gi|148531898|gb|ABQ83897.1| Inosine/uridine-preferring nucleoside hydrolase [Lactobacillus reuteri DSM 20016]	8	6	7	7	6	7	30.6	34.905	320
>gi|148530950|gb|ABQ82949.1| SSU ribosomal protein S2P [Lactobacillus reuteri DSM 20016]	20	17	15	15	14	16	78.2	29.657	262
>gi|148531171|gb|ABQ83170.1| L-lactate dehydrogenase [Lactobacillus reuteri DSM 20016]	19	15	14	15	14	13	45.7	35.005	324
>gi|337727880|emb|CCC02968.1| amino acid aminotransferase [Lactobacillus reuteri ATCC 53608]	18	11	12	13	14	14	47	43.078	394
>gi|148531464|gb|ABQ83463.1| glucokinase [Lactobacillus reuteri DSM 20016]	13	9	10	12	11	9	34.4	34.42	323
>gi|148530610|gb|ABQ82609.1| UDP-galactose 4-epimerase [Lactobacillus reuteri DSM 20016]	16	11	8	7	13	9	54.4	36.598	331

>gi|148531776|gb|ABQ83775.1| flavocytochrome c [Lactobacillus reuteri DSM 20016]	21	14	15	15	19	17	50	50.13	464
>gi|227186110|gb|EEI66181.1| protein of hypothetical function DUF964 [Lactobacillus reuteri CF48-3A]	12	12	11	9	12	10	91.9	14.126	123
>gi|148531709|gb|ABQ83708.1| Adenylate kinase [Lactobacillus reuteri DSM 20016]	16	12	12	12	10	10	73.5	24.609	219
>gi|148530419|gb|ABQ82418.1| phosphoglycerate mutase [Lactobacillus reuteri DSM 20016]	14	12	14	13	12	13	45.6	26.121	228
>gi|337728628|emb|CCC03738.1| aminopeptidase N [Lactobacillus reuteri ATCC 53608]	26	21	20	19	22	20	31.6	95.484	843
>gi|148530736|gb|ABQ82735.1| ATP synthase F1 subcomplex beta subunit [Lactobacillus reuteri DSM 20016]	21	17	14	13	17	15	58.5	51.631	475
>gi|148530959|gb|ABQ82958.1| prolyl-tRNA synthetase [Lactobacillus reuteri DSM 20016]	29	22	18	21	21	17	53.6	64.256	577
>gi|148530771|gb|ABQ82770.1| aminotransferase, class V [Lactobacillus reuteri DSM 20016]	22	13	17	16	13	16	49.5	42.097	382
>gi|148530951|gb|ABQ82950.1| translation elongation factor Ts (EF-Ts) [Lactobacillus reuteri DSM 20016]	23	14	14	15	16	19	57.7	31.962	291
>gi|148531543|gb|ABQ83542.1| ribokinase [Lactobacillus reuteri DSM 20016]	15	13	11	11	13	10	54.7	32.041	307
>gi|148531713|gb|ABQ83712.1| SSU ribosomal protein S5P [Lactobacillus reuteri DSM 20016]	18	16	17	17	17	17	82.2	17.646	169
>gi|148530918|gb|ABQ82917.1| trigger factor [Lactobacillus reuteri DSM 20016]	30	26	20	21	23	20	59.9	48.747	436
>gi|148531777|gb|ABQ83776.1| fumarate lyase [Lactobacillus reuteri DSM 20016]	23	17	17	14	16	18	61	50.312	462
>gi|148530389|gb|ABQ82388.1| inosine-5-monophosphate dehydrogenase [Lactobacillus reuteri DSM 20016]	16	14	12	11	14	12	56.1	39.373	380
>gi|148531734|gb|ABQ83733.1| SSU ribosomal protein S7P [Lactobacillus reuteri DSM 20016]	15	14	14	11	14	12	63.5	17.985	156
>gi|148531715|gb|ABQ83714.1| LSU ribosomal protein L6P [Lactobacillus reuteri DSM 20016]	16	14	15	14	13	12	58.4	19.635	178
>gi|148531102|gb|ABQ83101.1| hypothetical protein Lreu_0838 [Lactobacillus reuteri DSM 20016]	22	15	15	17	19	18	48.3	39.346	358
>gi|227070899|gb|EEI09222.1| D-lactate dehydrogenase [Lactobacillus reuteri MM2-3]	12	4	7	10	7	9	46.3	37.311	337
>gi|148532070|gb|ABQ84069.1| histidyl-tRNA synthetase [Lactobacillus reuteri DSM 20016]	21	17	15	15	18	17	41.6	49.318	433
>gi|148531517|gb|ABQ83516.1| YSIRK Gram-positive signal peptide [Lactobacillus reuteri DSM 20016]	10	8	9	9	9	9	33.8	35.991	328
>gi|148531730|gb|ABQ83729.1| LSU ribosomal protein L3P [Lactobacillus reuteri DSM 20016]	16	13	16	11	12	13	74	23.757	219
>gi|148530755|gb|ABQ82754.1| UspA domain protein [Lactobacillus reuteri DSM 20016]	17	11	16	12	11	11	87	18.017	162
>gi|148530642|gb|ABQ82641.1| UDP-glucose pyrophosphorylase [Lactobacillus reuteri DSM 20016]	12	10	9	10	10	11	44.1	34.101	304
>gi|148532032|gb|ABQ84031.1| cysteine synthase [Lactobacillus reuteri DSM 20016]	17	12	11	13	8	13	62.2	32.241	307
>gi|148530861|gb|ABQ82860.1| cell division protein FtsZ [Lactobacillus reuteri DSM 20016]	19	15	12	13	12	14	48.4	44.45	415
>gi|148530501|gb|ABQ82500.1| CTP synthase [Lactobacillus reuteri DSM 20016]	24	15	16	19	17	16	44.6	59.6	534
>gi|148531108|gb|ABQ83107.1| hypothetical protein Lreu_0844 [Lactobacillus reuteri DSM 20016]	10	6	9	8	9	10	71.3	20.426	181

>gi|337727883|emb|CCC02971.1| glycyl-tRNA synthase beta subunit [Lactobacillus reuteri ATCC 53608]	28	25	21	20	23	19	44	78.605	691
>gi|148531554|gb|ABQ83553.1| methionine adenosyltransferase [Lactobacillus reuteri DSM 20016]	16	8	10	11	8	11	39.7	43.195	395
>gi|183227647|dbj|BAG28163.1| dehydrogenase [Lactobacillus fermentum IFO 3956]	6	4	5	6	5	5	25.9	35.835	336
>gi|148530919|gb|ABQ82918.1| ATP-dependent Clp protease ATP-binding subunit ClpX [Lactobacillus reuteri DSM 20016]	24	14	13	14	15	18	58.4	45.969	416
>gi|148530340|gb|ABQ82339.1| 2,5-didehydrogluconate reductase [Lactobacillus reuteri DSM 20016]	15	11	10	13	10	13	49.7	32.62	288
>gi|227186142|gb|EEI66213.1| S-adenosylmethionine synthetase [Lactobacillus reuteri CF48-3A]	15	9	9	10	8	10	38	43.222	395
>gi|148530778|gb|ABQ82777.1| FAD-dependent pyridine nucleotide-disulfide oxidoreductase [Lactobacillus reuteri DSM 20016]	17	13	8	12	14	11	41.6	44.031	404
>gi|148530584|gb|ABQ82583.1| LSU ribosomal protein L10P [Lactobacillus reuteri DSM 20016]	11	10	11	11	11	10	68.7	18.139	166
>gi|148531929|gb|ABQ83928.1| dipeptidase A, Cysteine peptidase, MEROPS family C69 [Lactobacillus reuteri DSM 20016]	25	21	16	15	14	17	60.3	54.294	478
>gi|337729082|emb|CCC04205.1| acetate kinase [Lactobacillus reuteri ATCC 53608]	12	7	8	5	9	5	41.2	43.41	398
>gi|148530734|gb|ABQ82733.1| ATP synthase F1 subcomplex alpha subunit [Lactobacillus reuteri DSM 20016]	24	19	16	16	21	19	40.7	55.226	509
>gi|148530593|gb|ABQ82592.1| ribonucleoside-diphosphate reductase class Ib beta subunit [Lactobacillus reuteri DSM 20016]	15	12	11	11	11	13	44.5	39.248	339
>gi|227070649|gb|EEI08979.1| 1,3-propanediol dehydrogenase [Lactobacillus reuteri MM2-3]	19	14	15	11	17	14	57.8	44.01	405
>gi|148530684|gb|ABQ82683.1| Cof-like hydrolase [Lactobacillus reuteri DSM 20016]	10	8	7	5	8	7	44.8	30.206	268
>gi|148530579|gb|ABQ82578.1| LSU ribosomal protein L11P [Lactobacillus reuteri DSM 20016]	14	12	12	10	12	10	61.7	14.869	141
>gi|148530680|gb|ABQ82679.1| glutamine--fructose-6-phosphate transaminase [Lactobacillus reuteri DSM 20016]	24	18	17	20	18	14	46.9	66.611	606
>gi|148530542|gb|ABQ82541.1| lysyl-tRNA synthetase [Lactobacillus reuteri DSM 20016]	31	25	20	19	26	21	46.9	58.296	508
>gi|148531729|gb|ABQ83728.1| LSU ribosomal protein L4P [Lactobacillus reuteri DSM 20016]	9	7	8	6	5	6	53.6	22.29	207
>gi|148530491|gb|ABQ82490.1| UDP-N-acetylglucosamine pyrophosphorylase [Lactobacillus reuteri DSM 20016]	18	11	13	9	10	6	48.6	50.01	455
>gi|183225183|dbj|BAG25700.1| ligase [Lactobacillus reuteri JCM 1112]	17	13	13	13	12	11	48.1	49.294	441
>gi|227186160|gb|EEI66231.1| possible peptidoglycan-binding protein [Lactobacillus reuteri CF48-3A]	7	3	5	6	4	4	33.1	27.744	266
>gi|148530567|gb|ABQ82566.1| amino acid ABC transporter substrate-binding protein, PAAT family [Lactobacillus reuteri DSM 20016]	24	19	13	15	20	16	59.7	28.516	263
>gi|148530455|gb|ABQ82454.1| Substrate-binding region of ABC-type glycine betaine transport system [Lactobacillus reuteri DSM 20016]	20	13	10	10	12	12	61.2	32.953	299
>gi|148530288|gb|ABQ82287.1| LSU ribosomal protein L9P [Lactobacillus reuteri DSM 20016]	15	11	12	12	11	10	65.3	16.692	150

>gi|183226571|dbj|BAG27087.1| heat shock protein DnaK [Lactobacillus fermentum IFO 3956]	21	9	6	9	12	9	29	67.123	618
>gi|183226183|dbj|BAG26699.1| glyceraldehyde 3-phosphate dehydrogenase [Lactobacillus fermentum IFO 3956]	10	8	8	9	8	7	25.5	36.208	337
>gi|148531037|gb|ABQ83036.1| degV family protein [Lactobacillus reuteri DSM 20016]	16	11	14	12	11	13	60	30.742	280
>gi|148531050|gb|ABQ83049.1| Inorganic diphosphatase [Lactobacillus reuteri DSM 20016]	12	8	11	9	9	8	37	34.194	311
>gi|148531450|gb|ABQ83449.1| LSU ribosomal protein L21P [Lactobacillus reuteri DSM 20016]	10	8	6	5	8	9	73.5	11.256	102
>gi|148531692|gb|ABQ83691.1| ATP-dependent DNA helicase PcrA [Lactobacillus reuteri DSM 20016]	21	15	13	14	16	15	35.7	86.29	757
>gi|183225283|dbj|BAG25800.1| putative autolysin [Lactobacillus reuteri JCM 1112]	12	7	5	5	7	8	33.1	60.393	532
>gi|148530740|gb|ABQ82739.1| cell shape determining protein, MreB/Mrl family [Lactobacillus reuteri DSM 20016]	15	11	8	11	10	13	44.2	35.48	330
>gi|148531505|gb|ABQ83504.1| DNA polymerase I [Lactobacillus reuteri DSM 20016]	28	21	19	14	24	17	32.2	100.78	888
>gi|148531486|gb|ABQ83485.1| two component transcriptional regulator, winged helix family [Lactobacillus reuteri DSM 20016]	18	13	15	12	15	12	56.1	26.33	228
>gi|148530409|gb|ABQ82408.1| Adenylosuccinate lyase [Lactobacillus reuteri DSM 20016]	22	15	17	19	16	13	52.9	49.454	431
>gi|148531824|gb|ABQ83823.1| translation elongation factor P (EF-P) [Lactobacillus reuteri DSM 20016]	13	13	11	11	11	11	60	20.518	185
>gi|148531585|gb|ABQ83584.1| ATPase AAA-2 domain protein [Lactobacillus reuteri DSM 20016]	27	17	17	21	20	16	42.4	81.867	734
>gi|148530811|gb|ABQ82810.1| non-canonical purine NTP pyrophosphatase, rdgB/HAM1 family [Lactobacillus reuteri DSM 20016]	13	12	12	10	9	7	80.5	21.286	195
>gi|194454505|gb|EDX43402.1| 2,5-didehydrogluconate reductase [Lactobacillus reuteri 100-23]	14	10	9	12	9	12	43.8	32.469	288
>gi|183225729|dbj|BAG26246.1| hypothetical protein [Lactobacillus reuteri JCM 1112]	11	7	11	8	9	8	69.2	19.891	169
>gi|148530835|gb|ABQ82834.1| hypothetical protein Lreu_0566 [Lactobacillus reuteri DSM 20016]	11	9	9	8	8	8	52.1	18.628	163
>gi|148530865|gb|ABQ82864.1| DivIVA family protein [Lactobacillus reuteri DSM 20016]	17	10	9	8	9	11	47.2	27.591	246
>gi|148530646|gb|ABQ82645.1| thioredoxin reductase [Lactobacillus reuteri DSM 20016]	11	7	7	10	8	9	41.6	33.305	310
>gi|148530962|gb|ABQ82961.1| NusA antitermination factor [Lactobacillus reuteri DSM 20016]	11	9	8	9	9	8	31.4	44.525	395
>gi|148531641|gb|ABQ83640.1| oxidoreductase domain protein [Lactobacillus reuteri DSM 20016]	9	8	8	9	9	8	28.3	38.272	339
>gi|183226006|dbj|BAG26522.1| methionyl-tRNA synthase [Lactobacillus fermentum IFO 3956]	9	5	6	4	5	6	13.8	74.982	666
>gi|148530953|gb|ABQ82952.1| ribosome recycling factor [Lactobacillus reuteri DSM 20016]	12	8	10	9	11	6	67.4	20.783	187
>gi|227071142|gb|EEI09458.1| acetoin dehydrogenase [Lactobacillus reuteri MM2-3]	10	8	9	7	7	5	55.8	28.157	267
>gi|148530952|gb|ABQ82951.1| uridylate kinase [Lactobacillus reuteri DSM 20016]	12	10	10	10	8	8	43.8	25.876	240
>gi|148531003|gb|ABQ83002.1| GTP-binding protein Era [Lactobacillus reuteri DSM 20016]	10	9	8	7	7	8	36.2	33.79	301
>gi|148530283|gb|ABQ82282.1| DNA gyrase subunit A [Lactobacillus reuteri DSM 20016]	31	24	19	21	26	25	39.8	92.891	834

>gi|148531724|gb|ABQ83723.1| SSU ribosomal protein S3P [Lactobacillus reuteri DSM 20016]	17	16	13	13	14	13	58.4	24.696	221
>gi|148530413|gb|ABQ82412.1| phosphoribosylformylglycinamidine synthase subunit II [Lactobacillus reuteri DSM 20016]	17	10	9	10	13	4	30.9	80.944	742
>gi|148531658|gb|ABQ83657.1| nitroreductase [Lactobacillus reuteri DSM 20016]	9	5	6	6	8	6	49.3	24.752	217
>gi|183225222|dbj|BAG25739.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112]	11	9	11	10	8	9	67.6	18.557	170
>gi|148531704|gb|ABQ83703.1| DNA-directed RNA polymerase subunit alpha [Lactobacillus reuteri DSM 20016]	18	14	11	10	13	14	42	34.901	314
>gi|148530846|gb|ABQ82845.1| 6-phosphogluconolactonase [Lactobacillus reuteri DSM 20016]	15	9	10	12	14	12	52	37.901	342
>gi|183225901|dbj|BAG26417.1| inosine-5-monophosphate dehydrogenase [Lactobacillus fermentum IFO 3956]	7	6	7	5	6	4	22.4	39.708	380
>gi|227071794|gb|EEI10083.1| glycerone kinase [Lactobacillus reuteri MM2-3]	15	8	6	7	8	10	28.7	62.132	575
>gi|148531728|gb|ABQ83727.1| LSU ribosomal protein L23P [Lactobacillus reuteri DSM 20016]	11	8	11	9	10	9	79.6	11.25	98
>gi|183224807|dbj|BAG25324.1| hypothetical protein [Lactobacillus reuteri JCM 1112]	12	4	3	9	8	9	35.6	43.954	413
>gi|148530695|gb|ABQ82694.1| ornithine carbamoyltransferase [Lactobacillus reuteri DSM 20016]	17	12	11	11	12	12	43	37.559	335
>gi|183226422|dbj|BAG26938.1| GTP-binding protein [Lactobacillus fermentum IFO 3956]	15	12	11	12	15	13	22.4	68.695	615
>gi|194454225|gb|EDX43122.1| peptidase U34 dipeptidase [Lactobacillus reuteri 100-23]	22	18	14	12	13	15	49.6	54.429	478
>gi|148531722|gb|ABQ83721.1| LSU ribosomal protein L29P [Lactobacillus reuteri DSM 20016]	5	5	4	2	4	4	58.8	8.0041	68
>gi|183226702|dbj|BAG27218.1| DNA-binding protein [Lactobacillus fermentum IFO 3956]	6	6	5	4	6	5	63.7	9.5519	91
>gi|148531027|gb|ABQ83026.1| cytidylate kinase [Lactobacillus reuteri DSM 20016]	15	10	12	11	11	11	69.7	25.015	228
>gi|148530344|gb|ABQ82343.1| guanosine monophosphate reductase [Lactobacillus reuteri DSM 20016]	12	8	7	7	7	9	33.3	35.96	324
>gi|148530769|gb|ABQ82768.1| SSU ribosomal protein S4P [Lactobacillus reuteri DSM 20016]	20	17	16	15	17	18	66.7	22.943	201
>gi|227070613|gb|EEI08943.1| possible asparagine synthase (glutamine-hydrolyzing) [Lactobacillus reuteri MM2-3]	28	21	21	20	24	20	37.3	76.34	652
>gi|183225853|dbj|BAG26369.1| ATP-dependent Clp protease ATP-binding subunit [Lactobacillus fermentum IFO 3956]	9	8	6	5	9	6	11.8	76.678	697
>gi|148530284|gb|ABQ82283.1| SSU ribosomal protein S6P [Lactobacillus reuteri DSM 20016]	10	9	9	8	10	8	92.9	11.378	98
>gi|148531553|gb|ABQ83552.1| leucyl-tRNA synthetase [Lactobacillus reuteri DSM 20016]	23	17	16	18	21	16	28.8	92.918	806
>gi|183226626|dbj|BAG27142.1| L-lactate dehydrogenase [Lactobacillus fermentum IFO 3956]	6	6	5	5	5	5	18.3	33.736	317
>gi|148532158|gb|ABQ84157.1| hypoxanthine phosphoribosyltransferase [Lactobacillus reuteri DSM 20016]	9	7	9	8	6	5	47.2	20.407	178
>gi|148530724|gb|ABQ82723.1| serine hydroxymethyltransferase [Lactobacillus reuteri DSM 20016]	15	14	12	10	14	11	34.3	44.95	411
>gi|148531687|gb|ABQ83686.1| aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B [Lactobacillus reuteri DSM 20016]	22	14	13	13	14	13	48.3	53.424	474

>gi|148531720|gb|ABQ83719.1| LSU ribosomal protein L14P [Lactobacillus reuteri DSM 20016]	10	9	10	8	8	8	66.4	13.144	122
>gi|227071143|gb|EEI09459.1| ribosomal protein S9 [Lactobacillus reuteri MM2-3]	10	10	8	7	10	9	66.9	14.494	133
>gi|148530367|gb|ABQ82366.1| NADPH-dependent FMN reductase [Lactobacillus reuteri DSM 20016]	12	10	12	10	11	8	36.8	45.674	416
>gi|148531899|gb|ABQ83898.1| short-chain dehydrogenase/reductase SDR [Lactobacillus reuteri DSM 20016]	16	10	11	7	8	9	68.1	26.924	251
>gi|148530597|gb|ABQ82596.1| DNA polymerase III, subunits gamma and tau [Lactobacillus reuteri DSM 20016]	9	4	5	5	8	3	17.2	68.85	611
>gi|148531323|gb|ABQ83322.1| glycerol kinase [Lactobacillus reuteri DSM 20016]	9	6	6	5	5	7	19.2	55.388	500
>gi|148530634|gb|ABQ82633.1| SSU ribosomal protein S30P [Lactobacillus reuteri DSM 20016]	6	6	6	5	6	4	37.4	21.144	182
>gi|148530387|gb|ABQ82386.1| purine nucleoside phosphorylase [Lactobacillus reuteri DSM 20016]	8	8	6	7	5	7	41.1	25.751	236
>gi|227070055|gb|EEI08433.1| GTP-binding protein EngA [Lactobacillus reuteri MM2-3]	13	9	9	10	11	10	30.5	49.465	440
>gi|337728993|emb|CCC04113.1| hypoxanthine-guanine phosphoribosyltransferase [Lactobacillus reuteri ATCC 53608]	9	6	9	8	6	4	47.2	20.349	178
>gi|148530685|gb|ABQ82684.1| Peptidoglycan-binding LysM [Lactobacillus reuteri DSM 20016]	9	7	8	8	7	7	36.9	21.661	203
>gi|183224273|dbj|BAG24790.1| transcription accessory protein [Lactobacillus reuteri JCM 1112];	19	14	15	11	15	14	31.2	82.072	727
>gi|148531705|gb|ABQ83704.1| SSU ribosomal protein S11P [Lactobacillus reuteri DSM 20016];	5	5	5	5	4	5	32.6	13.759	129
>gi|148531448|gb|ABQ83447.1| LSU ribosomal protein L27P [Lactobacillus reuteri DSM 20016];	8	5	8	6	7	6	60.2	9.9211	93
>gi|148531114|gb|ABQ83113.1| hypothetical protein Lreu_0850 [Lactobacillus reuteri DSM 20016];	7	6	6	4	7	6	75.6	10.169	90
>gi|148530453|gb|ABQ82452.1| 3-beta hydroxysteroid dehydrogenase/isomerase [Lactobacillus reuteri DSM 20016];	9	8	9	7	8	8	40.1	31.632	284
>gi|148531583|gb|ABQ83582.1| Phosphotransferase system, phosphocarrier protein HPr [Lactobacillus reuteri DSM 20016];	7	6	7	4	6	4	56.8	9.4076	88
>gi|148531727|gb|ABQ83726.1| LSU ribosomal protein L2P [Lactobacillus reuteri DSM 20016];	21	17	18	16	17	19	69.8	30.47	281
>gi|148531731|gb|ABQ83730.1| SSU ribosomal protein S10P [Lactobacillus reuteri DSM 20016];>gi|148544697|ref|YP_001272067.1| 30S ribosomal protein S10 [Lactobacillus reuteri DSM 20016];>gi|166991569|sp|A5VLK6.1|RS10_LACRD RecName: Full=30S ribosomal protein	12	12	9	7	10	10	67.6	11.777	102
>gi|148530343|gb|ABQ82342.1| FAD-dependent pyridine nucleotide-disulfide oxidoreductase [Lactobacillus reuteri DSM 20016]	10	9	9	9	9	8	24.5	49.604	449
>gi|148530566|gb|ABQ82565.1| amino acid ABC transporter ATP-binding protein, PAAT family [Lactobacillus reuteri DSM 20016];	11	7	8	4	7	8	52.6	27.538	249
>gi|148531225|gb|ABQ83224.1| hypothetical protein Lreu_0962 [Lactobacillus reuteri DSM 20016]	16	9	10	9	12	7	23.8	88.735	774
>gi|227071698|gb|EEI09989.1| phosphoesterase [Lactobacillus reuteri MM2-3]	7	6	6	6	7	7	37.5	19.857	176
>gi|148530877|gb|ABQ82876.1| ribose-phosphate pyrophosphokinase [Lactobacillus reuteri DSM	13	10	10	12	12	10	38	36.06	324

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>gi|148530924|gb|ABQ82923.1| GTP-binding protein Obg/CgtA [Lactobacillus reuteri DSM 20016];	22	16	11	12	16	15	56.6	47.975	438
>gi|148532124|gb|ABQ84123.1| asparaginyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];	21	16	18	17	16	19	47	50.092	432
>gi|148531475|gb|ABQ83474.1| phenylalanyl-tRNA synthetase beta subunit [Lactobacillus reuteri DSM 20016];	14	10	9	13	12	9	19.4	89.003	805
>gi|227071324|gb|EEI09633.1| heat shock protein Hsp33 [Lactobacillus reuteri MM2-3];	14	10	9	9	9	12	53.9	35.232	323
>gi|148531494|gb|ABQ83493.1| protein of unknown function UPF0044 [Lactobacillus reuteri DSM 20016];	5	5	4	3	5	5	62.1	11.655	103
>gi|227071790|gb|EEI10079.1| ribulose-5-phosphate 3-epimerase [Lactobacillus reuteri MM2-3];	8	4	6	4	4	6	23.4	25.102	231
>gi|337729037|emb|CCC04160.1| aminotransferase [Lactobacillus reuteri ATCC 53608];	16	11	11	9	11	11	43.6	43.766	397
>gi|148530370|gb|ABQ82369.1| seryl-tRNA synthetase [Lactobacillus reuteri DSM 20016];	14	12	10	11	10	11	32.6	49.29	435
>gi|227070129|gb|EEI08505.1| D-tyrosyl-tRNA(Tyr) deacylase [Lactobacillus reuteri MM2-3];	7	7	7	3	5	3	57.9	17.733	159
>gi|148530819|gb|ABQ82818.1| transcriptional regulator, LacI family [Lactobacillus reuteri DSM 20016];	13	12	8	8	10	10	44.6	36.722	336
>gi|148531721|gb|ABQ83720.1| SSU ribosomal protein S17P [Lactobacillus reuteri DSM 20016];	8	7	7	6	7	6	67	10.168	88
>gi|148530382|gb|ABQ82381.1| GTP-binding protein YchF [Lactobacillus reuteri DSM 20016];	14	8	7	8	6	9	38.6	39.844	365
>gi|148530808|gb|ABQ82807.1| thioredoxin [Lactobacillus reuteri DSM 20016];	7	7	6	6	7	7	62.5	11.889	104
>gi|183224298|dbj|BAG24815.1| aminotransferase [Lactobacillus reuteri JCM 1112];	16	11	11	9	10	11	42	44.088	400
>gi|148530585|gb|ABQ82584.1| LSU ribosomal protein L12P [Lactobacillus reuteri DSM 20016];	18	13	16	8	14	13	95	12.356	121
>gi|148530810|gb|ABQ82809.1| glutamate racemase [Lactobacillus reuteri DSM 20016];	11	7	10	8	7	5	51.7	28.92	267
>gi|324978533|gb|EGC15482.1| cysteine--tRNA ligase [Lactobacillus reuteri MM4-1A];	16	8	13	10	8	9	37.2	54.852	478
>gi|148530870|gb|ABQ82869.1| methylthioadenosine nucleosidase [Lactobacillus reuteri DSM 20016];	8	3	7	4	3	4	39.4	24.687	231
>gi|183225282|dbj|BAG25799.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];	11	7	7	8	7	10	24.9	49.015	438
>gi|148531735|gb|ABQ83734.1| SSU ribosomal protein S12P [Lactobacillus reuteri DSM 20016];	9	9	6	6	8	8	38.8	15.503	139
>gi|148531549|gb|ABQ83548.1| peptidase V, Metallo peptidase, MEROPS family M20A [Lactobacillus reuteri DSM 20016];	20	14	15	16	15	10	53.3	51.22	467
>gi|183227319|dbj|BAG27835.1| 50S ribosomal protein L18 [Lactobacillus fermentum IFO 3956];	7	7	6	6	7	7	45.5	13.301	121
>gi|227185831|gb|EEI65902.1| nucleoside-triphosphatase [Lactobacillus reuteri CF48-3A]	9	8	7	8	5	4	60.5	21.213	195
>gi|148531869|gb|ABQ83868.1| GTP-binding protein, HSR1-related [Lactobacillus reuteri DSM 20016];	11	8	10	10	10	8	31.1	47.758	425
>gi|148532009|gb|ABQ84008.1| UspA domain protein [Lactobacillus reuteri DSM 20016];	12	9	12	9	9	8	74.7	17.199	154
>gi|194453762|gb|EDX42659.1| Peptidoglycan-binding LysM [Lactobacillus reuteri 100-23];	6	3	4	5	3	3	25.7	27.902	269
>gi|148532168|gb|ABQ84167.1| signal peptidase I [Lactobacillus reuteri DSM 20016];	9	7	7	6	7	5	41.3	22.703	201
>gi|148531417|gb|ABQ83416.1| RNAse III [Lactobacillus reuteri DSM 20016];	11	10	10	7	10	9	44.2	26.606	233

>gi|148530347|gb|ABQ82346.1| Uracil phosphoribosyltransferase [Lactobacillus reuteri DSM 20016];	10	9	8	8	8	8	69.3	20.252	179
>gi|148531497|gb|ABQ83496.1| LSU ribosomal protein L20P [Lactobacillus reuteri DSM 20016];	9	8	9	5	9	7	46.2	13.515	117
>gi|148531439|gb|ABQ83438.1| farnesyl-diphosphate synthase [Lactobacillus reuteri DSM 20016];	4	2	3	1	2	2	16.9	31.64	290
>gi|148530402|gb|ABQ82401.1| cyclopropane-fatty-acyl-phospholipid synthase [Lactobacillus reuteri DSM 20016]	14	10	11	7	9	10	33.3	46.488	403
>gi|337728387|emb|CCC03488.1| GTPases [Lactobacillus reuteri ATCC 53608]	10	7	8	8	9	7	28.7	47.753	425
>gi|194453681|gb|EDX42578.1| ribosomal protein L20 [Lactobacillus reuteri 100-23];	9	8	8	5	8	7	46.2	13.545	117
>gi|227186201|gb|EEI66272.1| response regulator [Lactobacillus reuteri CF48-3A];	9	7	7	5	7	7	43	26.602	237
>gi|148531061|gb|ABQ83060.1| hypothetical protein Lreu_0797 [Lactobacillus reuteri DSM 20016];	4	4	2	2	4	3	20.4	31.07	285
>gi|148532037|gb|ABQ84036.1| Endothelin-converting protein 1 [Lactobacillus reuteri DSM 20016];	22	10	10	13	11	14	30.4	72.39	634
>gi|148531183|gb|ABQ83182.1| penicillin-binding protein, 1A family [Lactobacillus reuteri DSM 20016];	10	9	7	6	9	10	14.1	82.075	754
>gi|148530721|gb|ABQ82720.1| bacterial peptide chain release factor 1 (bRF-1) [Lactobacillus reuteri DSM 20016];	18	11	13	15	12	14	47.5	41.287	362
>gi|148530772|gb|ABQ82771.1| thiamine biosynthesis/tRNA modification protein ThiI [Lactobacillus reuteri DSM 20016];	20	14	9	10	17	12	39.4	45.726	406
>gi|148531412|gb|ABQ83411.1| SSU ribosomal protein S16P [Lactobacillus reuteri DSM 20016];	7	7	7	5	7	6	62.6	10.449	91
>gi|148531107|gb|ABQ83106.1| hypothetical protein Lreu_0843 [Lactobacillus reuteri DSM 20016]	6	4	6	3	4	4	30.2	23.788	215
>gi|148530883|gb|ABQ82882.1| dihydrodipicolinate synthase [Lactobacillus reuteri DSM 20016]	6	5	5	5	5	4	20.5	33.823	307
>gi|183224279|dbj|BAG24796.1| putative lipase/esterase [Lactobacillus reuteri JCM 1112]	9	6	6	5	6	7	37.7	33.174	292
>gi|148530326|gb|ABQ82325.1| DNA helicase/exodeoxyribonuclease V, subunit B [Lactobacillus reuteri DSM 20016]	25	17	17	16	19	16	19.9	143.74	1260
>gi|183224869|dbj|BAG25386.1| cell division protein [Lactobacillus reuteri JCM 1112]	8	8	8	7	8	7	56.9	14.251	123
>gi|148531415|gb|ABQ83414.1| signal recognition particle-docking protein FtsY [Lactobacillus reuteri DSM 20016]	18	13	14	9	14	11	37	55.604	508
>gi|112943316|gb|ABI26304.1| predicted flavoprotein [Lactobacillus reuteri];	10	10	10	9	9	9	47.4	21.504	190
>gi|194452797|gb|EDX41695.1| Inorganic diphosphatase [Lactobacillus reuteri 100-23];>gi|194467944|ref|ZP_03073930.1| Inorganic diphosphatase [Lactobacillus reuteri 100-23];>gi|337727840|emb|CCC02928.1| inorganic pyrophosphatase [Lactobacillus reuteri ATCC	12	8	10	8	9	7	37	34.18	311
>gi|148530279|gb|ABQ82278.1| DNA polymerase III, beta subunit [Lactobacillus reuteri DSM 20016];	10	8	7	9	9	7	30.8	41.831	380
>gi|337728969|emb|CCC04089.1| two-component response regulator [Lactobacillus reuteri ATCC	9	7	6	4	7	6	44.5	25.811	229

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>gi|148530514|gb|ABQ82513.1| UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase [Lactobacillus reuteri DSM 20016];	10	5	7	5	6	7	28.5	50.527	459
>gi|183226118|dbj|BAG26634.1| ornithine carbamoyltransferase [Lactobacillus fermentum IFO 3956];	7	6	5	6	7	4	18.9	37.095	333
>gi|148531756|gb|ABQ83755.1| UspA domain protein [Lactobacillus reuteri DSM 20016];	9	7	8	6	7	6	55.1	17.57	158
>gi|148531407|gb|ABQ83406.1| LSU ribosomal protein L19P [Lactobacillus reuteri DSM 20016];	15	13	13	14	14	14	56.2	14.824	128
>gi|148530556|gb|ABQ82555.1| NH(3)-dependent NAD(+) synthetase [Lactobacillus reuteri DSM 20016];	10	6	7	6	6	6	47.3	30.54	275
>gi|148530564|gb|ABQ82563.1| cystathionine gamma-lyase [Lactobacillus reuteri DSM 20016];	11	5	5	5	7	5	31.8	41.498	380
>gi|148530664|gb|ABQ82663.1| RNAse R [Lactobacillus reuteri DSM 20016];	21	14	14	13	20	14	26.1	91.867	801
>gi|183224806|dbj|BAG25323.1| hypothetical protein [Lactobacillus reuteri JCM 1112];	8	3	6	3	8	8	49.7	21.08	183
>gi|148531690|gb|ABQ83689.1| CamS sex pheromone cAM373 family protein [Lactobacillus reuteri DSM 20016];	11	3	7	4	6	8	36.1	40.775	371
>gi|112943099|gb|ABI26296.1| single-stranded DNA-binding protein [Lactobacillus reuteri]	8	7	8	5	5	8	38.5	20.605	187
>gi|148530282|gb|ABQ82281.1| DNA gyrase subunit B [Lactobacillus reuteri DSM 20016];	18	13	10	13	13	12	29	72.528	649
>gi|148531419|gb|ABQ83418.1| phosphate:acyl-[acyl carrier protein] acyltransferase [Lactobacillus reuteri DSM 20016];	6	3	1	3	1	3	22.2	36.975	343
>gi|148530523|gb|ABQ82522.1| D-alanine-activating enzyme [Lactobacillus reuteri DSM 20016];	12	7	6	10	11	6	24.4	55.989	508
>gi|148531918|gb|ABQ83917.1| UspA domain protein [Lactobacillus reuteri DSM 20016];	7	4	6	4	5	5	55.5	17.168	155
>gi|148531570|gb|ABQ83569.1| Peptidoglycan-binding LysM [Lactobacillus reuteri DSM 20016];	6	3	4	4	4	5	34	24.901	235
>gi|227071637|gb|EEI09931.1| uracil phosphoribosyltransferase [Lactobacillus reuteri MM2-3];	11	9	10	9	9	8	48.9	24.512	221
>gi|148531434|gb|ABQ83433.1| guanylate kinase [Lactobacillus reuteri DSM 20016];	7	5	5	4	6	4	33.5	23.546	206
>gi|227071240|gb|EEI09554.1| possible glutathione-disulfide reductase [Lactobacillus reuteri MM2-3];	7	5	3	6	5	5	18.1	48.997	443
>gi|148530857|gb|ABQ82856.1| UDP-N-acetylmuramoylalanine--D-glutamate ligase [Lactobacillus reuteri DSM 20016];	10	5	6	4	5	3	34.9	50.332	456
>gi|148531033|gb|ABQ83032.1| ABC transporter related [Lactobacillus reuteri DSM 20016];	14	9	4	9	8	8	26.1	72.792	636
>gi|227071437|gb|EEI09740.1| transcriptional antiterminator NusG [Lactobacillus reuteri MM2-3];	9	6	9	4	5	6	59.1	20.875	186
>gi|148530327|gb|ABQ82326.1| Recombination helicase AddA [Lactobacillus reuteri DSM 20016];	32	22	26	25	25	21	23.9	159.71	1392
>gi|194453194|gb|EDX42092.1| glutamate racemase [Lactobacillus reuteri 100-23];	9	7	8	6	5	4	33	29	267
>gi|183224141|dbj|BAG24658.1| tyrosyl-tRNA synthase [Lactobacillus reuteri JCM 1112];	14	10	9	6	10	12	35	47.69	420
>gi|148530732|gb|ABQ82731.1| ATP synthase F0 subcomplex B subunit [Lactobacillus reuteri DSM 20016];	6	3	5	2	3	3	34.9	19.254	172
>gi|148530418|gb|ABQ82417.1| phosphoribosylamine--glycine ligase [Lactobacillus reuteri DSM 20016];	15	6	10	11	9	6	36.8	45.76	419

>gi|148530821|gb|ABQ82820.1| protein of unknown function DUF28 [Lactobacillus reuteri DSM 20016]	7	4	6	5	5	5	28.6	27.161	248
>gi|148530781|gb|ABQ82780.1| cell shape determining protein, MreB/Mrl family [Lactobacillus reuteri DSM 20016];	12	7	8	8	11	10	35.4	34.966	333
>gi|148531188|gb|ABQ83187.1| putative RNA methylase [Lactobacillus reuteri DSM 20016];	12	7	9	7	7	8	29.6	44.845	398
>gi|148530874|gb|ABQ82873.1| Phosphoglycerate mutase [Lactobacillus reuteri DSM 20016];	10	8	7	4	5	8	45	24.793	218
>gi|194454328|gb|EDX43225.1| histidyl-tRNA synthetase [Lactobacillus reuteri 100-23]	16	11	12	13	12	12	40.9	48.223	425
>gi|148530899|gb|ABQ82898.1| Dihydrolipoyllysine-residue succinyltransferase [Lactobacillus reuteri DSM 20016];	8	5	4	4	6	6	26.8	48.372	444
>gi|148530995|gb|ABQ82994.1| Choloylglycine hydrolase [Lactobacillus reuteri DSM 20016];	11	10	7	8	6	7	50.2	36.104	325
>gi|148531491|gb|ABQ83490.1| iojap-like protein [Lactobacillus reuteri DSM 20016];	5	3	4	1	3	4	30.5	13.309	118
>gi|148532087|gb|ABQ84086.1| histidyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];	16	12	13	14	11	12	40.9	48.236	425
>gi|1667472|gb|AAB53259.1| chloramphenicol acetyltransferase-TC [Lactobacillus reuteri]	5	5	4	4	4	4	22.7	27.351	238
>gi|148530526|gb|ABQ82525.1| alanine racemase [Lactobacillus reuteri DSM 20016];	4	3	3	3	3	4	13.3	41.162	375
>gi|148530412|gb|ABQ82411.1| phosphoribosylformylglycinamidine synthase subunit I [Lactobacillus reuteri DSM 20016];	7	5	6	5	5	2	42.5	24.732	226
>gi|148531495|gb|ABQ83494.1| Nitric-oxide synthase [Lactobacillus reuteri DSM 20016]	10	7	6	7	8	8	28.8	42.288	375
>gi|194453001|gb|EDX41899.1| sigma 54 modulation protein/ribosomal protein S30EA [Lactobacillus reuteri 100-23]	5	4	4	3	5	3	35.7	21.176	182
>gi|183224883|dbj|BAG25400.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112]	8	5	8	4	4	3	60.2	20.834	181
>gi|148531698|gb|ABQ83697.1| LSU ribosomal protein L13P [Lactobacillus reuteri DSM 20016];	14	11	12	10	13	12	65.3	16.304	147
>gi|148530415|gb|ABQ82414.1| phosphoribosylformylglycinamidine cyclo-ligase [Lactobacillus reuteri DSM 20016];	11	6	9	5	8	2	40.6	36.975	345
>gi|148530504|gb|ABQ82503.1| LSU ribosomal protein L31P [Lactobacillus reuteri DSM 20016];	9	7	8	5	8	7	95.1	9.123	81
>gi|148531691|gb|ABQ83690.1| DNA ligase, NAD-dependent [Lactobacillus reuteri DSM 20016]	20	13	11	13	14	18	35.9	76.002	680
>gi|227186428|gb|EEI66499.1| mannosyl-glycoprotein endo-beta-N-acetylglucosamidase [Lactobacillus reuteri CF48-3A];	8	5	5	5	6	4	37.4	25.86	222
>gi|337728946|emb|CCC04066.1| phosphoribosylformylglycinamidine synthase I [Lactobacillus reuteri ATCC 53608];	7	5	5	4	4	2	42.5	24.714	226
>gi|148531057|gb|ABQ83056.1| gluconate kinase, FGGY family [Lactobacillus reuteri DSM 20016]	13	10	9	11	11	9	31.2	55.417	509
>gi|148530885|gb|ABQ82884.1| aminotransferase [Lactobacillus reuteri DSM 20016];	11	8	5	6	8	7	36.3	42.991	394
>gi|148530622|gb|ABQ82621.1| CoA-binding domain protein [Lactobacillus reuteri DSM 20016];	14	10	12	8	8	9	57	23.939	214
>gi|183227331|dbj|BAG27847.1| 30S ribosomal protein S19 [Lactobacillus fermentum IFO 3956];	9	8	9	5	8	9	57	10.489	93
>gi|148530569|gb|ABQ82568.1| ribose-5-phosphate isomerase [Lactobacillus reuteri DSM 20016];	13	12	12	7	10	10	52	25.036	227
>gi|148530641|gb|ABQ82640.1| glycerol 3-phosphate dehydrogenase (NAD(P)+) [Lactobacillus reuteri DSM 20016];	17	16	11	11	12	13	46.7	36.905	338

>gi|148531075|gb|ABQ83074.1| RecT protein [Lactobacillus reuteri DSM 20016];	13	5	5	6	8	13	41.7	34.762	309
>gi|148530913|gb|ABQ82912.1| SSU ribosomal protein S20P [Lactobacillus reuteri DSM 20016];	4	2	3	2	4	2	36.9	9.2436	84
>gi|183225285|dbj|BAG25802.1| muramidase [Lactobacillus reuteri JCM 1112];	6	4	3	4	5	5	14.2	55.89	492
>gi|337727893|emb|CCC02982.1| conjugated bile salt hydrolase [Lactobacillus reuteri ATCC 53608]	9	8	4	6	5	4	36.9	36.06	325
>gi|148530832|gb|ABQ82831.1| HAD-superfamily subfamily IIA hydrolase like protein [Lactobacillus reuteri DSM 20016];	7	4	4	5	4	5	25.8	28.505	256
>gi|148530611|gb|ABQ82610.1| peptidase M22, glycoprotease [Lactobacillus reuteri DSM 20016];	5	4	4	2	3	3	28.2	26.806	241
>gi|148530799|gb|ABQ82798.1| protein translocase subunit yajC [Lactobacillus reuteri DSM 20016];	5	5	5	5	5	5	28.6	16.384	154
>gi|148530760|gb|ABQ82759.1| Glyoxalase/bleomycin resistance protein/dioxygenase [Lactobacillus reuteri DSM 20016];	5	5	5	4	4	5	45.2	15.166	135
>gi|148531622|gb|ABQ83621.1| lipopolysaccharide biosynthesis protein [Lactobacillus reuteri DSM 20016];	4	2	3	1	4	2	23.4	22.766	209
>gi|183224808|dbj|BAG25325.1| pantothenate metabolism flavoprotein [Lactobacillus reuteri JCM 1112];	5	2	1	2	3	4	17	44.905	401
>gi|148531723|gb|ABQ83722.1| LSU ribosomal protein L16P [Lactobacillus reuteri DSM 20016];	7	6	6	5	6	6	42.4	16.006	144
>gi|183225457|dbj|BAG25974.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];	5	5	4	4	5	4	26.8	20.101	179
>gi|148531131|gb|ABQ83130.1| hypothetical protein Lreu_0867 [Lactobacillus reuteri DSM 20016];	11	9	10	6	8	9	60.4	10.576	91
>gi|148531141|gb|ABQ83140.1| Purine nucleosidase [Lactobacillus reuteri DSM 20016];	5	3	4	3	4	4	23.9	34.734	314
>gi|148532166|gb|ABQ84165.1| aldo/keto reductase [Lactobacillus reuteri DSM 20016];	11	5	5	8	7	7	34.1	32.371	287
>gi|148530735|gb|ABQ82734.1| ATP synthase F1 subcomplex gamma subunit [Lactobacillus reuteri DSM 20016];	9	6	2	7	4	6	36	34.796	314
>gi|133930513|gb|ABO43828.1| triosephosphate isomerase [Lactobacillus reuteri];	7	6	4	4	4	4	31.1	29.214	264
>gi|148532120|gb|ABQ84119.1| NAD-dependent epimerase/dehydratase [Lactobacillus reuteri DSM 20016];	9	6	8	5	6	7	59.2	23.187	213
>gi|148530457|gb|ABQ82456.1| ABC transporter related [Lactobacillus reuteri DSM 20016];	11	7	8	6	6	8	49.6	29.359	256
>gi|148531523|gb|ABQ83522.1| PpiC-type peptidyl-prolyl cis-trans isomerase [Lactobacillus reuteri DSM 20016];	10	3	5	3	6	6	38.8	34.663	312
>gi|148530974|gb|ABQ82973.1| GTP-binding protein LepA [Lactobacillus reuteri DSM 20016];	12	6	8	7	8	8	25.5	68.063	611
>gi|148531054|gb|ABQ83053.1| protein of unknown function DUF322 [Lactobacillus reuteri DSM 20016]	10	9	9	4	7	7	81.9	13.85	127
>gi|148531034|gb|ABQ83033.1| thymidylate synthase [Lactobacillus reuteri DSM 20016];	9	5	7	4	7	7	30.6	36.982	320
>gi|148531575|gb|ABQ83574.1| ribonucleoside-triphosphate reductase class III catalytic subunit / ribonucleoside-triphosphate reductase [Lactobacillus reuteri DSM 20016]	24	10	14	6	18	17	32.3	85.159	744
>gi|148531479|gb|ABQ83478.1| tRNA/rRNA methyltransferase (SpoU) [Lactobacillus reuteri DSM	6	3	6	3	4	3	30.6	28.31	258

20016];									
>gi|227071710|gb|EEI10001.1| phosphoesterase [Lactobacillus reuteri MM2-3];	10	6	3	2	7	7	27.2	37.97	342
>gi|148532170|gb|ABQ84169.1| UDP-N-acetylmuramyl-tripeptide synthetase [Lactobacillus reuteri DSM 20016];	13	7	7	7	10	9	30.6	57.457	516
>gi|148531941|gb|ABQ83940.1| porphobilinogen synthase [Lactobacillus reuteri DSM 20016];	13	9	7	7	9	11	41.2	35.84	323
>gi|148531221|gb|ABQ83220.1| 1-deoxy-D-xylulose-5-phosphate synthase [Lactobacillus reuteri DSM 20016];	13	6	4	6	6	7	26.6	65.608	591
>gi|148532085|gb|ABQ84084.1| carbohydrate kinase, YjeF related protein [Lactobacillus reuteri DSM 20016];	6	4	6	2	4	3	34	22.43	212
>gi|148530512|gb|ABQ82511.1| LemA family protein [Lactobacillus reuteri DSM 20016];	9	9	8	5	7	6	50.3	21.33	189
>gi|148530623|gb|ABQ82622.1| chaperonin Cpn10 [Lactobacillus reuteri DSM 20016]	9	6	8	2	7	6	67	10.149	94
>gi|112943728|gb|ABI26320.1| fructose-2,6-bisphosphatase [Lactobacillus reuteri];	8	7	7	5	6	5	48.8	24.287	217
>gi|227070968|gb|EEI09291.1| glutamate-1-semialdehyde 2,1-aminomutase [Lactobacillus reuteri MM2-3]	6	4	4	3	5	6	19.2	48.026	443
>gi|148530654|gb|ABQ82653.1| Excinuclease ABC subunit A [Lactobacillus reuteri DSM 20016]	19	10	10	10	14	16	20.4	105.77	954
>gi|148530696|gb|ABQ82695.1| carbamate kinase [Lactobacillus reuteri DSM 20016];	12	6	7	6	6	8	41.6	33.008	310
>gi|183227302|dbj|BAG27818.1| 30S ribosomal protein S9 [Lactobacillus fermentum IFO 3956]	5	4	4	3	5	4	31.3	14.285	131
>gi|148531005|gb|ABQ83004.1| glycyl-tRNA synthetase alpha chain [Lactobacillus reuteri DSM 20016]	10	6	7	8	7	6	25.6	37.843	328
>gi|148532002|gb|ABQ84001.1| NrdI family protein [Lactobacillus reuteri DSM 20016]	6	4	5	4	5	3	41.9	17.501	155
>gi|227069775|gb|EEI08201.1| GDSL family lipase [Lactobacillus reuteri MM2-3];	7	4	5	5	5	6	24	42.852	384
>gi|148530668|gb|ABQ82667.1| phosphotransacetylase [Lactobacillus reuteri DSM 20016];	13	7	9	8	12	7	45.7	34.699	324
>gi|148530868|gb|ABQ82867.1| NUDIX hydrolase [Lactobacillus reuteri DSM 20016];	9	7	8	6	7	5	55.2	20.827	183
>gi|130893190|gb|ABO32597.1| ATP-dependent Clp protease ATP-binding subunit ClpC [Lactobacillus reuteri];	20	10	6	5	12	18	27.5	92.89	830
>gi|227184502|gb|EEI64573.1| phosphoribosylformylglycinamidine cyclo-ligase [Lactobacillus reuteri CF48-3A];	10	5	7	3	7	1	34.5	37.031	345
>gi|148530788|gb|ABQ82787.1| peptidase M16 domain protein [Lactobacillus reuteri DSM 20016];	8	4	6	4	6	6	24.5	49.689	432
>gi|194453205|gb|EDX42103.1| protein of unknown function DUF28 [Lactobacillus reuteri 100-23];	7	4	6	4	5	4	28.6	27.188	248
>gi|148530714|gb|ABQ82713.1| arginine deiminase [Lactobacillus reuteri DSM 20016]; 1	15	10	11	12	12	10	34.4	46.242	410
>gi|148530503|gb|ABQ82502.1| UDP-N-acetylglucosamine 1-carboxyvinyltransferase [Lactobacillus reuteri DSM 20016];	15	6	7	5	10	10	46.8	45.676	425
>gi|194454673|gb|EDX43570.1| conserved hypothetical protein [Lactobacillus reuteri 100-23];	5	3	3	4	3	3	26.8	25.988	246
>gi|183225058|dbj|BAG25575.1| phage major head protein [Lactobacillus reuteri JCM 1112];	7	4	3	4	3	5	19.3	43.887	394

>gi|148530963|gb|ABQ82962.1| protein of unknown function DUF448 [Lactobacillus reuteri DSM 20016];	7	5	5	4	5	4	46	11.662	100
>gi|183224780|dbj|BAG25297.1| hypothetical protein [Lactobacillus reuteri JCM 1112];	4	3	1	0	2	1	49.1	12.105	106
>gi|148531719|gb|ABQ83718.1| LSU ribosomal protein L24P [Lactobacillus reuteri DSM 20016];	4	4	4	2	4	4	47.1	10.975	102
>gi|148531712|gb|ABQ83711.1| LSU ribosomal protein L30P [Lactobacillus reuteri DSM 20016];	5	4	5	3	4	4	76.7	6.5566	60
>gi|227070988|gb|EEI09311.1| precorrin-8X methylmutase [Lactobacillus reuteri MM2-3];	6	2	6	1	1	1	34.3	25.708	230
>gi|194453265|gb|EDX42163.1| NLP/P60 protein [Lactobacillus reuteri 100-23];	4	3	3	3	4	3	7.8	48.822	477
>gi|148530538|gb|ABQ82537.1| hypoxanthine phosphoribosyltransferase [Lactobacillus reuteri DSM 20016];	10	5	8	7	6	6	53.3	20.464	180
>gi|227071376|gb|EEI09682.1| malate dehydrogenase (NAD) [Lactobacillus reuteri MM2-3];	10	5	4	5	4	8	28	35.211	321
>gi|148531436|gb|ABQ83435.1| DNA replication and repair protein RecN [Lactobacillus reuteri DSM 20016];	6	5	2	5	3	3	12.3	62.895	559
>gi|133930481|gb|ABO43812.1| RuvA [Lactobacillus reuteri];	3	0	2	0	2	2	16.6	22.369	199
>gi|148530310|gb|ABQ82309.1| pyrroline-5-carboxylate reductase [Lactobacillus reuteri DSM 20016];	5	1	5	1	3	2	24.1	29.38	270
>gi|148531444|gb|ABQ83443.1| protein of unknown function DUF322 [Lactobacillus reuteri DSM 20016]	10	7	7	2	6	5	73.8	15.835	145
>gi|296313256|gb|ACZ97544.2| D-alanine-D-alanine ligase [Lactobacillus reuteri]	4	3	3	3	3	3	16.6	33.684	301
>gi|183226359|dbj|BAG26875.1| catabolite control protein [Lactobacillus fermentum IFO 3956]	5	4	3	4	3	5	14.1	37.084	340
>gi|227071420|gb|EEI09725.1| FMN reductase [Lactobacillus reuteri MM2-3];	7	7	6	4	7	7	31.9	26.152	238
>gi|148531276|gb|ABQ83275.1| thiamine-phosphate diphosphorylase [Lactobacillus reuteri DSM 20016]	10	6	9	4	5	5	40.5	23.849	215
>gi|148531008|gb|ABQ83007.1| RNA polymerase, sigma 70 subunit, RpoD [Lactobacillus reuteri DSM 20016]	15	10	8	9	8	12	38.9	43.385	380
>gi|148530920|gb|ABQ82919.1| small GTP-binding protein [Lactobacillus reuteri DSM 20016]	7	6	6	5	5	6	38.3	22.641	196
>gi|148530377|gb|ABQ82376.1| 16S rRNA m(7)G-527 methyltransferase [Lactobacillus reuteri DSM 20016];	9	6	7	4	7	6	41.1	26.539	241
>gi|148531801|gb|ABQ83800.1| TrkA-N domain protein [Lactobacillus reuteri DSM 20016];	5	4	5	4	2	3	27.9	24.531	222
>gi|183225758|dbj|BAG26275.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];	7	6	6	4	6	7	32.5	22.577	203
>gi|148531442|gb|ABQ83441.1| methenyltetrahydrofolate cyclohydrolase / 5,10-methylenetetrahydrofolate dehydrogenase (NADP+) [Lactobacillus reuteri DSM 20016];	4	3	4	3	3	3	16.8	30.424	286
>gi|148532172|gb|ABQ84171.1| glucose inhibited division protein A [Lactobacillus reuteri DSM 20016];	8	5	5	5	5	7	15.9	71.918	647
>gi|183227567|dbj|BAG28083.1| phosphoketolase [Lactobacillus fermentum IFO 3956];]	7	4	5	4	6	5	8.1	90.724	799
>gi|148530828|gb|ABQ82827.1| Adenine-specific DNA methylase-like protein [Lactobacillus reuteri DSM 20016];	5	3	4	4	4	3	18.8	31.155	277

>gi|148530723|gb|ABQ82722.1| translation factor SUA5 [Lactobacillus reuteri DSM 20016];	11	6	4	5	6	6	39.2	37.261	342
>gi|148530659|gb|ABQ82658.1| ATP-dependent Clp protease proteolytic subunit ClpP [Lactobacillus reuteri DSM 20016];	6	5	5	5	5	4	26.9	21.433	197
>gi|148531956|gb|ABQ83955.1| precorrin-4 C11-methyltransferase [Lactobacillus reuteri DSM 20016];	8	4	4	2	3	2	33.2	27.872	253
>gi|148530886|gb|ABQ82885.1| aspartate semialdehyde dehydrogenase [Lactobacillus reuteri DSM 20016];	7	4	2	3	3	5	29.5	38.274	352
>gi|194454462|gb|EDX43359.1| peptidase S1 and S6 chymotrypsin/Hap [Lactobacillus reuteri 100-23];	6	1	2	3	2	5	20.2	43.882	425
>gi|148531416|gb|ABQ83415.1| condensin subunit Smc [Lactobacillus reuteri DSM 20016];	18	13	13	13	12	12	16.4	135.2	1187
>gi|183227323|dbj|BAG27839.1| 50S ribosomal protein L5 [Lactobacillus fermentum IFO 3956];	5	5	4	4	5	5	27.8	20.091	180
>gi|148531330|gb|ABQ83329.1| Peroxiredoxin [Lactobacillus reuteri DSM 20016];	6	5	5	3	4	5	38.5	21.041	187
>gi|227184503|gb|EEI64574.1| amidophosphoribosyltransferase [Lactobacillus reuteri CF48-3A];	12	5	9	9	8	4	26.9	53.593	490
>gi|227071347|gb|EEI09654.1| DNA-directed RNA polymerase delta subunit [Lactobacillus reuteri MM2-3];	4	2	4	1	1	1	36.5	21.639	189
>gi|148530787|gb|ABQ82786.1| peptidase M16 domain protein [Lactobacillus reuteri DSM 20016];	10	8	6	5	5	8	24.3	47.131	415
>gi|148530614|gb|ABQ82613.1| pyrroline-5-carboxylate reductase [Lactobacillus reuteri DSM 20016]	7	3	5	3	2	3	37	26.78	257
>gi|148530971|gb|ABQ82970.1| GrpE protein [Lactobacillus reuteri DSM 20016];	9	5	7	5	5	4	44.7	21.43	190
>gi|148530601|gb|ABQ82600.1| thymidylate kinase [Lactobacillus reuteri DSM 20016];	9	6	8	3	5	6	46.5	24.198	213
>gi|148531757|gb|ABQ83756.1| amino acid ABC transporter ATP-binding protein, PAAT family [Lactobacillus reuteri DSM 20016];	10	6	8	3	5	5	37.8	27.486	246
>gi|148530925|gb|ABQ82924.1| RNAse Z [Lactobacillus reuteri DSM 20016];	5	1	3	3	4	4	22.7	34.498	309
>gi|148530374|gb|ABQ82373.1| Na+ dependent nucleoside transporter domain protein [Lactobacillus reuteri DSM 20016];	7	6	6	6	7	6	16.6	44.04	403
>gi|148531352|gb|ABQ83351.1| N-acetylmuramoyl-L-alanine amidase, family 2 [Lactobacillus reuteri DSM 20016];	3	2	1	1	3	2	10.8	44.687	399
>gi|148530635|gb|ABQ82634.1| protein translocase subunit secA [Lactobacillus reuteri DSM 20016];	15	7	9	6	8	12	20.8	90.351	787
>gi|148530576|gb|ABQ82575.1| RNA methyltransferase, TrmH family, group 3 [Lactobacillus reuteri DSM 20016];	8	7	6	4	3	5	33.7	27.28	249
>gi|183227087|dbj|BAG27603.1| 50S ribosomal protein L27 [Lactobacillus fermentum IFO 3956];>gi|184155743|ref|YP_001844083.1| 50S ribosomal protein L27 [Lactobacillus fermentum IFO 3956];>gi|226737943|sp|B2GD71.1|RL27_LACF3 RecName: Full=50S ribosomal prote	5	2	4	3	3	3	40.9	9.9871	93
>gi|148532139|gb|ABQ84138.1| protein of unknown function DUF1002 [Lactobacillus reuteri DSM 20016];	10	2	4	5	4	8	35.1	35.908	333

>gi|148530405|gb|ABQ82404.1| acetolactate synthase, catabolic [Lactobacillus reuteri DSM 20016]	8	6	3	5	6	3	16.3	60.994	559
>gi|183225693|dbj|BAG26210.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];	7	3	5	2	3	3	22.3	41.737	364
>gi|148531530|gb|ABQ83529.1| malate dehydrogenase (NAD) [Lactobacillus reuteri DSM 20016]	6	3	3	3	3	5	26.3	33.353	312
>gi|148531170|gb|ABQ83169.1| LrgB family protein [Lactobacillus reuteri DSM 20016];	4	4	3	2	4	3	22.8	26.306	246
>gi|183226402|dbj|BAG26918.1| isoleucyl-tRNA synthase [Lactobacillus fermentum IFO 3956];	7	6	4	5	6	4	6.5	105.63	930
>gi|148531897|gb|ABQ83896.1| FAD-dependent pyridine nucleotide-disulfide oxidoreductase [Lactobacillus reuteri DSM 20016]	5	4	2	4	2	3	22	35.791	332
>gi|148531115|gb|ABQ83114.1| hypothetical protein Lreu_0851 [Lactobacillus reuteri DSM 20016]	7	4	3	0	5	4	61.1	10.116	90
>gi|148531196|gb|ABQ83195.1| degV family protein [Lactobacillus reuteri DSM 20016] J	4	1	4	2	1	3	17.5	32.228	292
>gi|148531360|gb|ABQ83359.1| hypothetical protein Lreu_1102 [Lactobacillus reuteri DSM 20016]	6	3	4	0	3	2	61.8	10.015	89
>gi|148531143|gb|ABQ83142.1| riboflavin synthase, alpha subunit [Lactobacillus reuteri DSM 20016]	6	4	4	4	4	4	37	21.593	200
>gi|183225127|dbj|BAG25644.1| hypothetical protein [Lactobacillus reuteri JCM 1112];>	7	5	4	5	6	6	21.6	40.161	352
>gi|148531613|gb|ABQ83612.1| hypothetical protein Lreu_1363 [Lactobacillus reuteri DSM 20016];	3	3	1	3	3	3	15.9	19.693	189
>gi|227070209|gb|EEI08583.1| diaminopimelate decarboxylase [Lactobacillus reuteri MM2-3];	5	4	3	2	4	2	15.2	48.355	441
>gi|194453840|gb|EDX42737.1| conserved hypothetical protein [Lactobacillus reuteri 100-23]	5	5	4	3	4	4	24.1	28.451	245
>gi|148531348|gb|ABQ83347.1| exodeoxyribonuclease III Xth [Lactobacillus reuteri DSM 20016	7	3	5	5	7	5	28.7	31.516	275
>gi|148531041|gb|ABQ83040.1| Ras superfamily GTP-binding protein YlqF [Lactobacillus reuteri DSM 20016]	5	2	2	2	1	2	23.8	32.562	290
>gi|148531780|gb|ABQ83779.1| Peptidylprolyl isomerase [Lactobacillus reuteri DSM 20016];	5	4	5	3	3	3	32	22.199	197
>gi|183227321|dbj|BAG27837.1| 30S ribosomal protein S8 [Lactobacillus fermentum IFO 3956];	6	6	5	4	5	6	35.6	14.515	132
>gi|183225444|dbj|BAG25961.1| hypothetical protein [Lactobacillus reuteri JCM 1112	8	5	5	6	5	4	18.1	62.062	536
>gi|148530896|gb|ABQ82895.1| peptide deformylase [Lactobacillus reuteri DSM 20016	9	8	7	8	8	9	48.4	20.898	186
>gi|148530878|gb|ABQ82877.1| diaminopimelate epimerase [Lactobacillus reuteri DSM 20016	4	3	3	4	2	3	16.5	36.381	333
>gi|227071504|gb|EEI09804.1| glutamate-5-semialdehyde dehydrogenase [Lactobacillus reuteri MM2-3];	7	2	4	4	4	6	16.3	45.499	416
>gi|148530690|gb|ABQ82689.1| glucose-6-phosphate isomerase [Lactobacillus reuteri DSM 20016	11	5	6	5	11	6	29.9	50.35	452
>gi|183224052|dbj|BAG24569.1| beta-phosphoglucomutase [Lactobacillus reuteri JCM 1112	11	7	8	6	3	6	52.4	24.587	225
>gi|148530375|gb|ABQ82374.1| Inosine/uridine-preferring nucleoside hydrolase [Lactobacillus reuteri DSM 20016]	5	4	3	5	4	4	21.5	32.573	302
>gi|148530984|gb|ABQ82983.1| protein of unknown function DUF558 [Lactobacillus reuteri DSM 20016];	5	3	3	3	5	3	20.7	26.768	246

>gi|148530483|gb|ABQ82482.1| RNAse M5 [Lactobacillus reuteri DSM 20016];	4	1	4	0	2	0	28.7	21.042	188
>gi|148530478|gb|ABQ82477.1| hypothetical protein Lreu_0207 [Lactobacillus reuteri DSM 20016];	6	5	2	1	5	3	25.1	36.908	319
>gi|227070615|gb|EEI08945.1| tRNA modification GTPase TrmE [Lactobacillus reuteri MM2-3];>	7	4	4	4	5	4	17.2	52.527	477
>gi|148530926|gb|ABQ82925.1| short-chain dehydrogenase/reductase SDR [Lactobacillus reuteri DSM 20016]	7	6	4	3	5	5	26.4	31.787	284
>gi|148530761|gb|ABQ82760.1| hypothetical protein Lreu_0492 [Lactobacillus reuteri DSM 20016];	5	3	4	2	3	3	16.6	27.759	247
>gi|148531462|gb|ABQ83461.1| hypothetical protein Lreu_1204 [Lactobacillus reuteri DSM 20016]	4	2	4	2	3	2	69	6.3153	58
>gi|148530860|gb|ABQ82859.1| cell division protein FtsA [Lactobacillus reuteri DSM 20016];	11	5	3	5	10	6	25.2	50.485	457
>gi|227071203|gb|EEI09517.1| UDP-galactopyranose mutase [Lactobacillus reuteri MM2-3	9	5	5	3	4	6	21.1	44.975	384
>gi|183224788|dbj|BAG25305.1| hypothetical phage protein [Lactobacillus reuteri JCM 1112]	12	8	9	9	10	9	58.9	25.075	219
>gi|183226202|dbj|BAG26718.1| glutamine-fructose-6-phosphate transaminase [Lactobacillus fermentum IFO 3956];	5	4	2	4	4	5	7.8	66.739	606
>gi|227070517|gb|EEI08850.1| deoxyribose-phosphate aldolase [Lactobacillus reuteri MM2-3];>	6	5	4	3	3	3	23.2	25.76	237
>gi|148531361|gb|ABQ83360.1| hypothetical protein Lreu_1103 [Lactobacillus reuteri DSM 20016	5	5	5	2	4	3	60	9.0878	80
>gi|148530541|gb|ABQ82540.1| tRNA-U20-dihydrouridine synthase [Lactobacillus reuteri DSM 20016]	8	5	7	3	4	7	29.7	37.061	333
>gi|227070638|gb|EEI08968.1| DNA-binding response regulator [Lactobacillus reuteri MM2-3]	7	4	5	3	4	4	32.4	27.524	241
>gi|183225783|dbj|BAG26300.1| putative aminotransferase [Lactobacillus reuteri JCM 1112];	10	6	7	3	4	7	33	44.073	400
>gi|183224121|dbj|BAG24638.1| hypothetical protein [Lactobacillus reuteri JCM 1112];	4	1	4	3	3	2	21.5	24.277	219
>gi|227070121|gb|EEI08497.1| possible malate dehydrogenase [Lactobacillus reuteri MM2-3];>	6	2	2	2	2	2	24.2	39.19	360
>gi|337729312|emb|CCC04441.1| thymidylate kinase [Lactobacillus reuteri ATCC 53608];>	8	7	6	3	5	5	36.6	24.299	213
>gi|148530673|gb|ABQ82672.1| UDP-N-acetylmuramate dehydrogenase [Lactobacillus reuteri DSM 20016]	9	4	3	3	6	6	38.6	32.389	298
>gi|148530879|gb|ABQ82878.1| aspartate kinase [Lactobacillus reuteri DSM 20016	6	2	3	3	3	4	13.3	50.12	452
>gi|148531987|gb|ABQ83986.1| Glycerol dehydratase [Lactobacillus reuteri DSM 20016]	10	2	3	5	10	4	22.4	62.092	558
>gi|148530435|gb|ABQ82434.1| short-chain dehydrogenase/reductase SDR [Lactobacillus reuteri DSM 20016]	6	5	4	3	3	4	32.5	26.39	246
>gi|146749585|gb|ABQ44378.1| deoxynucleoside kinase [Lactobacillus reuteri];	5	3	4	3	3	4	31	24.773	210
>gi|148530473|gb|ABQ82472.1| nucleoside diphosphate kinase [Lactobacillus reuteri DSM 20016];	5	5	4	3	5	4	38.1	16.772	147
>gi|148530817|gb|ABQ82816.1| hypothetical protein Lreu_0548 [Lactobacillus reuteri DSM 20016];	5	2	4	2	2	2	52	16.479	152
>gi|133930437|gb|ABO43790.1| cytosine deaminase [Lactobacillus reuteri];	5	4	4	5	4	4	17.7	46.441	412

>gi|148531474|gb|ABQ83473.1| NLP/P60 protein [Lactobacillus reuteri DSM 20016];	4	3	2	2	3	3	22.6	22.822	212
>gi|227070071|gb|EEI08449.1| aldose 1-epimerase [Lactobacillus reuteri MM2-3]	3	2	3	3	3	2	15.5	34.581	304
>gi|148530286|gb|ABQ82285.1| SSU ribosomal protein S18P [Lactobacillus reuteri DSM 20016]	11	8	9	5	8	8	70.5	9.1536	78
>gi|148530311|gb|ABQ82310.1| aldehyde dehydrogenase [Lactobacillus reuteri DSM 20016];	6	1	5	2	3	5	18.4	50.505	457
>gi|148531138|gb|ABQ83137.1| type I site-specific deoxyribonuclease, HsdR family [Lactobacillus reuteri DSM 20016];	17	11	9	9	12	5	17.2	118.77	1041
>gi|148530873|gb|ABQ82872.1| tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase [Lactobacillus reuteri DSM 20016];	10	7	6	5	6	6	28.4	42.769	377
>gi|183224607|dbj|BAG25124.1| pyruvate dehydrogenase complex E1 component alpha subunit [Lactobacillus reuteri JCM 1112	8	7	4	6	5	5	21.3	41.361	371
>gi|133930439|gb|ABO43791.1| 2-keto-4-pentenoate hydratase [Lactobacillus reuteri];	5	4	2	3	2	4	17.5	29.068	263
>gi|148532084|gb|ABQ84083.1| ErfK/YbiS/YcfS/YnhG family protein [Lactobacillus reuteri DSM 20016	5	4	3	3	3	3	24.8	24.726	218
>gi|148530720|gb|ABQ82719.1| thymidine kinase [Lactobacillus reuteri DSM 20016];	12	9	11	8	7	7	58.6	22.171	191
>gi|148531875|gb|ABQ83874.1| phage shock protein C, PspC [Lactobacillus reuteri DSM 20016];	2	2	2	2	2	2	18.1	9.3228	83
>gi|148531473|gb|ABQ83472.1| uridine kinase [Lactobacillus reuteri DSM 20016	6	5	6	3	5	4	33.9	25.134	218
>gi|148531098|gb|ABQ83097.1| phage portal protein, SPP1 family [Lactobacillus reuteri DSM 20016];	14	4	3	5	9	12	27.3	63.32	553
>gi|227070201|gb|EEI08575.1| conserved hypothetical protein [Lactobacillus reuteri MM2-3	7	4	6	3	3	4	60.7	13.567	117
>gi|148531447|gb|ABQ83446.1| alanine racemase domain protein [Lactobacillus reuteri DSM 20016];>	6	4	5	3	4	3	32.8	26.995	235
>gi|148532169|gb|ABQ84168.1| ATP-grasp protein-like protein [Lactobacillus reuteri DSM 20016	9	7	4	7	6	7	19.6	48.604	414
>gi|183227039|dbj|BAG27555.1| 50S Ribosomal protein L19 [Lactobacillus fermentum IFO 3956];	5	4	4	3	4	4	25.2	13.668	119
>gi|148530494|gb|ABQ82493.1| Cof-like hydrolase [Lactobacillus reuteri DSM 20016	7	2	6	4	3	5	28.2	30.465	273
>gi|148530507|gb|ABQ82506.1| sortase family protein [Lactobacillus reuteri DSM 20016];>	5	5	3	2	2	2	26.5	26.348	234
>gi|337727992|emb|CCC03081.1| diaminopimelate epimerase [Lactobacillus reuteri ATCC 53608]	2	1	1	1	1	1	9.5	35.836	328
>gi|148530602|gb|ABQ82601.1| protein of unknown function DUF970 [Lactobacillus reuteri DSM 20016];>	5	5	5	2	4	4	69.2	11.874	107
>gi|183224278|dbj|BAG24795.1| phosphoglycerate mutase [Lactobacillus reuteri JCM 1112];>	7	5	5	4	5	3	30.6	29.591	255
>gi|148530464|gb|ABQ82463.1| NLPA lipoprotein [Lactobacillus reuteri DSM 20016	8	5	4	5	4	7	31.4	31.931	283
>gi|148530378|gb|ABQ82377.1| Effector of nucleoid occlusion Noc [Lactobacillus reuteri DSM 20016];>	9	6	4	2	5	6	28.3	36.94	322
>gi|148531122|gb|ABQ83121.1| holin, Cph1 family [Lactobacillus reuteri DSM 20016];>	2	2	2	0	2	2	10.1	17.139	149
>gi|148530319|gb|ABQ82318.1| OsmC family protein [Lactobacillus reuteri DSM 20016];>	5	4	5	4	3	5	29.6	15.724	142
>gi|148530539|gb|ABQ82538.1| membrane protease FtsH catalytic subunit [Lactobacillus reuteri DSM 20016	14	5	5	6	8	10	24.8	77.238	702

>gi|148531693|gb|ABQ83692.1| 5-(carboxyamino)imidazole ribonucleotide synthase [Lactobacillus reuteri DSM 20016	7	6	6	3	7	4	21.5	42.001	377
>gi|183225042|dbj|BAG25559.1| hypothetical phage protein [Lactobacillus reuteri JCM 1112];>gi|184153698|ref|YP_001842039.1| hypothetical protein LAR_1043 [Lactobacillus reuteri JCM 1112];>gi|227069922|gb|EEI08309.1| conserved hypothetical protein [Lactobac	6	2	2	5	4	4	15.8	41.121	386
>gi|148531022|gb|ABQ83021.1| ribosomal large subunit pseudouridine synthase B [Lactobacillus reuteri DSM 20016];>	6	3	6	3	4	3	29.8	26.693	238
>gi|148531286|gb|ABQ83285.1| Phosphoglycerate mutase [Lactobacillus reuteri DSM 20016	7	3	4	5	4	5	31.3	30.347	278
>gi|227070559|gb|EEI08890.1| possible gamma-glutamyl-gamma-aminobutyrate hydrolase [Lactobacillus reuteri MM2-3];	4	3	4	3	4	3	23.9	27.281	247
>gi|148531976|gb|ABQ83975.1| ATP:cob(I)alamin adenosyltransferase [Lactobacillus reuteri DSM 20016];>	4	3	3	2	2	2	28	16.903	157
>gi|148530321|gb|ABQ82320.1| aromatic amino acid aminotransferase apoenzyme [Lactobacillus reuteri DSM 20016];>	8	5	4	5	5	6	25.6	42.52	395
>gi|183224315|dbj|BAG24832.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	4	4	3	0	2	2	55.7	11.883	106
>gi|183226271|dbj|BAG26787.1| D-alanine-D-alanine ligase [Lactobacillus fermentum IFO 3956];>	2	2	2	2	2	2	7.2	42.344	377
>gi|148530914|gb|ABQ82913.1| SSU ribosomal protein S15P [Lactobacillus reuteri DSM 20016	7	7	7	5	6	6	64	10.416	89
>gi|148531241|gb|ABQ83240.1| DSBA oxidoreductase [Lactobacillus reuteri DSM 20016];	5	3	3	1	2	1	27.4	24.566	215
>gi|227071111|gb|EEI09429.1| Dyp family peroxidase family protein [Lactobacillus reuteri MM2-3];>	8	6	5	7	6	5	24	36.463	317
>gi|148531694|gb|ABQ83693.1| xanthine phosphoribosyltransferase [Lactobacillus reuteri DSM 20016]	5	4	3	1	3	2	24.6	21.113	191
>gi|112943339|gb|ABI26305.1| sphingosine kinase [Lactobacillus reuteri];>	8	4	2	4	4	3	26.1	37.14	337
>gi|148530942|gb|ABQ82941.1| hydroxymethylglutaryl-CoA synthase [Lactobacillus reuteri DSM 20016];>	4	3	3	3	3	4	10.4	42.757	385
>gi|227070978|gb|EEI09301.1| cobalt-precorrin-2 C(20)-methyltransferase [Lactobacillus reuteri MM2-3	5	3	4	1	2	3	26.7	26.806	240
>gi|148531000|gb|ABQ82999.1| PhoH family protein [Lactobacillus reuteri DSM 20016];	7	3	3	5	3	7	19.7	37.467	335
>gi|148531487|gb|ABQ83486.1| LSU ribosomal protein L32P [Lactobacillus reuteri DSM 20016	5	4	4	2	4	4	64.4	6.5727	59
>gi|148530956|gb|ABQ82955.1| Undecaprenyl pyrophosphate synthetase [Lactobacillus reuteri DSM 20016]	7	5	6	5	4	2	28.1	30.635	270
>gi|148530459|gb|ABQ82458.1| methionine-R-sulfoxide reductase [Lactobacillus reuteri DSM 20016];	6	4	4	2	3	6	40.1	16.101	142
>gi|148531657|gb|ABQ83656.1| methylated-DNA--protein-cysteine methyltransferase [Lactobacillus reuteri DSM 20016];	2	1	2	0	1	0	13.8	17.204	152
>gi|227185629|gb|EEI65700.1| SMC structural maintenance of chromosomes partitioning protein [Lactobacillus reuteri CF48-3A]	13	10	8	9	7	8	12	135.26	1187

>gi|148531334|gb|ABQ83333.1| hypothetical protein Lreu_1076 [Lactobacillus reuteri DSM 20016	3	2	3	1	2	2	21.4	17.798	154
>gi|183225185|dbj|BAG25702.1| thioredoxin [Lactobacillus reuteri JCM 1112	4	3	2	2	3	2	27.5	12.828	109
>gi|194453683|gb|EDX42580.1| translation initiation factor IF-3 [Lactobacillus reuteri 100-23	6	3	5	3	4	4	40	19.488	170
>gi|183227137|dbj|BAG27653.1| DNA-directed DNA polymerase I [Lactobacillus fermentum IFO 3956	6	4	5	3	6	3	7.2	99.23	886
>gi|148530407|gb|ABQ82406.1| 5-(carboxyamino)imidazole ribonucleotide mutase [Lactobacillus reuteri DSM 20016];>	6	3	6	2	4	2	59	16.958	161
>gi|148530785|gb|ABQ82784.1| amino acid ABC transporter ATP-binding protein, PAAT family [Lactobacillus reuteri DSM 20016];	6	6	5	4	5	5	29.2	23.926	216
>gi|148531433|gb|ABQ83432.1| DNA-directed RNA polymerase subunit omega [Lactobacillus reuteri DSM 20016];	6	4	4	0	6	2	87.5	7.9219	72
>gi|227070037|gb|EEI08415.1| S-adenosyl-L-methionine-dependent methyltransferase [Lactobacillus reuteri MM2-3];	6	6	6	4	5	3	33.2	26.69	238
>gi|112943293|gb|ABI26303.1| pyruvate/2-oxoglutarate dehydrogenase complex dihydrolipoamide dehydrogenase (E3) component [Lactobacillus reuteri];>	8	7	6	6	7	7	21.3	50.725	475
>gi|148530921|gb|ABQ82920.1| MazG nucleotide pyrophosphohydrolase [Lactobacillus reuteri DSM 20016];>	2	1	2	0	1	1	20.2	11.475	99
>gi|148530490|gb|ABQ82489.1| purine operon repressor, PurR [Lactobacillus reuteri DSM 20016	5	5	2	3	5	5	22.4	30.926	281
>gi|130893136|gb|ABO32595.1| D-lactate dehydrogenase [Lactobacillus reuteri];>	6	2	2	5	1	5	23.3	36.867	330
>gi|148530385|gb|ABQ82384.1| phosphopentomutase [Lactobacillus reuteri DSM 20016];>	7	3	3	2	7	5	25.2	44.002	397
>gi|148531430|gb|ABQ83429.1| methionyl-tRNA formyltransferase [Lactobacillus reuteri DSM 20016];>	9	4	4	6	3	7	27.4	34.729	317
>gi|148530733|gb|ABQ82732.1| ATP synthase F1 subcomplex delta subunit [Lactobacillus reuteri DSM 20016];>	2	2	1	1	1	2	10.6	20.468	180
>gi|148531544|gb|ABQ83543.1| transcriptional regulator, LacI family [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	5	37.693	338
>gi|148531488|gb|ABQ83487.1| protein of unknown function DUF177 [Lactobacillus reuteri DSM 20016];>	3	0	2	1	2	2	19.5	20.961	185
>gi|227070644|gb|EEI08974.1| phosphatidylglycerophosphatase [Lactobacillus reuteri MM2-3];>	7	5	7	4	5	5	36.7	18.978	169
>gi|148531067|gb|ABQ83066.1| prophage antirepressor [Lactobacillus reuteri DSM 20016];	10	2	2	3	3	9	37	28.734	257
>gi|183224776|dbj|BAG25293.1| phage replication protein [Lactobacillus reuteri JCM 1112	7	2	3	2	3	4	23.5	34.382	294
>gi|183224461|dbj|BAG24978.1| cell division membrane protein [Lactobacillus reuteri JCM 1112];>	1	1	0	1	0	1	4	44.8	399
>gi|183225137|dbj|BAG25654.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	2	2	2	2	2	2	13.7	16.242	139
>gi|148531954|gb|ABQ83953.1| precorrin-3 methyltransferase [Lactobacillus reuteri DSM 20016];>	10	7	8	5	7	6	32.4	26.233	241

>gi|148531795|gb|ABQ83794.1| Esterase/lipase-like protein [Lactobacillus reuteri DSM 20016];>	5	0	4	1	4	0	32.8	26.628	238
>gi|148531986|gb|ABQ83985.1| dehydratase, medium subunit [Lactobacillus reuteri DSM 20016];	5	2	3	1	2	5	24.2	25.808	236
>gi|148532132|gb|ABQ84131.1| hypothetical protein Lreu_1894 [Lactobacillus reuteri DSM 20016	4	3	3	1	2	2	45.8	13.741	120
>gi|133930453|gb|ABO43798.1| DnaB [Lactobacillus reuteri];>	2	1	1	1	1	2	5.2	51.331	463
>gi|183225054|dbj|BAG25571.1| hypothetical phage protein [Lactobacillus reuteri JCM 1112];>	5	4	1	0	3	4	38.6	14.835	132
>gi|148531744|gb|ABQ83743.1| Deoxyadenosine kinase [Lactobacillus reuteri DSM 20016	8	4	6	0	3	1	53.1	24.647	213
>gi|227184813|gb|EEI64884.1| aspartate racemase [Lactobacillus reuteri CF48-3A];>	5	4	4	4	4	5	17.2	27.131	239
>gi|148530653|gb|ABQ82652.1| Excinuclease ABC subunit B [Lactobacillus reuteri DSM 20016];>	11	5	2	6	4	5	17.1	79.421	690
>gi|227070656|gb|EEI08986.1| ABC superfamily ATP binding cassette transporter, ABC protein [Lactobacillus reuteri MM2-3];>g	2	1	2	0	1	1	12.2	26.94	238
>gi|148531689|gb|ABQ83688.1| aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit C [Lactobacillus reuteri DSM 20016];	3	3	3	1	3	3	43.8	11.572	105
>gi|183225864|dbj|BAG26380.1| adenylosuccinate synthase [Lactobacillus fermentum IFO 3956];>	2	1	2	1	1	1	5.6	47.282	429
>gi|227185635|gb|EEI65706.1| protein of hypothetical function DUF322 [Lactobacillus reuteri CF48-3A];>	4	3	4	3	3	3	36.1	13.014	122
>gi|336449796|gb|AEI58411.1| HD domain protein [Lactobacillus reuteri SD2112];>	1	1	1	1	1	1	3.5	29.851	257
>gi|148530379|gb|ABQ82378.1| chromosome segregation ATPase [Lactobacillus reuteri DSM 20016];>	4	1	2	1	0	0	21.1	28.007	256
>gi|227070139|gb|EEI08514.1| MazG nucleotide pyrophosphohydrolase [Lactobacillus reuteri MM2-3];>	2	1	1	1	1	1	13.3	13.023	113
>gi|227070646|gb|EEI08976.1| SPOUT methyltransferase superfamily protein [Lactobacillus reuteri MM2-3	6	2	3	3	3	3	35.3	19.733	173
>gi|148530662|gb|ABQ82661.1| preprotein translocase, SecG subunit [Lactobacillus reuteri DSM 20016];>	1	1	1	0	1	0	24.1	8.5972	79
>gi|183227656|dbj|BAG28172.1| asparagine synthase [Lactobacillus fermentum IFO 3956];>	7	5	6	6	6	6	8.8	73.993	636
>gi|148531843|gb|ABQ83842.1| Peptidoglycan-binding LysM [Lactobacillus reuteri DSM 20016];>	3	1	2	3	2	2	17.5	37.509	355
>gi|148530941|gb|ABQ82940.1| RNase HI [Lactobacillus reuteri DSM 20016];>	7	2	5	2	4	2	39.6	24.902	222
>gi|148531708|gb|ABQ83707.1| bacterial translation initiation factor 1 (bIF-1) [Lactobacillus reuteri DSM 20016];	5	3	4	4	3	3	66.7	8.1615	72
>gi|148530520|gb|ABQ82519.1| DltD, C-terminal domain protein [Lactobacillus reuteri DSM 20016];>	5	4	3	4	5	5	15.2	49.455	429
>gi|183225184|dbj|BAG25701.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	5	3	3	0	2	2	33.8	23.595	216
>gi|227070541|gb|EEI08874.1| phosphoribosylaminoimidazole carboxylase [Lactobacillus reuteri MM2-3];	4	1	4	2	2	2	10.4	42.428	384
>gi|148530742|gb|ABQ82741.1| hypothetical protein Lreu_0473 [Lactobacillus reuteri DSM	3	2	3	2	2	2	50	8.7648	74

20016];									
>gi|148530901|gb|ABQ82900.1| protein of unknown function UPF0223 [Lactobacillus reuteri DSM 20016];	5	4	5	1	5	3	37.3	11.867	102
>gi|148531400|gb|ABQ83399.1| phage antirepressor protein [Lactobacillus reuteri DSM 20016];>	10	3	2	2	4	10	36.7	30.203	267
>gi|227186252|gb|EEI66323.1| possible aspartate transaminase [Lactobacillus reuteri CF48-3A	7	5	3	4	4	4	23	42.745	395
>gi|148531531|gb|ABQ83530.1| hypothetical protein Lreu_1273 [Lactobacillus reuteri DSM 20016];>	4	3	4	3	3	2	48.9	10.018	90
>gi|227070870|gb|EEI09194.1| iron-containing alcohol dehydrogenase [Lactobacillus reuteri MM2-3	3	2	3	0	2	3	11.9	40.929	379
>gi|148530842|gb|ABQ82841.1| NAD(+) kinase [Lactobacillus reuteri DSM 20016];>	7	4	6	4	5	4	29.3	30.666	270
>gi|183225766|dbj|BAG26283.1| aspartate-ammonia ligase [Lactobacillus reuteri JCM 1112];	13	7	6	5	7	8	42.8	39.72	339
>gi|148530479|gb|ABQ82478.1| Silent information regulator protein Sir2 [Lactobacillus reuteri DSM 20016];>	5	2	4	1	1	1	23.7	26.454	232
>gi|148530853|gb|ABQ82852.1| S-adenosyl-methyltransferase MraW [Lactobacillus reuteri DSM 20016];>	3	3	3	3	3	3	11.6	35.666	318
>gi|337728180|emb|CCC03271.1| Mannosyl-glycoprotein endo-beta-N-acetylglucosaminidase [Lactobacillus reuteri ATCC 53608]	3	2	2	1	3	2	8.8	55.966	499
>gi|183225556|dbj|BAG26073.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	4	3	3	2	1	1	16.1	33.021	286
>gi|148530571|gb|ABQ82570.1| deoxyuridine 5-triphosphate nucleotidohydrolase [Lactobacillus reuteri DSM 20016];	5	4	4	1	3	3	29.2	20.3	178
>gi|148531772|gb|ABQ83771.1| Uracil-DNA glycosylase superfamily [Lactobacillus reuteri DSM 20016];>	2	1	2	1	1	1	12	24.505	217
>gi|148531752|gb|ABQ83751.1| iron dependent repressor [Lactobacillus reuteri DSM 20016]	7	6	5	5	5	4	31.7	25.242	224
>gi|148530329|gb|ABQ82328.1| acetyl-CoA acetyltransferase [Lactobacillus reuteri DSM 20016];>	6	4	2	4	2	5	17.9	41.055	392
>gi|183226447|dbj|BAG26963.1| metal-dependent hydrolase of the beta-lactamase superfamily III [Lactobacillus fermentum IFO 3956	2	1	1	1	1	1	6.8	34.44	310
>gi|183225182|dbj|BAG25699.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112	1	1	1	1	1	1	5.9	25.715	238
>gi|148530346|gb|ABQ82345.1| hypothetical protein Lreu_0070 [Lactobacillus reuteri DSM 20016	3	2	2	2	2	2	13.4	29.491	261
>gi|133930447|gb|ABO43795.1| E1 component beta subunit [Lactobacillus reuteri];>	5	2	1	2	3	3	18.5	35.243	325
>gi|148531423|gb|ABQ83422.1| LSU ribosomal protein L28P [Lactobacillus reuteri DSM 20016	5	3	5	3	4	4	45.9	6.9271	61
>gi|148530383|gb|ABQ82382.1| membrane protein of unknown function UCP033111 [Lactobacillus reuteri DSM 20016];>	5	4	4	2	2	4	24.6	28.788	256
>gi|148531981|gb|ABQ83980.1| microcompartments protein [Lactobacillus reuteri DSM 20016];>	5	1	3	2	3	3	81.2	9.7479	96
>gi|148530744|gb|ABQ82743.1| glycine cleavage H-protein [Lactobacillus reuteri DSM 20016];>	6	4	3	0	4	2	83.5	10.641	97
>gi|148531828|gb|ABQ83827.1| quorum-sensing autoinducer 2 (AI-2), LuxS [Lactobacillus reuteri DSM 20016];>	7	6	6	4	4	3	41.8	17.695	158

>gi|148531111|gb|ABQ83110.1| protein of unknown function DUF1306 [Lactobacillus reuteri DSM 20016];>	6	3	3	3	4	6	22	33.264	295
>gi|148531643|gb|ABQ83642.1| conserved hypothetical protein 730 [Lactobacillus reuteri DSM 20016];>	3	3	3	2	3	3	14.9	21.895	195
>gi|227070830|gb|EEI09155.1| BS_ykrK family protein [Lactobacillus reuteri MM2-3];>	5	4	4	1	3	4	23.3	21.839	193
>gi|148531602|gb|ABQ83601.1| bacterial peptide chain release factor 3 (bRF-3) [Lactobacillus reuteri DSM 20016];>	9	6	5	6	6	5	19.6	59.477	525
>gi|148531012|gb|ABQ83011.1| protein of unknown function DUF34 [Lactobacillus reuteri DSM 20016];>	5	4	4	2	3	3	20.4	30.534	274
>gi|148532059|gb|ABQ84058.1| ABC transporter related [Lactobacillus reuteri DSM 20016];>	6	2	5	0	2	1	33.9	28.077	254
>gi|148531953|gb|ABQ83952.1| precorrin-6A reductase [Lactobacillus reuteri DSM 20016	6	5	4	4	4	4	29	28.054	252
>gi|148530871|gb|ABQ82870.1| aminotransferase, class V [Lactobacillus reuteri DSM 20016];>	4	3	3	4	3	4	11.2	42.481	384
>gi|183227153|dbj|BAG27669.1| arginyl-tRNA synthase [Lactobacillus fermentum IFO 3956];	2	2	1	1	1	1	4.6	63.149	562
>gi|183224876|dbj|BAG25393.1| carbamoyl-phosphate synthase large subunit [Lactobacillus reuteri JCM 1112	12	4	7	5	8	4	16.7	92.789	825
>gi|148532146|gb|ABQ84145.1| hypothetical protein Lreu_1908 [Lactobacillus reuteri DSM 20016];>	2	1	2	1	1	1	13	18.28	161
>gi|154705574|gb|ABS84213.1| AsnC-type transcription regulator [Lactobacillus reuteri];>	6	5	4	3	3	3	40.4	17.782	156
>gi|148531797|gb|ABQ83796.1| cystathionine beta-synthase (acetylserine-dependent) [Lactobacillus reuteri DSM 20016]	8	5	3	3	4	6	28.6	32.602	304
>gi|183224612|dbj|BAG25129.1| myo-inositol-1(or 4)-monophosphatase [Lactobacillus reuteri JCM 1112];>	8	5	7	6	4	4	26.1	28.91	261
>gi|148530548|gb|ABQ82547.1| amino acid/peptide transporter [Lactobacillus reuteri DSM 20016];>	2	1	1	2	1	1	4.4	54.667	499
>gi|148531329|gb|ABQ83328.1| FAD-dependent pyridine nucleotide-disulfide oxidoreductase [Lactobacillus reuteri DSM 20016];	4	3	2	1	2	2	10.8	60.056	555
>gi|183225947|dbj|BAG26463.1| phosphoribosylformylglycinamidine synthase II [Lactobacillus fermentum IFO 3956];>	3	2	2	3	2	1	4.7	79.944	741
>gi|148530906|gb|ABQ82905.1| putative methyltransferase [Lactobacillus reuteri DSM 20016];>	7	5	5	4	4	4	33.7	20.85	187
>gi|148530816|gb|ABQ82815.1| protein of unknown function DUF948 [Lactobacillus reuteri DSM 20016];	2	2	2	2	2	2	11	16.569	146
>gi|227071772|gb|EEI10061.1| transcription antitermination protein NusB [Lactobacillus reuteri MM2-3];>	7	5	6	1	4	4	41.1	16.148	141
>gi|148531472|gb|ABQ83471.1| transcription elongation factor GreA [Lactobacillus reuteri DSM 20016];>	7	3	6	4	5	2	47.5	18.019	158
>gi|148530351|gb|ABQ82350.1| peptidase C26 [Lactobacillus reuteri DSM 20016];>	5	3	3	3	4	4	20.1	26.977	244
>gi|148531326|gb|ABQ83325.1| dipeptidase A, Cysteine peptidase, MEROPS family C69 [Lactobacillus reuteri DSM 20016]	8	5	6	4	5	4	17.8	52.667	467

>gi|183226119|dbj|BAG26635.1| carbamate kinase [Lactobacillus fermentum IFO 3956];>	4	3	1	1	2	2	12.3	32.714	309
>gi|148531458|gb|ABQ83457.1| aminotransferase [Lactobacillus reuteri DSM 20016];>	9	4	5	5	3	7	23.9	43.367	393
>gi|148531418|gb|ABQ83417.1| acyl carrier protein [Lactobacillus reuteri DSM 20016];	4	4	2	1	3	2	52.4	9.4942	84
>gi|194454108|gb|EDX43005.1| conserved hypothetical protein [Lactobacillus reuteri 100-23];>	3	2	0	1	3	2	11.7	38.01	350
>gi|148531410|gb|ABQ83409.1| 16S rRNA processing protein RimM [Lactobacillus reuteri DSM 20016];>	4	4	2	1	2	1	23.2	19.219	168
>gi|183224799|dbj|BAG25316.1| hypothetical protein [Lactobacillus reuteri JCM 1112];>	11	2	5	4	9	8	9.5	132.13	1198
>gi|227070039|gb|EEI08417.1| peptidase T [Lactobacillus reuteri MM2-3];>	5	3	1	3	2	3	16.2	46.484	419
>gi|227071446|gb|EEI09749.1| possible acetyltransferase [Lactobacillus reuteri MM2-3	7	5	6	2	5	4	79.8	12.089	104
>gi|148530794|gb|ABQ82793.1| DNA mismatch repair protein MutS [Lactobacillus reuteri DSM 20016	8	5	3	1	7	6	8.5	99.674	881
>gi|148531477|gb|ABQ83476.1| transcriptional regulator, HxlR family [Lactobacillus reuteri DSM 20016	5	3	4	2	3	3	33	13.039	112
>gi|148531144|gb|ABQ83143.1| GTP cyclohydrolase II [Lactobacillus reuteri DSM 20016];>	7	2	3	2	5	3	25.7	43.927	393
>gi|194454301|gb|EDX43198.1| protein of unknown function DUF534 [Lactobacillus reuteri 100-23];>	2	0	1	0	0	1	7.5	37.857	345
>gi|148531619|gb|ABQ83618.1| hypothetical protein Lreu_1369 [Lactobacillus reuteri DSM 20016	5	5	3	3	3	2	11.2	38.188	338
>gi|148531536|gb|ABQ83535.1| GTP cyclohydrolase I [Lactobacillus reuteri DSM 20016	3	1	2	1	2	2	16.7	22.03	192
>gi|146345339|gb|ABQ23681.1| Zn-dependent alcohol dehydrogenase [Lactobacillus reuteri	5	4	4	5	4	4	14.7	37.938	348
>gi|148530718|gb|ABQ82717.1| CobB/CobQ domain protein glutamine amidotransferase [Lactobacillus reuteri DSM 20016];>	6	5	4	2	2	4	30.6	26.134	235
>gi|183227318|dbj|BAG27834.1| 30S ribosomal protein S5 [Lactobacillus fermentum IFO 3956	5	5	4	3	4	5	18.9	17.581	169
>gi|148531533|gb|ABQ83532.1| Dihydropteroate synthase [Lactobacillus reuteri DSM 20016	6	2	5	3	4	2	18.3	43.584	387
>gi|148531039|gb|ABQ83038.1| Uncharacterized protein [Lactobacillus reuteri DSM 20016	5	4	2	3	3	3	20.5	22.591	200
>gi|148531975|gb|ABQ83974.1| LSU ribosomal protein L29P [Lactobacillus reuteri DSM 20016	6	3	2	3	3	4	15.7	51.127	477
>gi|148530997|gb|ABQ82996.1| argininosuccinate lyase [Lactobacillus reuteri DSM 20016	3	0	2	1	2	1	5	51.92	461
>gi|148530789|gb|ABQ82788.1| Uncharacterized protein [Lactobacillus reuteri DSM 20016	4	3	3	1	3	3	14.5	38.296	345
>gi|148531438|gb|ABQ83437.1| hemolysin A [Lactobacillus reuteri DSM 20016	5	4	3	5	3	3	20.5	30.273	273
>gi|227069946|gb|EEI08331.1| conserved hypothetical protein [Lactobacillus reuteri MM2-3	5	3	1	0	4	0	33.1	19.78	175
>gi|148530657|gb|ABQ82656.1| protein of unknown function UPF0052 and CofD [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	4.6	36.198	328
>gi|183225679|dbj|BAG26196.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112	4	2	3	1	2	3	24.7	18.055	170
>gi|183224651|dbj|BAG25168.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112	4	4	4	4	4	4	42.7	8.6646	75
>gi|148532042|gb|ABQ84041.1| protein of unknown function DUF488 [Lactobacillus reuteri DSM 20016	3	2	3	1	2	2	33.1	14.61	124

>gi|148531026|gb|ABQ83025.1| hypothetical protein Lreu_0761 [Lactobacillus reuteri DSM 20016];>	3	2	3	3	2	3	17.9	21.977	190
>gi|148530739|gb|ABQ82738.1| UDP-N-acetylglucosamine 1-carboxyvinyltransferase [Lactobacillus reuteri DSM 20016	5	1	2	2	4	2	16.4	47.242	438
>gi|148531404|gb|ABQ83403.1| protein of unknown function DUF1535 [Lactobacillus reuteri DSM 20016];>	3	2	1	2	1	2	23.7	18.631	169
>gi|148530475|gb|ABQ82474.1| tryptophanyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	6	4	3	1	3	4	18.8	38.062	340
>gi|227070750|gb|EEI09077.1| conserved hypothetical protein [Lactobacillus reuteri MM2-3];>	10	7	8	5	6	5	37.1	25.173	221
>gi|183224190|dbj|BAG24707.1| DNA helicase [Lactobacillus reuteri JCM 1112];>	8	1	4	2	5	7	12.2	88.988	773
>gi|227070893|gb|EEI09216.1| DNA helicase [Lactobacillus reuteri MM2-3	11	7	6	6	7	8	14.7	90.014	775
>gi|227071654|gb|EEI09947.1| oxalyl-CoA decarboxylase [Lactobacillus reuteri MM2-3	6	3	3	6	5	4	12.3	63.052	583
>gi|148531126|gb|ABQ83125.1| GCN5-related N-acetyltransferase [Lactobacillus reuteri DSM 20016	3	2	1	1	2	2	22.6	20.109	177
>gi|148530420|gb|ABQ82419.1| hypothetical protein Lreu_0147 [Lactobacillus reuteri DSM 20016	7	4	5	3	3	3	15.8	40.003	349
>gi|148532108|gb|ABQ84107.1| Methionine synthase, vitamin-B12 independent [Lactobacillus reuteri DSM 20016	6	4	2	5	5	4	21.7	42.352	378
>gi|183226173|dbj|BAG26689.1| excinuclease ABC subunit B [Lactobacillus fermentum IFO 3956	5	3	2	3	2	3	7.2	76.358	671
>gi|148531106|gb|ABQ83105.1| hypothetical protein Lreu_0842 [Lactobacillus reuteri DSM 20016	5	3	5	3	3	4	43.4	15.738	136
>gi|183224586|dbj|BAG25103.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112	3	2	2	1	3	1	13	26.479	230
>gi|148532020|gb|ABQ84019.1| PEBP family protein [Lactobacillus reuteri DSM 20016	5	4	4	3	4	3	29.8	18.294	168
>gi|148531367|gb|ABQ83366.1| phage major tail protein [Lactobacillus reuteri DSM 20016	2	1	2	1	1	1	14.8	25.3	236
>gi|148531440|gb|ABQ83439.1| Exodeoxyribonuclease VII small subunit [Lactobacillus reuteri DSM 20016	1	1	1	0	1	1	18.3	10.268	93
>gi|148532023|gb|ABQ84022.1| hypothetical protein Lreu_1783 [Lactobacillus reuteri DSM 20016	5	1	1	1	2	4	14.7	43.26	373
>gi|148531467|gb|ABQ83466.1| 5-formyltetrahydrofolate cyclo-ligase [Lactobacillus reuteri DSM 20016	3	2	3	2	2	2	18.5	21.115	184
>gi|148532091|gb|ABQ84090.1| transcriptional regulator, LacI family [Lactobacillus reuteri DSM 20016	7	6	5	3	6	3	20.7	35.062	314
>gi|148531468|gb|ABQ83467.1| LSU ribosomal protein L33P [Lactobacillus reuteri DSM 20016	2	2	2	2	2	2	46.9	5.9998	49
>gi|148530805|gb|ABQ82804.1| protein of unknown function DUF1292 [Lactobacillus reuteri DSM 20016	2	2	1	0	2	2	35.7	11.401	98
>gi|227070920|gb|EEI09243.1| possible phosphatase [Lactobacillus reuteri MM2-3	7	4	3	3	4	2	28.9	24.08	218
>gi|148531215|gb|ABQ83214.1| Glyoxalase/bleomycin resistance protein/dioxygenase [Lactobacillus reuteri DSM 20016	5	5	5	1	4	4	36.9	14.777	130
>gi|148530484|gb|ABQ82483.1| dimethyladenosine transferase [Lactobacillus reuteri DSM 20016	8	5	6	3	6	5	31.6	33.039	297

>gi|148530964|gb|ABQ82963.1| LSU ribosomal protein L7AE [Lactobacillus reuteri DSM 20016 DSM 20016];>	3	3	3	3	2	3	33	11.651	103
>gi|183224803|dbj|BAG25320.1| hypothetical protein [Lactobacillus reuteri JCM 1112	4	1	1	3	3	4	12.1	40.672	371
>gi|148530978|gb|ABQ82977.1| hypothetical protein Lreu_0712 [Lactobacillus reuteri DSM 20016	3	3	2	3	3	3	19.7	17.36	152
>gi|148531985|gb|ABQ83984.1| dehydratase, small subunit [Lactobacillus reuteri DSM 20016	4	2	4	2	2	1	27.9	19.319	172
>gi|148531413|gb|ABQ83412.1| signal recognition particle subunit FFH/SRP54 (srp54) [Lactobacillus reuteri DSM 20016];>	6	2	4	1	2	4	14.1	54.114	481
>gi|148531627|gb|ABQ83626.1| hypothetical protein Lreu_1377 [Lactobacillus reuteri DSM 20016	4	3	3	1	1	2	20.4	30.386	255
>gi|227070716|gb|EEI09044.1| NAD-dependent epimerase/dehydratase [Lactobacillus reuteri MM2-3	6	5	3	4	4	4	22.7	27.234	242
>gi|148531500|gb|ABQ83499.1| replicative DNA helicase loader DnaI [Lactobacillus reuteri DSM 20016	2	1	2	1	0	1	8.6	35.847	313
>gi|227070756|gb|EEI09083.1| ABC superfamily ATP binding cassette transporter, ABC/membrane protein [Lactobacillus reuteri MM2-3	5	2	1	1	2	4	7.4	72.445	666
>gi|148531133|gb|ABQ83132.1| N-6 DNA methylase [Lactobacillus reuteri DSM 20016	5	2	3	4	3	2	10	58.018	510
>gi|148531233|gb|ABQ83232.1| nucleotide deoxyribosyltransferase [Lactobacillus reuteri DSM 20016];>	5	3	5	3	4	2	33.8	18.201	160
>gi|148530565|gb|ABQ82564.1| amino acid ABC transporter membrane protein, PAAT family [Lactobacillus reuteri DSM 20016];>	5	5	5	4	4	4	21	26.062	233
>gi|148530719|gb|ABQ82718.1| domain of unknown function DUF1727 [Lactobacillus reuteri DSM 20016];>	6	4	4	2	2	4	14.4	50.191	445
>gi|148531973|gb|ABQ83972.1| acetate kinase [Lactobacillus reuteri DSM 20016	6	5	4	4	5	6	18.5	43.802	394
>gi|112943862|gb|ABI26325.1| tetrahydrodipicolinate N-succinyltransferase [Lactobacillus reuteri	4	1	3	2	2	2	18.6	24.845	236
>gi|148530534|gb|ABQ82533.1| RNA-binding S4 domain protein [Lactobacillus reuteri DSM 20016];>	3	3	3	1	3	2	31.1	10.249	90
>gi|183224722|dbj|BAG25239.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	4	2	2	3	3	4	16.6	33.759	296
>gi|148530568|gb|ABQ82567.1| aminopeptidase C, Cysteine peptidase, MEROPS family C01B [Lactobacillus reuteri DSM 20016];>	5	4	2	4	1	5	13.2	51.985	446
>gi|148531846|gb|ABQ83845.1| tRNA/rRNA methyltransferase (SpoU) [Lactobacillus reuteri DSM 20016	4	1	3	2	3	1	16.6	31.804	290
>gi|148531889|gb|ABQ83888.1| prolinase, Serine peptidase, MEROPS family S33 [Lactobacillus reuteri DSM 20016];>	2	1	1	2	1	1	8.6	34.841	303
>gi|148531496|gb|ABQ83495.1| HAD superfamily (subfamily IIIA) phosphatase, TIGR01668 [Lactobacillus reuteri DSM 20016	2	2	2	1	2	2	13.1	20.274	176
>gi|148531368|gb|ABQ83367.1| protein of unknown function DUF806 [Lactobacillus reuteri DSM 20016	4	3	2	1	3	4	26.8	14.543	127
>gi|148531335|gb|ABQ83334.1| SSU ribosomal protein S14P [Lactobacillus reuteri DSM 20016	5	5	2	1	2	3	30.3	10.399	89

>gi|227071316|gb|EEI09625.1| peptidyl-tRNA hydrolase [Lactobacillus reuteri MM2-3];>	5	3	4	2	1	3	32.7	22.79	205
>gi|148530966|gb|ABQ82965.1| ribosome-binding factor A [Lactobacillus reuteri DSM 20016	6	5	3	2	5	3	32.8	13.412	119
>gi|227071224|gb|EEI09538.1| glutamate-cysteine ligase [Lactobacillus reuteri MM2-3	6	5	4	3	4	4	12	60.334	517
>gi|148531529|gb|ABQ83528.1| phosphomethylpyrimidine kinase [Lactobacillus reuteri DSM 20016	3	2	2	1	2	3	14.8	29.317	271
>gi|148532138|gb|ABQ84137.1| asparaginase [Lactobacillus reuteri DSM 20016	3	2	2	2	1	3	10	35.705	329
>gi|183224804|dbj|BAG25321.1| hypothetical protein [Lactobacillus reuteri JCM 1112	2	1	2	1	2	2	21.2	15.336	137
>gi|148530837|gb|ABQ82836.1| Negative regulator of genetic competence [Lactobacillus reuteri DSM 20016	3	2	3	1	2	1	19.7	25.701	223
>gi|148530994|gb|ABQ82993.1| GatB/Yqey domain protein [Lactobacillus reuteri DSM 20016	6	5	4	1	4	1	47.3	16.725	148
>gi|148530371|gb|ABQ82370.1| amino acid ABC transporter membrane protein, PAAT family / amino acid ABC transporter substrate-binding protein, PAAT family [Lactobacillus reuteri DSM 20016];>	3	0	0	0	1	2	8.6	53.65	487
>gi|148532131|gb|ABQ84130.1| glutamine amidotransferase class-I [Lactobacillus reuteri DSM 20016];>	5	3	2	2	2	1	25.1	25.353	223
>gi|148531683|gb|ABQ83682.1| N-6 DNA methylase [Lactobacillus reuteri DSM 20016	6	3	3	3	3	2	13.9	59.038	517
>gi|148530993|gb|ABQ82992.1| SSU ribosomal protein S21P [Lactobacillus reuteri DSM 20016];>	4	3	4	2	4	3	44.4	7.7178	63
>gi|227184649|gb|EEI64720.1| alcohol dehydrogenase class IV [Lactobacillus reuteri CF48-3A];>	3	3	2	0	2	3	11.3	40.915	379
>gi|133930561|gb|ABO43851.1| TetW [Lactobacillus reuteri];>	2	1	1	0	0	0	2.2	71.362	639
>gi|194453331|gb|EDX42228.1| cell envelope-related transcriptional attenuator [Lactobacillus reuteri 100-23];>	5	4	2	4	4	4	20.1	38.126	334
>gi|148530463|gb|ABQ82462.1| ABC transporter related [Lactobacillus reuteri DSM 20016	5	3	2	0	4	1	10.5	39.639	354
>gi|227070164|gb|EEI08539.1| protein of hypothetical function DUF896 [Lactobacillus reuteri MM2-3];>	2	2	2	1	2	2	25.6	10.422	86
>gi|148531087|gb|ABQ83086.1| hypothetical protein Lreu_0823 [Lactobacillus reuteri DSM 20016	2	1	2	1	1	2	24.4	10.395	86
>gi|148531103|gb|ABQ83102.1| hypothetical protein Lreu_0839 [Lactobacillus reuteri DSM 20016	2	1	1	0	1	1	16.5	13.733	121
>gi|227184567|gb|EEI64638.1| possible calcium-transporting ATPase [Lactobacillus reuteri CF48-3A	6	3	4	3	4	2	7.6	100.19	911
>gi|183226408|dbj|BAG26924.1| tRNA (5-methylaminomethyl-2-thiouridylate)- methyltransferase [Lactobacillus fermentum IFO 3956	2	1	1	2	1	0	5	42.445	377
>gi|6707064|gb|AAF25576.1|AF120104_1 mucus binding protein precursor [Lactobacillus reuteri ATCC 53608];>	5	3	0	0	3	0	9.9	357.95	3269
>gi|148530803|gb|ABQ82802.1| protein of unknown function DUF965 [Lactobacillus reuteri DSM 20016];>	3	3	3	1	3	2	37.5	10.353	88
>gi|148531074|gb|ABQ83073.1| putative transcriptional regulator, XRE family [Lactobacillus reuteri DSM 20016	3	1	2	1	1	1	36.7	10.465	90
>gi|148531375|gb|ABQ83374.1| phage Terminase [Lactobacillus reuteri DSM 20016	5	2	0	0	1	5	8.7	72.459	629

>gi|183227399|dbj|BAG27915.1| alcohol dehydrogenase [Lactobacillus fermentum IFO 3956	3	1	2	2	1	2	8.6	37.11	347
>gi|148531370|gb|ABQ83369.1| phage head-tail adaptor, putative [Lactobacillus reuteri DSM 20016];>	5	3	3	3	4	3	48.3	13.324	116
>gi|148531476|gb|ABQ83475.1| phenylalanyl-tRNA synthetase, alpha subunit [Lactobacillus reuteri DSM 20016	5	1	1	3	1	1	16.4	39.026	348
>gi|148531362|gb|ABQ83361.1| Chromosome segregation ATPase-like protein [Lactobacillus reuteri DSM 20016	5	1	4	2	4	4	4.5	149.31	1359
>gi|148530554|gb|ABQ82553.1| transcriptional regulator, GntR family [Lactobacillus reuteri DSM 20016	7	3	7	2	3	3	35.2	26.59	233
>gi|148530960|gb|ABQ82959.1| DNA polymerase III catalytic subunit, PolC type [Lactobacillus reuteri DSM 20016	5	1	1	0	4	4	3.9	163.27	1443
>gi|148531168|gb|ABQ83167.1| two component transcriptional regulator, LytTR family [Lactobacillus reuteri DSM 20016	5	3	4	4	2	2	21.4	27.714	243
>gi|148531521|gb|ABQ83520.1| histidine triad (HIT) protein [Lactobacillus reuteri DSM 20016	6	3	3	0	2	0	43.8	16.333	144
>gi|227070057|gb|EEI08435.1| tetratricopeptide repeat family protein [Lactobacillus reuteri MM2-3];>	3	2	1	2	1	2	10.8	50.211	434
>gi|148531165|gb|ABQ83164.1| Fmu (Sun) domain protein [Lactobacillus reuteri DSM 20016];>	2	0	1	2	0	0	6.4	51.193	455
>gi|227071872|gb|EEI10159.1| DNA-3-methyladenine glycosylase I [Lactobacillus reuteri MM2-3];>	3	1	2	2	2	2	14.8	23.171	196
>gi|227070793|gb|EEI09119.1| NADPH dehydrogenase [Lactobacillus reuteri MM2-3];>	2	1	2	1	1	1	10.9	26.711	230
>gi|148531635|gb|ABQ83634.1| methionine aminopeptidase, type I [Lactobacillus reuteri DSM 20016	6	3	3	3	4	4	16.1	31.665	285
>gi|183225571|dbj|BAG26088.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112	4	3	3	2	2	1	18.1	27.624	249
>gi|148530588|gb|ABQ82587.1| large conductance mechanosensitive channel protein [Lactobacillus reuteri DSM 20016];>	4	1	3	1	1	1	40.7	13.773	123
>gi|148530784|gb|ABQ82783.1| amino acid ABC transporter membrane protein, PAAT family [Lactobacillus reuteri DSM 20016	3	2	1	0	1	0	17.7	24.169	215
>gi|227184608|gb|EEI64679.1| ribonucleotide reductase, alpha subunit [Lactobacillus reuteri CF48-3A	4	3	1	1	0	1	6.1	82.149	734
>gi|148530458|gb|ABQ82457.1| binding-protein-dependent transport systems inner membrane component [Lactobacillus reuteri DSM 20016	1	1	1	0	1	1	8.5	23.399	212
>gi|183224626|dbj|BAG25143.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	3	1	1	1	1	3	10.5	35.43	304
>gi|148532063|gb|ABQ84062.1| thioredoxin [Lactobacillus reuteri DSM 20016];>	5	2	3	1	3	1	42.9	12.064	105
>gi|148531426|gb|ABQ83425.1| ribosome small subunit-dependent GTPase A [Lactobacillus reuteri DSM 20016	3	2	1	2	2	3	12.5	33.257	296
>gi|183226120|dbj|BAG26636.1| arginine deiminase [Lactobacillus fermentum IFO 3956];>	3	2	3	2	3	2	8.4	45.948	407
>gi|148530884|gb|ABQ82883.1| dihydrodipicolinate reductase [Lactobacillus reuteri DSM 20016	3	1	0	2	1	2	18.5	27.932	259

>gi|227071059|gb|EEI09378.1| phosphate regulatory protein [Lactobacillus reuteri MM2-3	3	2	1	1	1	1	13.2	27.712	243
>gi|148530686|gb|ABQ82685.1| two component transcriptional regulator, winged helix family [Lactobacillus reuteri DSM 20016];>	3	1	3	2	2	2	16.7	27.76	239
>gi|148531492|gb|ABQ83491.1| metal dependent phosphohydrolase [Lactobacillus reuteri DSM 20016];>	2	2	1	1	0	1	9.8	23.131	204
>gi|148531710|gb|ABQ83709.1| protein translocase subunit secY/sec61 alpha [Lactobacillus reuteri DSM 20016	5	1	1	2	3	4	12.6	47.772	438
>gi|148530858|gb|ABQ82857.1| UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase [Lactobacillus reuteri DSM 20016];>	5	1	2	1	4	2	18.6	40.606	370
>gi|148531274|gb|ABQ83273.1| Hydroxyethylthiazole kinase [Lactobacillus reuteri DSM 20016];>	2	0	1	1	1	0	11.2	29.028	269
>gi|148531574|gb|ABQ83573.1| ribonucleoside-triphosphate reductase class III activase subunit [Lactobacillus reuteri DSM 20016];>	3	2	2	2	3	3	15.1	22.234	192
>gi|183224837|dbj|BAG25354.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112	6	3	3	3	3	3	17	48.212	430
>gi|148531338|gb|ABQ83337.1| demethylmenaquinone methyltransferase [Lactobacillus reuteri DSM 20016];>	3	0	3	0	0	0	14.2	26.083	233
>gi|148531151|gb|ABQ83150.1| 1,4-Dihydroxy-2-naphthoate synthase [Lactobacillus reuteri DSM 20016	4	3	2	3	3	2	17.9	30.019	273
>gi|227186158|gb|EEI66229.1| ribonucleoside-triphosphate reductase class III activase subunit [Lactobacillus reuteri CF48-3A	3	2	2	3	2	2	15.6	22.224	192
>gi|227070128|gb|EEI08504.1| GTP diphosphokinase [Lactobacillus reuteri MM2-3	4	1	0	2	3	2	5.3	85.34	751
>gi|148531503|gb|ABQ83502.1| dephospho-CoA kinase [Lactobacillus reuteri DSM 20016]	4	2	3	1	2	3	24.1	21.94	199
>gi|148531891|gb|ABQ83890.1| Ferritin, Dps family protein [Lactobacillus reuteri DSM 20016	4	4	2	2	3	4	31	18.223	155
>gi|148530416|gb|ABQ82415.1| formyltetrahydrofolate-dependent phosphoribosylglycinamide formyltransferase [Lactobacillus reuteri DSM 20016	4	2	3	0	2	0	27.9	21.163	190
>gi|148530943|gb|ABQ82942.1| SOS-response transcriptional repressor, LexA [Lactobacillus reuteri DSM 20016	3	3	2	1	2	2	18.3	23.184	208
>gi|148531520|gb|ABQ83519.1| ABC transporter related [Lactobacillus reuteri DSM 20016];>	5	2	4	3	2	3	26.5	27.345	245
>gi|148531166|gb|ABQ83165.1| band 7 protein [Lactobacillus reuteri DSM 20016	5	3	3	3	2	4	17.7	31.856	288
>gi|148532015|gb|ABQ84014.1| transcriptional regulator, LacI family [Lactobacillus reuteri DSM 20016	3	2	0	3	3	3	9.1	36.929	330
>gi|148531145|gb|ABQ83144.1| 6,7-dimethyl-8-ribityllumazine synthase [Lactobacillus reuteri DSM 20016	2	1	1	1	1	2	22.4	16.637	152
>gi|148531193|gb|ABQ83192.1| carbamoyl-phosphate synthase small subunit [Lactobacillus reuteri DSM 20016];>	3	2	3	3	3	3	11.6	39.871	361
>gi|148530977|gb|ABQ82976.1| hypothetical protein Lreu_0711 [Lactobacillus reuteri DSM 20016];>	1	1	1	0	1	1	8.7	16.649	149

>gi|148531429|gb|ABQ83428.1| sun protein [Lactobacillus reuteri DSM 20016	4	2	3	2	2	2	11.6	50.377	449
>gi|148531957|gb|ABQ83956.1| precorrin-6Y C5,15-methyltransferase (decarboxylating), CbiT subunit [Lactobacillus reuteri DSM 20016];>	4	3	3	1	3	3	20.7	20.387	184
>gi|183225646|dbj|BAG26163.1| hypothetical protein [Lactobacillus reuteri JCM 1112];>	4	2	3	2	2	1	18.5	19.84	178
>gi|148530983|gb|ABQ82982.1| [LSU ribosomal protein L11P]-lysine N-methyltransferase [Lactobacillus reuteri DSM 20016	5	3	3	3	2	2	17.6	34.966	319
>gi|148531099|gb|ABQ83098.1| phage putative head morphogenesis protein, SPP1 gp7 family [Lactobacillus reuteri DSM 20016];>	2	1	2	1	1	2	7.6	35.825	316
>gi|227071855|gb|EEI10143.1| penicillin-binding protein [Lactobacillus reuteri MM2-3];>	3	1	2	2	2	2	5.3	76.108	699
>gi|183224027|dbj|BAG24544.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	6	4	1	1	4	3	29.4	20.228	180
>gi|227071501|gb|EEI09801.1| LL-diaminopimelate aminotransferase [Lactobacillus reuteri MM2-3	4	2	1	2	2	4	10.6	43.448	395
>gi|336448856|gb|AEI57471.1| sensor histidine kinase [Lactobacillus reuteri SD2112	5	2	0	1	0	2	17.9	44.299	390
>gi|227071080|gb|EEI09398.1| metallo-beta-lactamase [Lactobacillus reuteri MM2-3];>	4	2	2	1	1	2	7.3	70.828	628
>gi|148531079|gb|ABQ83078.1| hypothetical protein Lreu_0815 [Lactobacillus reuteri DSM 20016	4	3	3	2	3	4	24.4	14.305	123
>gi|336448233|gb|AEI56848.1| ribosomal large subunit pseudouridine synthase D [Lactobacillus reuteri SD2112	3	0	3	1	0	0	11.1	37.027	325
>gi|148531769|gb|ABQ83768.1| DJ-1 family protein [Lactobacillus reuteri DSM 20016	2	1	2	0	1	1	17.9	20.995	190
>gi|148531874|gb|ABQ83873.1| transcriptional regulator, TetR family [Lactobacillus reuteri DSM 20016];>	2	2	1	1	1	1	15.2	20.576	178
>gi|183224587|dbj|BAG25104.1| exodeoxyribonuclease V alpha subunit [Lactobacillus reuteri JCM 1112	6	3	2	0	5	2	7.6	91.732	828
>gi|148531084|gb|ABQ83083.1| hypothetical protein Lreu_0820 [Lactobacillus reuteri DSM 20016];>	2	0	2	0	1	2	27.9	9.6179	86
>gi|148531409|gb|ABQ83408.1| tRNA (Guanine37-N(1)-) methyltransferase [Lactobacillus reuteri DSM 20016];>	2	2	1	1	1	1	6.7	28.476	252
>gi|227070907|gb|EEI09230.1| possible Bis(5-nucleosyl)-tetraphosphatase (asymmetrical) [Lactobacillus reuteri MM2-3];>	3	0	3	1	2	1	24.4	19.718	168
>gi|148530818|gb|ABQ82817.1| Xaa-Pro aminopeptidase, Metallo peptidase, MEROPS family M24B [Lactobacillus reuteri DSM 20016];>	6	1	4	2	2	0	24.9	40.615	369
>gi|148530572|gb|ABQ82571.1| DNA replication and repair protein RadA [Lactobacillus reuteri DSM 20016	3	2	1	1	2	2	9.4	49.96	457
>gi|227071188|gb|EEI09504.1| transcriptional regulator CtsR [Lactobacillus reuteri MM2-3];>	3	3	1	1	1	1	17.4	19.037	167
>gi|148530278|gb|ABQ82277.1| chromosomal replication initiator protein DnaA [Lactobacillus reuteri DSM 20016	3	1	1	1	1	3	8.4	49.942	440
>gi|148530753|gb|ABQ82752.1| aldo/keto reductase [Lactobacillus reuteri DSM 20016];>	4	0	0	0	2	3	18.2	32.082	280

>gi|148531032|gb|ABQ83031.1| Polynucleotide adenylyltransferase region [Lactobacillus reuteri DSM 20016	2	2	1	2	1	2	6.7	45.099	403
>gi|227185796|gb|EEI65867.1| rod shape-determining protein MreC [Lactobacillus reuteri CF48-3A];>	4	2	0	2	1	3	15.5	31.961	291
>gi|148531235|gb|ABQ83234.1| hypothetical protein Lreu_0972 [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	10	13.528	120
>gi|148531292|gb|ABQ83291.1| SMC domain protein [Lactobacillus reuteri DSM 20016	3	2	1	1	1	1	3.3	118.56	1033
>gi|148531095|gb|ABQ83094.1| ParB domain protein nuclease [Lactobacillus reuteri DSM 20016];>	4	1	2	1	2	3	13.9	29.81	259
>gi|148531518|gb|ABQ83517.1| tRNA (guanine-N(7)-)-methyltransferase [Lactobacillus reuteri DSM 20016];>	2	1	0	1	2	2	8.5	24.675	213
>gi|148531324|gb|ABQ83323.1| 4-oxalocrotonate tautomerase [Lactobacillus reuteri DSM 20016	1	1	1	1	1	1	18	6.8159	61
>gi|148531516|gb|ABQ83515.1| LPXTG-motif cell wall anchor domain [Lactobacillus reuteri DSM 20016	2	0	1	2	0	2	18.5	81.784	745
>gi|148530737|gb|ABQ82736.1| ATP synthase F1 subcomplex epsilon subunit [Lactobacillus reuteri DSM 20016	2	1	1	0	1	1	17.5	15.452	143
>gi|148531100|gb|ABQ83099.1| hypothetical protein Lreu_0836 [Lactobacillus reuteri DSM 20016	3	2	2	0	3	3	42	9.5449	81
>gi|148530907|gb|ABQ82906.1| Phosphopantetheine adenylyltransferase [Lactobacillus reuteri DSM 20016	3	3	2	2	2	3	15	19.361	173
>gi|148531640|gb|ABQ83639.1| DEAD/DEAH box helicase domain protein [Lactobacillus reuteri DSM 20016	3	3	1	0	2	1	8.1	48.772	433
>gi|148530656|gb|ABQ82655.1| uncharacterized P-loop ATPase protein UPF0042 [Lactobacillus reuteri DSM 20016	3	1	2	0	2	1	12.1	33.671	297
>gi|148531339|gb|ABQ83338.1| Methionine synthase, vitamin-B12 independent [Lactobacillus reuteri DSM 20016	4	1	2	2	1	3	11.6	42.705	379
>gi|148532077|gb|ABQ84076.1| Phosphoglycerate mutase [Lactobacillus reuteri DSM 20016	2	2	0	0	1	1	10.6	24.661	217
>gi|227185170|gb|EEI65241.1| amidohydrolase [Lactobacillus reuteri CF48-3A	2	1	1	1	1	0	5.7	42.662	383
>gi|148531787|gb|ABQ83786.1| penicillin-binding protein, transpeptidase [Lactobacillus reuteri DSM 20016	2	1	2	2	0	1	6.8	29.751	265
>gi|148530613|gb|ABQ82612.1| O-sialoglycoprotein endopeptidase [Lactobacillus reuteri DSM 20016	3	3	2	1	2	3	11.1	37.453	343
>gi|148530631|gb|ABQ82630.1| protein of unknown function UPF0029 [Lactobacillus reuteri DSM 20016	3	2	1	0	2	2	19.5	23.548	210
>gi|148530910|gb|ABQ82909.1| ComE operon protein 2 [Lactobacillus reuteri DSM 20016];>	3	2	2	1	3	1	18	18.085	161
>gi|183224312|dbj|BAG24829.1| putative glutaredoxin [Lactobacillus reuteri JCM 1112	2	1	1	0	1	2	40	8.662	75
>gi|148530511|gb|ABQ82510.1| protein tyrosine phosphatase [Lactobacillus reuteri DSM 20016[	3	3	1	1	0	1	33.1	17.398	154
>gi|148532003|gb|ABQ84002.1| 3-demethylubiquinone-9 3-methyltransferase [Lactobacillus reuteri DSM 20016];>	2	1	2	1	1	1	15.6	15.941	141

>gi|148531566|gb|ABQ83565.1| aldo/keto reductase [Lactobacillus reuteri DSM 20016	2	1	1	2	1	1	7.1	30.851	282
>gi|148531980|gb|ABQ83979.1| Propanediol utilization protein [Lactobacillus reuteri DSM 20016	5	2	3	2	5	3	25.2	23.962	214
>gi|148531174|gb|ABQ83173.1| protein of unknown function DUF1440 [Lactobacillus reuteri DSM 20016	3	3	3	2	3	3	20.5	18.375	166
>gi|183226397|dbj|BAG26913.1| cell division protein FtsZ [Lactobacillus fermentum IFO 3956	3	2	1	3	0	1	6.1	45.111	429
>gi|336448991|gb|AEI57606.1| zinc/iron ABC superfamily ATP binding cassette transporter, ABC protein [Lactobacillus reuteri SD2112	3	3	2	1	2	2	13.2	27.995	250
>gi|183226087|dbj|BAG26603.1| transcription antitermination protein [Lactobacillus fermentum IFO 3956	2	1	2	1	2	1	21	20.377	181
>gi|183224767|dbj|BAG25284.1| hypothetical protein [Lactobacillus reuteri JCM 1112	1	0	0	1	1	1	13.7	20.916	190
>gi|148530495|gb|ABQ82494.1| metal dependent phosphohydrolase [Lactobacillus reuteri DSM 20016];>	4	1	3	2	2	0	9.7	53.542	455
>gi|148530334|gb|ABQ82333.1| two component transcriptional regulator, winged helix family [Lactobacillus reuteri DSM 20016];>	2	1	1	1	1	1	11.3	24.908	221
>gi|148531751|gb|ABQ83750.1| cold-shock DNA-binding protein family [Lactobacillus reuteri DSM 20016];>	2	2	1	1	1	1	34.8	7.3269	66
>gi|148530599|gb|ABQ82598.1| DNA replication and repair protein RecR [Lactobacillus reuteri DSM 20016	4	1	3	1	1	1	15.5	22.089	200
>gi|148530841|gb|ABQ82840.1| RelA/SpoT domain protein [Lactobacillus reuteri DSM 20016];>	4	3	2	1	1	2	17.5	25.826	217
>gi|227070166|gb|EEI08541.1| 1-acyl-sn-glycerol-3-phosphate acyltransferase [Lactobacillus reuteri MM2-3	2	1	2	1	1	1	9.5	28.013	243
>gi|148531758|gb|ABQ83757.1| amino acid ABC transporter substrate-binding protein, PAAT family [Lactobacillus reuteri DSM 20016];>	3	2	2	3	2	2	12.3	30.556	276
>gi|183224605|dbj|BAG25122.1| hypothetical protein [Lactobacillus reuteri JCM 1112];>	1	1	0	0	1	1	9.3	18.717	161
>gi|227071315|gb|EEI09624.1| cystathionine beta-synthase (CBS) domain protein [Lactobacillus reuteri MM2-3];>	3	3	1	1	2	1	15.8	24.393	215
>gi|148530804|gb|ABQ82803.1| Holliday junction resolvase YqgF [Lactobacillus reuteri DSM 20016];>	3	2	2	1	2	0	23.1	16.525	147
>gi|148530575|gb|ABQ82574.1| ribonuclease III [Lactobacillus reuteri DSM 20016	2	1	1	1	2	1	16.8	15.515	137
>gi|183224778|dbj|BAG25295.1| hypothetical protein [Lactobacillus reuteri JCM 1112];>	6	0	3	0	1	4	44.5	15.26	128
>gi|148531760|gb|ABQ83759.1| amino acid ABC transporter membrane protein 2, PAAT family [Lactobacillus reuteri DSM 20016];>	2	1	1	1	1	2	13.8	24.332	217
>gi|148531927|gb|ABQ83926.1| transcriptional regulator, GntR family [Lactobacillus reuteri DSM 20016	2	2	1	1	2	2	9.5	27.747	243
>gi|148530515|gb|ABQ82514.1| DEAD/DEAH box helicase domain protein [Lactobacillus reuteri DSM 20016];>	4	3	1	0	1	3	8.4	56.522	498
>gi|148530636|gb|ABQ82635.1| bacterial peptide chain release factor 2 (bRF-2) [Lactobacillus reuteri DSM 20016];>	2	2	1	2	0	1	8.4	37.463	332

>gi|227185380|gb|EEI65451.1| site-specific DNA-methyltransferase (adenine-specific) [Lactobacillus reuteri CF48-3A];>	2	1	1	1	2	1	3.3	57.986	512
>gi|148530461|gb|ABQ82460.1| acetylornithine deacetylase [Lactobacillus reuteri DSM 20016];>	2	1	1	0	0	0	6	41.681	381
>gi|148531774|gb|ABQ83773.1| transcriptional regulator, LysR family [Lactobacillus reuteri DSM 20016];>	4	1	3	1	2	0	11.9	33.598	295
>gi|148531428|gb|ABQ83427.1| Protein phosphatase 2C-like protein [Lactobacillus reuteri DSM 20016];>	3	2	3	1	2	2	12.2	26.923	246
>gi|183224761|dbj|BAG25278.1| hypothetical protein [Lactobacillus reuteri JCM 1112];>	2	2	1	1	1	2	10.9	21.003	184
>gi|148530813|gb|ABQ82812.1| CBS domain containing protein [Lactobacillus reuteri DSM 20016];>	3	3	2	1	2	2	17	16.967	147
>gi|227070532|gb|EEI08865.1| orotidine-5-phosphate decarboxylase [Lactobacillus reuteri MM2-3];>	2	1	2	1	1	1	8.5	26.968	248
>gi|148530848|gb|ABQ82847.1| RNA methyltransferase, TrmH family, group 2 [Lactobacillus reuteri DSM 20016];>	3	1	3	0	1	1	20.7	19.557	169
>gi|194453849|gb|EDX42746.1| cell envelope-related transcriptional attenuator [Lactobacillus reuteri 100-23	2	1	1	1	1	1	5.3	55.148	489
>gi|119390607|pdb|2NT8|A Chain A, Atp Bound At The Active Site Of A Pduo Type Atp:co(I) Rrinoid Adenosyltransferase From Lactobacillus Reuteri;>	2	1	1	0	1	2	9.9	25.598	223
>gi|148531071|gb|ABQ83070.1| transcriptional regulator, XRE family [Lactobacillus reuteri DSM 20016];>	3	1	1	0	3	3	37.8	9.7862	82
>gi|148530790|gb|ABQ82789.1| CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase [Lactobacillus reuteri DSM 20016];>	2	1	1	0	1	0	11.8	21.79	195
>gi|227070045|gb|EEI08423.1| possible reductase [Lactobacillus reuteri MM2-3	2	1	1	1	1	2	20.8	14.924	125
>gi|148531512|gb|ABQ83511.1| L-glutaminase [Lactobacillus reuteri DSM 20016];>	2	1	1	2	1	1	6.9	33.392	306
>gi|148532176|gb|ABQ84175.1| ribonuclease P protein component [Lactobacillus reuteri DSM 20016	2	2	2	0	2	1	18.8	13.651	117
>gi|148531992|gb|ABQ83991.1| major intrinsic protein [Lactobacillus reuteri DSM 20016	1	1	1	1	1	1	4.7	25.304	235
>gi|183225835|dbj|BAG26351.1| two-component response regulator [Lactobacillus fermentum IFO 3956	3	1	1	2	1	3	9.8	26.747	235
>gi|148530393|gb|ABQ82392.1| branched-chain amino acid transport [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	8.8	12.954	114
>gi|300379508|gb|ADK08386.1| alkyl hydroperoxide reductase F subunit [Lactobacillus reuteri]	2	1	1	0	1	0	4.9	55.347	512
>gi|148531175|gb|ABQ83174.1| isopentenyl-diphosphate delta-isomerase, type 2 [Lactobacillus reuteri DSM 20016];>	3	2	1	2	3	2	10.3	38.769	348
>gi|148531449|gb|ABQ83448.1| protein of unknown function DUF464 [Lactobacillus reuteri DSM 20016];>	2	1	2	0	1	1	32.7	11.705	107
>gi|227070762|gb|EEI09089.1| NRAMP family manganese (Mn2+) transporter [Lactobacillus reuteri MM2-3	3	1	1	1	3	2	6.4	59.447	546

>gi|148530836|gb|ABQ82835.1| arsenate reductase-like protein [Lactobacillus reuteri DSM 20016	4	2	3	0	3	3	34.6	15.539	133
>gi|183224289|dbj|BAG24806.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112	2	1	1	1	2	2	6.5	43.741	383
>gi|148530487|gb|ABQ82486.1| periplasmic solute binding protein [Lactobacillus reuteri DSM 20016];>	2	1	2	1	0	0	7.3	34.078	303
>gi|148530499|gb|ABQ82498.1| Domain of unknown function DUF1934 [Lactobacillus reuteri DSM 20016];>	3	1	1	1	2	0	22.7	15.33	132
>gi|227070084|gb|EEI08461.1| metal-dependent hydrolase [Lactobacillus reuteri MM2-3	2	1	1	1	1	2	11.2	18.134	160
>gi|337729366|emb|CCC04496.1| conserved hypothetical protein [Lactobacillus reuteri ATCC 53608]	2	2	0	0	0	0	4.7	44.72	402
>gi|227071130|gb|EEI09446.1| TrmA family tRNA (uracil-5-)-methyltransferase [Lactobacillus reuteri MM2-3	3	1	2	0	1	1	7.9	53.815	480
>gi|148530400|gb|ABQ82399.1| orotate phosphoribosyltransferase [Lactobacillus reuteri DSM 20016];>	2	2	1	0	1	1	8	23.434	213
>gi|227184596|gb|EEI64667.1| transcriptional regulator [Lactobacillus reuteri CF48-3A	2	1	1	1	2	1	9.1	27.898	243
>gi|148531537|gb|ABQ83536.1| 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase [Lactobacillus reuteri DSM 20016	3	1	2	2	1	1	14.1	19.815	170
>gi|183225948|dbj|BAG26464.1| amidophosphoribosyltransferase [Lactobacillus fermentum IFO 3956	3	1	2	1	1	1	6.2	53.518	487
>gi|148530649|gb|ABQ82648.1| thioesterase superfamily protein [Lactobacillus reuteri DSM 20016	2	2	2	2	1	1	13.7	19.242	168
>gi|148531190|gb|ABQ83189.1| hypothetical protein Lreu_0927 [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	8.1	15.956	135
>gi|148531700|gb|ABQ83699.1| cobalt transport protein [Lactobacillus reuteri DSM 20016	3	1	2	0	1	1	12.4	30.347	267
>gi|183226825|dbj|BAG27341.1| 1-deoxy-D-xylulose-5-phosphate synthase [Lactobacillus fermentum IFO 3956	2	2	0	1	0	1	5.4	65.874	592
>gi|183225944|dbj|BAG26460.1| phosphoribosylaminoimidazole-succinocarboxamide synthase [Lactobacillus fermentum IFO 3956	1	1	1	1	1	0	5.4	27.483	240
>gi|148532142|gb|ABQ84141.1| Hydratase/decarboxylase [Lactobacillus reuteri DSM 20016	2	2	2	2	2	2	6.3	29.863	270
>gi|148530456|gb|ABQ82455.1| binding-protein-dependent transport systems inner membrane component [Lactobacillus reuteri DSM 20016	1	1	1	0	1	1	5	24.06	221
>gi|148530651|gb|ABQ82650.1| hydrolase of HD superfamily-like protein [Lactobacillus reuteri DSM 20016];>gi|148543617|ref|YP_001270987.1| HD superfamily hydrolase-like protein [Lactobacillus reuteri DSM 20016];>	4	0	2	1	2	0	18.9	24.304	212
>gi|148532000|gb|ABQ83999.1| ABC-type multidrug transport system ATPase component-like protein [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	3.5	31.737	287
>gi|148531803|gb|ABQ83802.1| 67 kDa myosin-cross-reactive antigen family protein [Lactobacillus reuteri DSM 20016];>	4	2	3	1	3	3	8.1	67.715	590
>gi|227184772|gb|EEI64843.1| protein-tyrosine-phosphatase [Lactobacillus reuteri CF48-3A];>	2	1	1	0	1	2	9.8	30.178	265

>gi|148531939|gb|ABQ83938.1| adenosylcobinamide kinase [Lactobacillus reuteri DSM 20016];>	2	2	0	0	0	1	12.2	22.192	196
>gi|148530976|gb|ABQ82975.1| hypothetical protein Lreu_0710 [Lactobacillus reuteri DSM 20016];>	2	0	1	1	0	1	8.2	28.409	244
>gi|194453872|gb|EDX42769.1| hypothetical protein Lreu23DRAFT_4288 [Lactobacillus reuteri 100-23	1	1	0	1	1	1	1.6	69.515	629
>gi|148531699|gb|ABQ83698.1| tRNA pseudouridine synthase A [Lactobacillus reuteri DSM 20016];>	4	1	2	0	1	1	15.2	29.138	256
>gi|148531457|gb|ABQ83456.1| NLPA lipoprotein [Lactobacillus reuteri DSM 20016];>	1	1	0	0	0	1	4.2	31.575	283
>gi|148531446|gb|ABQ83445.1| peptidase M24 [Lactobacillus reuteri DSM 20016	2	1	1	1	2	1	6.1	39.767	358
>gi|148531411|gb|ABQ83410.1| hypothetical protein Lreu_1153 [Lactobacillus reuteri DSM 20016];>	3	1	2	2	2	2	34.9	9.7331	86
>gi|148531080|gb|ABQ83079.1| hypothetical protein Lreu_0816 [Lactobacillus reuteri DSM 20016	1	1	1	0	0	0	16.3	10.515	92
>gi|148530356|gb|ABQ82355.1| Protein of unknown function DUF1975 [Lactobacillus reuteri DSM 20016];>	2	1	0	1	0	1	4.5	59.726	513
>gi|148531785|gb|ABQ83784.1| hypothetical protein Lreu_1539 [Lactobacillus reuteri DSM 20016];>	2	1	1	1	1	1	15.1	16.827	146
>gi|148530965|gb|ABQ82964.1| bacterial translation initiation factor 2 (bIF-2) [Lactobacillus reuteri DSM 20016];>	2	1	0	0	1	2	3.1	83.537	752
>gi|148531636|gb|ABQ83635.1| flavodoxin [Lactobacillus reuteri DSM 20016];>	2	2	2	1	1	2	12.5	16.373	152
>gi|148531996|gb|ABQ83995.1| B3/4 domain protein [Lactobacillus reuteri DSM 20016	3	1	2	1	2	2	12.4	27.29	241
>gi|148532096|gb|ABQ84095.1| ABC transporter related [Lactobacillus reuteri DSM 20016	2	1	2	0	0	0	11.4	25.064	229
>gi|148530932|gb|ABQ82931.1| phage head-tail adaptor, putative [Lactobacillus reuteri DSM 20016	1	1	1	1	1	1	10.1	13.175	119
>gi|148531401|gb|ABQ83400.1| transcriptional regulator, XRE family [Lactobacillus reuteri DSM 20016	1	1	1	1	1	1	14.9	7.715	67
>gi|148531835|gb|ABQ83834.1| cytochrome b5 [Lactobacillus reuteri DSM 20016	1	1	0	0	1	0	21.2	8.6688	80
>gi|148531212|gb|ABQ83211.1| protein of unknown function DUF21 [Lactobacillus reuteri DSM 20016	1	1	1	1	1	1	2.4	52.119	453
>gi|148531490|gb|ABQ83489.1| Methyltransferase type 12 [Lactobacillus reuteri DSM 20016];>	2	2	1	2	1	1	7.7	28.291	246
>gi|183225246|dbj|BAG25763.1| hypothetical protein [Lactobacillus reuteri JCM 1112	2	1	1	1	2	1	6.4	33.488	297
>gi|148531620|gb|ABQ83619.1| glycosyl transferase, group 1 [Lactobacillus reuteri DSM 20016	2	1	1	1	0	0	7.4	40.131	349
>gi|148531272|gb|ABQ83271.1| hypothetical protein Lreu_1011 [Lactobacillus reuteri DSM 20016	3	1	0	0	1	1	5.2	72.576	629
>gi|227070859|gb|EEI09184.1| nicotinamide mononucleotide transporter [Lactobacillus reuteri MM2-3];>	2	2	1	2	2	2	6.2	30.903	276
>gi|148530683|gb|ABQ82682.1| transcriptional regulator, MarR family [Lactobacillus reuteri DSM 20016];>	2	2	1	0	0	0	12.6	17.828	151
>gi|194453686|gb|EDX42583.1| ATP-cone domain protein [Lactobacillus reuteri 100-23];>	2	1	2	1	1	2	15.5	18.122	155

>gi|183227385|dbj|BAG27901.1| malolactic regulator [Lactobacillus fermentum IFO 3956	2	2	1	0	1	0	8.4	33.124	297
>gi|148531137|gb|ABQ83136.1| restriction modification system DNA specificity domain [Lactobacillus reuteri DSM 20016];>	2	0	0	1	1	1	4.8	42.885	375
>gi|337729524|emb|CCC04655.1| conserved hypothetical protein [Lactobacillus reuteri ATCC 53608]	3	2	0	0	0	1	3.8	94.907	821
>gi|148531374|gb|ABQ83373.1| phage portal protein, HK97 family [Lactobacillus reuteri DSM 20016];>	2	1	1	1	2	1	6.8	43.309	396
>gi|148531707|gb|ABQ83706.1| LSU ribosomal protein L36P [Lactobacillus reuteri DSM 20016];>	1	0	0	1	0	1	25.6	4.5345	39
>gi|227185263|gb|EEI65334.1| aldo/keto reductase [Lactobacillus reuteri CF48-3A	2	1	2	0	2	0	6.4	35.985	326
>gi|194454517|gb|EDX43414.1| Protein of unknown function DUF1975 [Lactobacillus reuteri 100-23];>	2	0	0	1	0	2	6.2	59.739	513
>gi|148530372|gb|ABQ82371.1| amino acid ABC transporter ATP-binding protein, PAAT family [Lactobacillus reuteri DSM 20016	3	3	2	1	2	1	15	27.521	247
>gi|227071659|gb|EEI09952.1| conserved hypothetical protein [Lactobacillus reuteri MM2-3	2	1	1	0	0	2	11	19.844	173
>gi|337727943|emb|CCC03032.1| adenine phosphoribosyltransferase [Lactobacillus reuteri ATCC 53608];>	3	3	0	0	0	0	19.2	18.664	172
>gi|148531452|gb|ABQ83451.1| hypothetical protein Lreu_1194 [Lactobacillus reuteri DSM 20016];>	2	0	1	0	2	0	8.3	19.53	168
>gi|148531179|gb|ABQ83178.1| DnaQ family exonuclease/DinG family helicase, putative [Lactobacillus reuteri DSM 20016];>	3	0	0	0	3	0	4	108.2	954
>gi|227070539|gb|EEI08872.1| acetolactate decarboxylase [Lactobacillus reuteri MM2-3];>	3	1	1	2	2	2	9.3	27.238	246
>gi|148531565|gb|ABQ83564.1| heat shock protein Hsp20 [Lactobacillus reuteri DSM 20016];>	2	0	2	0	2	0	16.1	16.747	143
>gi|148531789|gb|ABQ83788.1| major facilitator superfamily MFS_1 [Lactobacillus reuteri DSM 20016	1	1	1	1	1	1	2.2	43.836	405
>gi|183227479|dbj|BAG27995.1| transposase [Lactobacillus fermentum IFO 3956];>]	2	1	0	0	1	0	5.9	29.427	256
>gi|148531804|gb|ABQ83803.1| nitroreductase [Lactobacillus reuteri DSM 20016	2	1	1	0	0	2	8.7	28.863	253
>gi|183224791|dbj|BAG25308.1| hypothetical phage protein [Lactobacillus reuteri JCM 1112];>	1	1	1	0	1	1	15.9	12.096	107
>gi|148531759|gb|ABQ83758.1| amino acid ABC transporter membrane protein 1, PAAT family [Lactobacillus reuteri DSM 20016];>	2	2	2	1	1	2	11.3	23.543	212
>gi|148531353|gb|ABQ83352.1| hypothetical protein Lreu_1095 [Lactobacillus reuteri DSM 20016];>	1	1	0	0	1	1	13	13.383	123
>gi|227071585|gb|EEI09882.1| uracil-DNA glycosylase [Lactobacillus reuteri MM2-3	1	1	1	1	1	1	6.9	27.942	245
>gi|148530839|gb|ABQ82838.1| dipeptidase A, Cysteine peptidase, MEROPS family C69 [Lactobacillus reuteri DSM 20016];>	2	2	1	2	2	2	4.6	51.591	458
>gi|148531952|gb|ABQ83951.1| uroporphyrinogen-III C-methyltransferase [Lactobacillus reuteri DSM 20016];>	2	0	0	1	1	0	6	51.349	464
>gi|148530411|gb|ABQ82410.1| phosphoribosylformylglycinamidine synthase, purS [Lactobacillus	2	0	1	0	1	0	22	9.3323	82

reuteri DSM 20016									
>gi|148530320|gb|ABQ82319.1| D-isomer specific 2-hydroxyacid dehydrogenase, NAD-binding protein [Lactobacillus reuteri DSM 20016	1	1	1	0	0	1	4.2	36.471	331
>gi|183225293|dbj|BAG25810.1| galactosyltransferase [Lactobacillus reuteri JCM 1112 [	4	2	1	3	2	2	16.1	25.338	218
>gi|148531784|gb|ABQ83783.1| aldose 1-epimerase [Lactobacillus reuteri DSM 20016	1	1	0	1	0	1	2.9	37.927	345
>gi|148530754|gb|ABQ82753.1| transcriptional regulator, GntR family [Lactobacillus reuteri DSM 20016	2	1	1	0	1	2	4.9	41.571	365
>gi|148532052|gb|ABQ84051.1| Mannosyl-glycoprotein endo-beta-N-acetylglucosamidase [Lactobacillus reuteri DSM 20016	2	1	1	1	2	1	14.7	22.38	197
>gi|148530678|gb|ABQ82677.1| YbbR family protein [Lactobacillus reuteri DSM 20016 J	3	0	0	2	1	0	13.4	32.748	299
>gi|337728183|emb|CCC03274.1| hypothetical protein LRATCC53608_0523 [Lactobacillus reuteri ATCC 53608]	3	2	1	0	2	1	13.4	22.62	209
>gi|227185256|gb|EEI65327.1| peptidase M10A and M12B, matrixin and adamalysin [Lactobacillus reuteri CF48-3A];>	2	1	2	1	2	1	7.8	26.997	244
>gi|148530694|gb|ABQ82693.1| response regulator receiver protein [Lactobacillus reuteri DSM 20016];>	1	1	1	0	0	0	5.4	31.663	279
>gi|148531048|gb|ABQ83047.1| DNA topoisomerase IV subunit A [Lactobacillus reuteri DSM 20016];>	4	1	1	2	4	1	6.1	92.718	821
>gi|49242758|emb|CAG41483.1| transport system membrane protein [Staphylococcus aureus subsp. aureus MRSA252];>	2	1	1	2	1	1	9.2	26.496	239
>gi|183224486|dbj|BAG25003.1| cell division regulatory protein [Lactobacillus reuteri JCM 1112];>	3	1	0	2	0	1	6.3	66.585	570
>gi|148532145|gb|ABQ84144.1| transcriptional regulator [Lactobacillus reuteri DSM 20016];>	2	2	0	1	1	0	6.9	32.842	289
>gi|148531083|gb|ABQ83082.1| hypothetical protein Lreu_0819 [Lactobacillus reuteri DSM 20016	1	1	0	0	1	1	17.2	7.5354	64
>gi|148531988|gb|ABQ83987.1| microcompartments protein [Lactobacillus reuteri DSM 20016	3	1	0	0	0	2	18.5	24.945	238
>gi|112944062|gb|ABI26332.1| phosphoglycerate mutase [Lactobacillus reuteri	2	0	1	1	1	1	7.8	24.692	218
>gi|194454116|gb|EDX43013.1| hypothetical protein Lreu23DRAFT_4532 [Lactobacillus reuteri 100-23	1	0	0	1	1	0	19.5	4.7396	41
>gi|227070651|gb|EEI08981.1| rhodanese domain protein [Lactobacillus reuteri MM2-3];>	2	1	2	1	2	1	8.8	29.725	261
>gi|148531180|gb|ABQ83179.1| Uncharacterized protein [Lactobacillus reuteri DSM 20016];>	2	1	0	0	1	2	12.2	19.912	172
>gi|227070790|gb|EEI09116.1| possible DNA-3-methyladenine glycosylase II [Lactobacillus reuteri MM2-3];>	2	1	1	0	1	1	9	24.938	222
>gi|337728658|emb|CCC03769.1| putative lipoprotein [Lactobacillus reuteri ATCC 53608	2	2	0	0	0	0	10.2	24.095	216
>gi|148530603|gb|ABQ82602.1| DNA polymerase III, delta prime subunit [Lactobacillus reuteri DSM 20016	1	1	1	0	1	0	4.5	37.892	337
>gi|148530535|gb|ABQ82534.1| Septum formation initiator [Lactobacillus reuteri DSM 20016];>	1	0	1	1	0	1	8.5	14.01	118
>gi|148530915|gb|ABQ82914.1| beta-lactamase domain protein [Lactobacillus reuteri DSM	2	1	1	1	1	2	3.5	66.72	593

20016];>									
>gi|148532105|gb|ABQ84104.1| ketopantoate reductase [Lactobacillus reuteri DSM 20016];>	2	1	0	0	1	2	5.3	36.285	318
>gi|227184639|gb|EEI64710.1| propanediol dehydratase, large subunit [Lactobacillus reuteri CF48-3A];>	2	1	2	1	1	2	2.3	65.4	615
>gi|148531951|gb|ABQ83950.1| anaerobic cobaltochelatase [Lactobacillus reuteri DSM 20016];>	3	1	0	1	1	2	16.6	29.176	259
>gi|194454644|gb|EDX43541.1| hypothetical protein Lreu23DRAFT_5063 [Lactobacillus reuteri 100-23	1	1	1	1	1	0	5.9	19.747	169
>gi|148532114|gb|ABQ84113.1| transcriptional regulator, HxlR family [Lactobacillus reuteri DSM 20016];>	2	0	2	0	1	0	11.5	14.22	122
>gi|148530779|gb|ABQ82778.1| FolC bifunctional protein [Lactobacillus reuteri DSM 20016];>	1	1	1	0	1	0	3.2	47.999	437
>gi|227071709|gb|EEI10000.1| ATP-dependent RNA helicase [Lactobacillus reuteri MM2-3	2	0	1	1	0	2	4.7	53.802	467
>gi|148531437|gb|ABQ83436.1| transcriptional regulator, ArgR family [Lactobacillus reuteri DSM 20016	1	1	1	1	1	0	8.7	16.881	150
>gi|148530337|gb|ABQ82336.1| glutamate-cysteine ligase [Lactobacillus reuteri DSM 20016	2	0	1	0	1	0	5.8	51.01	446
>gi|337728749|emb|CCC03868.1| conserved hypothetical protein [Lactobacillus reuteri ATCC 53608	3	0	1	1	1	1	13.7	33.684	292
>gi|148532016|gb|ABQ84015.1| UTP-hexose-1-phosphate uridylyltransferase [Lactobacillus reuteri DSM 20016	1	1	1	1	1	1	2.3	54.926	483
>gi|148531876|gb|ABQ83875.1| hypothetical protein Lreu_1635 [Lactobacillus reuteri DSM 20016];>	2	2	2	2	2	2	5.4	31.711	279
>gi|148531224|gb|ABQ83223.1| DNA/RNA non-specific endonuclease [Lactobacillus reuteri DSM 20016	2	0	2	1	1	1	8.8	31.236	284
>gi|228752976|gb|EEM02516.1| Transposase IS116/IS110/IS902 [Bacillus mycoides Rock1-4];>	2	1	2	1	2	0	5.7	47.304	403
>gi|148531644|gb|ABQ83643.1| malate dehydrogenase (NAD) [Lactobacillus reuteri DSM 20016	3	1	1	0	2	1	10.7	33.353	307
>gi|227070914|gb|EEI09237.1| ABC superfamily ATP binding cassette transporter, ABC protein [Lactobacillus reuteri MM2-3];>	2	1	0	1	2	1	6.8	41.798	370
>gi|148531298|gb|ABQ83297.1| hypothetical protein Lreu_1037 [Lactobacillus reuteri DSM 20016];>	1	1	0	0	1	0	2.1	69.932	630
>gi|148530908|gb|ABQ82907.1| secreted protein containing a PDZ domain-like protein [Lactobacillus reuteri DSM 20016	1	1	1	0	1	0	4.3	38.633	350
>gi|183224812|dbj|BAG25329.1| putative amidase [Lactobacillus reuteri JCM 1112];>	2	1	1	2	1	1	2.6	70.612	645
>gi|148531534|gb|ABQ83533.1| Ham1 family protein [Lactobacillus reuteri DSM 20016];>	1	1	1	1	0	1	6.2	21.804	195
>gi|183227658|dbj|BAG28174.1| glucose inhibited division protein [Lactobacillus fermentum IFO 3956];>	2	1	2	1	1	1	3.2	70.176	634
>gi|148531919|gb|ABQ83918.1| translation elongation factor 2 (EF-2/EF-G) [Lactobacillus reuteri DSM 20016];>	2	2	1	2	1	1	2.6	71.217	642
>gi|194453810|gb|EDX42707.1| VRR-NUC domain protein [Lactobacillus reuteri 100-23];>	1	1	1	1	1	1	7.1	12.669	112

>gi|148530713|gb|ABQ82712.1| beta-lactamase [Lactobacillus reuteri DSM 20016	2	0	0	0	2	0	6.5	38.305	339
>gi|133930445|gb|ABO43794.1| AraA [Lactobacillus reuteri];>gi|148530751|gb|ABQ82750.1| L-arabinose isomerase [Lactobacillus reuteri DSM 20016];>	2	1	0	1	0	0	4	53.759	473
>gi|337728903|emb|CCC04023.1| conserved hypothetical protein [Lactobacillus reuteri ATCC 53608];>	2	1	0	2	1	2	5.6	30.777	286
>gi|183225651|dbj|BAG26168.1| putative transport protein [Lactobacillus reuteri JCM 1112	2	1	1	0	0	0	4.3	42.512	392
>gi|183224728|dbj|BAG25245.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	1	1	1	1	1	1	2.6	40.889	344
>gi|227186022|gb|EEI66093.1| recombination regulator RecX [Lactobacillus reuteri CF48-3A];>	2	0	1	0	1	1	8.5	31.88	271
>gi|148530715|gb|ABQ82714.1| transcriptional regulator, ArgR family [Lactobacillus reuteri DSM 20016];>	2	1	2	0	2	0	14.4	17.482	153
>gi|148532081|gb|ABQ84080.1| protein of unknown function DUF915, hydrolase family protein [Lactobacillus reuteri DSM 20016];>	1	1	1	0	1	0	3.2	31.277	284
>gi|148531717|gb|ABQ83716.1| SSU ribosomal protein S14P [Lactobacillus reuteri DSM 20016	3	2	3	1	3	1	27.9	7.1065	61
>gi|324978619|gb|EGC15568.1| APC family amino acid transporter [Lactobacillus reuteri MM4-1A];> [	4	2	0	1	2	1	7.2	49.893	460
>gi|148530702|gb|ABQ82701.1| hypothetical protein Lreu_0432 [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	7	18.525	158
>gi|148531105|gb|ABQ83104.1| hypothetical protein Lreu_0841 [Lactobacillus reuteri DSM 20016];>	2	0	0	0	0	2	9.7	20.695	186
>gi|183226724|dbj|BAG27240.1| topoisomerase IV subunit A [Lactobacillus fermentum IFO 3956]	3	2	2	1	2	1	3.4	92.09	825
>gi|148531178|gb|ABQ83177.1| mevalonate kinase [Lactobacillus reuteri DSM 20016	3	2	0	0	1	1	13.3	34.209	316
>gi|227185375|gb|EEI65446.1| type I site-specific deoxyribonuclease [Lactobacillus reuteri CF48-3A];>	2	1	1	2	2	1	1.5	118.02	1038
>gi|183224941|dbj|BAG25458.1| molybdopterin biosynthesis protein MoeA [Lactobacillus reuteri JCM 1112];>	1	1	0	1	1	1	3.2	44.877	405
>gi|148530373|gb|ABQ82372.1| amino acid/polyamine/organocation transporter, APC superfamily [Lactobacillus reuteri DSM 20016	1	1	1	0	1	1	2.7	49.337	449
>gi|148531063|gb|ABQ83062.1| hypothetical protein Lreu_0799 [Lactobacillus reuteri DSM 20016];>	2	0	0	0	1	1	20.9	14.8	134
>gi|148530513|gb|ABQ82512.1| Heat shock protein, Metallo peptidase, MEROPS family M48B [Lactobacillus reuteri DSM 20016];>gi|	1	1	1	0	0	0	3.7	32.779	298
>gi|183224263|dbj|BAG24780.1| hypothetical protein [Lactobacillus reuteri JCM 1112];>	2	0	1	1	2	1	11	25.982	237
>gi|148532141|gb|ABQ84140.1| malate dehydrogenase (NAD) [Lactobacillus reuteri DSM 20016];>	3	0	0	0	1	2	9.5	34.793	316
>gi|148530922|gb|ABQ82921.1| hypothetical protein Lreu_0656 [Lactobacillus reuteri DSM 20016];>	1	1	1	0	0	0	22.2	5.307	45
>gi|148530863|gb|ABQ82862.1| protein of unknown function YGGT [Lactobacillus reuteri DSM	1	1	1	1	1	1	8.1	9.7797	86

20016];>									
>gi|148531540|gb|ABQ83539.1| hypothetical protein Lreu_1282 [Lactobacillus reuteri DSM 20016];>	1	1	0	0	1	0	14.1	10.138	92
>gi|227185036|gb|EEI65107.1| DNA primase [Lactobacillus reuteri CF48-3A];>	1	1	1	1	1	1	2.1	59.979	522
>gi|148531397|gb|ABQ83396.1| RecT protein [Lactobacillus reuteri DSM 20016];>	1	1	0	0	0	1	4.3	36.317	329
>gi|227071516|gb|EEI09815.1| APC family amino acid-polyamine-organocation transporter [Lactobacillus reuteri MM2-3];>	4	1	0	1	3	1	7.6	48.252	445
>gi|133930409|gb|ABO43776.1| triosephosphate isomerase [Lactobacillus reuteri	1	1	1	1	0	0	4.4	27.379	249
>gi|148530376|gb|ABQ82375.1| 3-hydroxyacyl-CoA dehydrogenase [Lactobacillus reuteri DSM 20016	1	0	1	0	0	1	3.7	32.931	294
>gi|183224401|dbj|BAG24918.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112	1	1	1	1	0	1	4.9	32.584	287
>gi|148532054|gb|ABQ84053.1| ABC transporter related [Lactobacillus reuteri DSM 20016	2	0	0	1	1	1	3.3	58.081	513
>gi|227071701|gb|EEI09992.1| conserved hypothetical protein [Lactobacillus reuteri MM2-3];>	1	0	1	0	1	0	5.2	22.201	193
>gi|112943598|gb|ABI26315.1| modification methylase HemK [Lactobacillus reuteri	1	1	1	0	0	0	3.1	32.592	288
>gi|227069913|gb|EEI08301.1| P27 family phage terminase small subunit [Lactobacillus reuteri MM2-3	1	1	0	0	1	0	6.3	18.213	158
>gi|148531525|gb|ABQ83524.1| metallophosphoesterase [Lactobacillus reuteri DSM 20016];>	1	0	0	1	0	1	2	45.056	394
>gi|183225267|dbj|BAG25784.1| dextransucrase [Lactobacillus reuteri JCM 1112	3	3	2	1	1	3	2.1	167.91	1488
>gi|148530536|gb|ABQ82535.1| RNA binding S1 domain protein [Lactobacillus reuteri DSM 20016];> p	2	0	2	1	1	0	11	18.376	164
>gi|183224509|dbj|BAG25026.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112	1	1	0	1	0	0	1.7	57.982	517
>gi|183225365|dbj|BAG25882.1| cobalt ABC transporter ATP-binding component [Lactobacillus reuteri JCM 1112];>	2	0	2	1	1	1	7.5	31.093	281
>gi|148530666|gb|ABQ82665.1| hypothetical protein Lreu_0396 [Lactobacillus reuteri DSM 20016];>	1	1	1	1	0	1	5.2	22.065	194
>gi|148531021|gb|ABQ83020.1| condensin subunit ScpB [Lactobacillus reuteri DSM 20016];>	2	1	2	1	1	0	8.5	22.54	201
>gi|148531177|gb|ABQ83176.1| diphosphomevalonate decarboxylase [Lactobacillus reuteri DSM 20016];>	2	1	1	1	2	2	7.4	35.168	323
>gi|183225010|dbj|BAG25527.1| hypothetical protein [Lactobacillus reuteri JCM 1112	2	1	1	0	1	0	13.5	20.282	170
>gi|148531200|gb|ABQ83199.1| protein of unknown function DUF915, hydrolase family protein [Lactobacillus reuteri DSM 20016];>	2	1	1	0	0	0	7.5	31.402	281
>gi|148531569|gb|ABQ83568.1| exodeoxyribonuclease III Xth [Lactobacillus reuteri DSM 20016];>	2	1	0	2	0	0	8.3	29.639	253
>gi|183226310|dbj|BAG26826.1| thiamine biosynthesis protein ThiI [Lactobacillus fermentum IFO 3956];>	2	1	0	0	1	0	4.2	45.199	406
>gi|183225665|dbj|BAG26182.1| hypothetical protein [Lactobacillus reuteri JCM 1112	2	0	2	0	0	0	25.6	10.074	86
>gi|148530665|gb|ABQ82664.1| SsrA-binding protein [Lactobacillus reuteri DSM 20016];>]	2	1	1	0	1	2	9.6	18.262	157

>gi|148530982|gb|ABQ82981.1| hypothetical protein Lreu_0717 [Lactobacillus reuteri DSM 20016];>	2	0	2	1	0	1	17.9	13.889	112
>gi|337729355|emb|CCC04485.1| conserved hypothetical protein [Lactobacillus reuteri ATCC 53608]	2	1	0	1	1	0	6.4	58.193	517
>gi|148530791|gb|ABQ82790.1| competence/damage-inducible protein cinA [Lactobacillus reuteri DSM 20016];>	2	1	0	1	1	1	5.8	45.373	415
>gi|183226723|dbj|BAG27239.1| topoisomerase IV subunit B [Lactobacillus fermentum IFO 3956];>	2	1	0	2	2	2	2.4	73.864	665
>gi|337729337|emb|CCC04466.1| putative phage minor tail protein [Lactobacillus reuteri ATCC 53608]	2	1	1	1	2	1	0.8	335.06	3023
>gi|148530533|gb|ABQ82532.1| transcription-repair coupling factor [Lactobacillus reuteri DSM 20016];>	2	1	2	0	1	1	1.6	133.43	1179
>gi|133930577|gb|ABO43859.1| putative replication protein [Lactobacillus reuteri];>	2	1	1	2	1	1	2.1	80.698	705
>gi|183226575|dbj|BAG27091.1| histidyl-tRNA synthase [Lactobacillus fermentum IFO 3956];>	2	0	1	0	0	1	5.2	43.193	386
>gi|183227095|dbj|BAG27611.1| glutamine synthase [Lactobacillus fermentum IFO 3956	2	1	0	0	0	1	4	50.271	450
>gi|194453843|gb|EDX42740.1| ABC transporter related [Lactobacillus reuteri 100-23];>	1	0	0	1	1	0	3.5	32.935	288
>gi|134081895|emb|CAK42150.1| unnamed protein product [Aspergillus niger]	2	0	1	1	0	0	2.8	60.461	541
>gi|148532031|gb|ABQ84030.1| Homoserine O-succinyltransferase [Lactobacillus reuteri DSM 20016	2	1	1	2	1	1	5.8	32.139	275
>gi|227185207|gb|EEI65278.1| ATPase family protein [Lactobacillus reuteri CF48-3A];>	2	0	1	0	1	0	1.4	139.08	1221
>gi|194454127|gb|EDX43024.1| GCN5-related N-acetyltransferase [Lactobacillus reuteri 100-23	2	1	0	0	0	1	10.4	20.084	173
>gi|183224053|dbj|BAG24570.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	2	1	2	1	1	1	8.7	24.002	206
>gi|183225802|dbj|BAG26319.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	2	1	1	0	0	0	7.1	24.47	225
>gi|194454399|gb|EDX43296.1| aminotransferase class I and II [Lactobacillus reuteri 100-23];>	2	0	1	1	2	1	3.6	43.686	386
>gi|148531015|gb|ABQ83014.1| DNA polymerase III catalytic subunit, DnaE type [Lactobacillus reuteri DSM 20016];>	3	0	1	0	2	1	2.5	126.23	1115

Cell surface proteome of *L. reuteri* MM4KO

Appendix 2 - CSP Protein Identified	Peptides	Peptides sample 1	Peptides sample 2	Peptides sample 3	Peptides sample 4	Peptides sample 5	Sequence Coverage [%]	Mol. Weight [kDa]	Sequence Length
>gi|148531475|gb|ABQ83474.1| phenylalanyl-tRNA synthetase beta subunit [Lactobacillus reuteri DSM 20016	6	4	3	3	1	5	11.7	89.003	805
>gi|183226183|dbj|BAG26699.1| glyceraldehyde 3-phosphate dehydrogenase [Lactobacillus fermentum IFO 3956	7	4	4	4	3	5	28.8	36.208	337
>gi|227186429|gb|EEI66500.1| acetoin dehydrogenase [Lactobacillus reuteri CF48-3A];>	7	6	5	4	4	5	42.7	28.27	267
>gi|227071142|gb|EEI09458.1| acetoin dehydrogenase [Lactobacillus reuteri MM2-3	8	6	6	6	6	7	65.5	28.157	267
>gi|133930435|gb|ABO43789.1| DNA-binding protein [Lactobacillus reuteri];>	8	8	6	6	7	6	72.5	9.5238	91
>gi|148531729|gb|ABQ83728.1| LSU ribosomal protein L4P [Lactobacillus reuteri DSM 20016	8	8	7	7	7	7	49.8	22.29	207
>gi|183224383|dbj|BAG24900.1| enolase [Lactobacillus reuteri JCM 1112	15	9	11	14	15	12	47.3	49.951	457
>gi|148530951|gb|ABQ82950.1| translation elongation factor Ts (EF-Ts) [Lactobacillus reuteri DSM 20016	15	12	12	11	11	13	66	31.962	291
>gi|148531718|gb|ABQ83717.1| LSU ribosomal protein L5P [Lactobacillus reuteri DSM 20016];>	17	12	14	13	11	13	87.8	20.171	180
>gi|148532099|gb|ABQ84098.1| Alcohol dehydrogenase GroES domain protein [Lactobacillus reuteri DSM 20016	17	14	14	12	14	13	74.4	35.918	336
>gi|148530650|gb|ABQ82649.1| alpha-phosphoglucomutase [Lactobacillus reuteri DSM 20016	19	15	15	13	11	13	40.8	63.706	574
>gi|227070122|gb|EEI08498.1| malate dehydrogenase (NAD) [Lactobacillus reuteri MM2-3];>gi|227185101|gb|EEI65172.1| L-lactate dehydrogenase [Lactobacillus reuteri CF48-3A];>gi|227364859|ref|ZP_03848906.1| malate dehydrogenase (NAD) [Lactobacillus reuteri MM	20	16	16	15	16	16	74.2	34.901	326
>gi|148531743|gb|ABQ83742.1| Alcohol dehydrogenase GroES domain protein [Lactobacillus reuteri DSM 20016];>gi|148544709|ref|YP_001272079.1| alcohol dehydrogenase [Lactobacillus reuteri DSM 20016];>gi|183225405|dbj|BAG25922.1| alcohol dehydrogenase [Lactoba	22	17	18	18	20	17	87.4	36.124	342
>gi|148531460|gb|ABQ83459.1| L-glutamine synthetase [Lactobacillus reuteri DSM 20016	23	14	18	15	18	18	66.2	50.835	447
>gi|148530455|gb|ABQ82454.1| Substrate-binding region of ABC-type glycine betaine transport system [Lactobacillus reuteri DSM 20016];>	24	17	20	20	13	15	59.9	32.953	299
>gi|148530917|gb|ABQ82916.1| translation elongation factor 1A (EF-1A/EF-Tu) [Lactobacillus reuteri DSM 20016];>gi|148543883|ref|YP_001271253.1| elongation factor Tu [Lactobacillus reuteri DSM 20016];>gi|183224627|dbj|BAG25144.1| elongation factor Tu [Lacto	24	22	22	23	22	22	75.8	43.432	396
>gi|227186481|gb|EEI66552.1| D-alanine--D-alanine ligase [Lactobacillus reuteri CF48-3A];>	25	24	18	19	16	13	70.9	42.569	378
>gi|183224464|dbj|BAG24981.1| D-alanine--D-alanine ligase [Lactobacillus reuteri JCM 1112	25	24	19	20	17	14	72	43.001	382
>gi|227071557|gb|EEI09855.1| glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) [Lactobacillus reuteri MM2-3	26	23	23	22	21	22	77.7	37.062	345

>gi|148530918|gb|ABQ82917.1| trigger factor [Lactobacillus reuteri DSM 20016	27	18	18	15	19	18	64.4	48.747	436
>gi|143798699|gb|ABF06644.2| sucrose phosphorylase [Lactobacillus reuteri	27	21	18	21	20	20	66.8	55.969	485
>gi|183224381|dbj|BAG24898.1| phosphoglycerate kinase [Lactobacillus reuteri JCM 1112];>	27	25	23	19	14	12	82.3	42.96	401
>gi|148530972|gb|ABQ82971.1| chaperone protein DnaK [Lactobacillus reuteri DSM 20016	29	14	18	18	21	20	65.9	67.211	621
>gi|148530417|gb|ABQ82416.1| IMP cyclohydrolase [Lactobacillus reuteri DSM 20016	30	22	25	23	21	10	65.2	57.032	512
>gi|148530345|gb|ABQ82344.1| Adenylosuccinate synthetase [Lactobacillus reuteri DSM 20016	30	23	20	22	21	22	75.5	47.725	432
>gi|145202295|gb|ABF06651.2| pyruvate kinase [Lactobacillus reuteri	31	23	28	26	24	22	73.6	51.853	473
>gi|227184598|gb|EEI64669.1| phosphoketolase [Lactobacillus reuteri CF48-3A];>	32	31	26	27	29	25	41.2	91.435	803
>gi|337728287|emb|CCC03382.1| translation elongation factor G [Lactobacillus reuteri ATCC 53608]	33	26	28	26	25	27	67.2	76.758	695
>gi|148531028|gb|ABQ83027.1| SSU ribosomal protein S1P [Lactobacillus reuteri DSM 20016	33	27	27	28	24	27	85.6	45.995	416
>gi|148531528|gb|ABQ83527.1| arginyl-tRNA synthetase [Lactobacillus reuteri DSM 20016	34	26	25	28	23	22	67.4	63.878	562
>gi|337727862|emb|CCC02950.1| 30S ribosomal protein S1 [Lactobacillus reuteri ATCC 53608]	34	28	26	27	24	26	88.9	45.982	416
>gi|337728521|emb|CCC03625.1| glucose-6-phosphate 1-dehydrogenase [Lactobacillus reuteri ATCC 53608]	36	28	29	31	28	27	73.8	56.355	493
>gi|148530624|gb|ABQ82623.1| chaperonin GroEL [Lactobacillus reuteri DSM 20016];>	36	28	30	29	30	26	75.5	57.123	542
>gi|148531733|gb|ABQ83732.1| translation elongation factor 2 (EF-2/EF-G) [Lactobacillus reuteri DSM 20016	37	27	35	33	31	33	74.2	76.77	695
>gi|227070837|gb|EEI09162.1| glucose-6-phosphate 1-dehydrogenase [Lactobacillus reuteri MM2-3	38	30	32	34	30	29	77.2	56.713	496
>gi|148531571|gb|ABQ83570.1| hypothetical protein Lreu_1313 [Lactobacillus reuteri DSM 20016	39	26	31	32	29	25	79.5	64.749	566
>gi|183225653|dbj|BAG26170.1| 6-phosphogluconate dehydrogenase [Lactobacillus reuteri JCM 1112	41	38	37	38	36	34	87.9	53.417	478
>gi|337729321|emb|CCC04450.1| aldehyde-alcohol dehydrogenase [Lactobacillus reuteri ATCC 53608];>	43	37	36	34	27	34	61.5	97.181	878
>gi|148530322|gb|ABQ82321.1| ATPase AAA-2 domain protein [Lactobacillus reuteri DSM 20016];>	50	44	37	32	44	38	71.4	82.305	745
>gi|337728440|emb|CCC03541.1| xylulose 5-phosphate phosphoketolase [Lactobacillus reuteri ATCC 53608]	51	49	42	43	45	41	70.2	91.372	803
>gi|194454221|gb|EDX43118.1| Phosphoketolase [Lactobacillus reuteri 100-23];>	52	50	43	45	46	42	70.2	91.374	803
>gi|148531926|gb|ABQ83925.1| Phosphoketolase [Lactobacillus reuteri DSM 20016	52	50	44	45	46	44	71.2	91.403	803
>gi|148530592|gb|ABQ82591.1| alcohol dehydrogenase AdhE / acetaldehyde dehydrogenase [Lactobacillus reuteri DSM 20016];>gi|148543558|ref|YP_001270928.1| bifunctional acetaldehyde-CoA/alcohol dehydrogenase [Lactobacillus reuteri DSM 20016];>gi|183224309|dbj	55	46	50	46	39	47	74.8	97.187	878
>gi|337727906|emb|CCC02995.1| L-lactate dehydrogenase [Lactobacillus reuteri ATCC 53608]	19	15	15	14	15	15	75.2	34.069	319
>gi|269930626|gb|ACZ53583.1| phosphoketolase [Lactobacillus reuteri]	14	14	11	12	13	12	89.9	21.656	189

>gi|183227647|dbj|BAG28163.1| dehydrogenase [Lactobacillus fermentum IFO 3956];>]	7	5	5	4	4	5	25.9	35.835	336
>gi|183224518|dbj|BAG25035.1| alanyl-tRNA synthase [Lactobacillus reuteri JCM 1112	22	20	16	15	15	14	36.3	98.648	885
>gi|148530453|gb|ABQ82452.1| 3-beta hydroxysteroid dehydrogenase/isomerase [Lactobacillus reuteri DSM 20016];>	11	7	8	10	8	7	51.8	31.632	284
>gi|148532121|gb|ABQ84120.1| lysyl aminopeptidase, Metallo peptidase, MEROPS family M01 [Lactobacillus reuteri DSM 20016];>	23	13	14	15	11	8	37.1	95.323	843
>gi|148531698|gb|ABQ83697.1| LSU ribosomal protein L13P [Lactobacillus reuteri DSM 20016];>	13	13	12	10	9	8	80.3	16.304	147
>gi|148532092|gb|ABQ84091.1| Mannosyl-glycoprotein endo-beta-N-acetylglucosamidase [Lactobacillus reuteri DSM 20016];>	21	17	14	16	15	16	51.8	60.446	568
>gi|148531290|gb|ABQ83289.1| LPXTG-motif cell wall anchor domain [Lactobacillus reuteri DSM 20016];>	22	12	19	15	15	13	45.7	80.889	752
>gi|148530866|gb|ABQ82865.1| Isoleucyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>gi|148543832|ref|YP_001271202.1| isoleucyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	33	22	16	21	15	17	48.3	109.95	960
>gi|148530340|gb|ABQ82339.1| 2,5-didehydrogluconate reductase [Lactobacillus reuteri DSM 20016];>	15	10	11	9	7	7	72.6	32.62	288
>gi|148530567|gb|ABQ82566.1| amino acid ABC transporter substrate-binding protein, PAAT family [Lactobacillus reuteri DSM 20016];>	19	14	18	15	11	15	57.8	28.516	263
>gi|337729108|emb|CCC04231.1| alanyl-tRNA synthase [Lactobacillus reuteri ATCC 53608]	21	19	13	15	14	12	35.5	98.679	884
>gi|183225729|dbj|BAG26246.1| hypothetical protein [Lactobacillus reuteri JCM 1112];>	10	8	4	3	9	7	68	19.891	169
>gi|148531932|gb|ABQ83931.1| (R)-2-hydroxyisocaproate dehydrogenase [Lactobacillus reuteri DSM 20016];>	21	14	16	13	14	12	80.5	37.358	334
>gi|148530447|gb|ABQ82446.1| pyridine nucleotide-disulfide oxidoreductase dimerization region [Lactobacillus reuteri DSM 20016];>	16	16	13	13	11	14	50.3	49.944	451
>gi|148531711|gb|ABQ83710.1| LSU ribosomal protein L15P [Lactobacillus reuteri DSM 20016];>	14	12	12	12	10	11	72.9	15.447	144
>gi|227186263|gb|EEI66334.1| maltose phosphorylase [Lactobacillus reuteri CF48-3A];>	24	15	19	15	14	10	40	87.449	757
>gi|148531526|gb|ABQ83525.1| protein of unknown function DUF964 [Lactobacillus reuteri DSM 20016];>	12	9	8	10	8	8	88.6	14.096	123
>gi|148530584|gb|ABQ82583.1| LSU ribosomal protein L10P [Lactobacillus reuteri DSM 20016];>	11	8	9	10	8	9	66.3	18.139	166
>gi|337728005|emb|CCC03094.1| isoleucyl-tRNA synthase [Lactobacillus reuteri ATCC 53608]	29	20	14	18	13	14	43	106.67	931
>gi|148531713|gb|ABQ83712.1| SSU ribosomal protein S5P [Lactobacillus reuteri DSM 20016];>	16	14	15	14	14	13	81.1	17.646	169
>gi|148530953|gb|ABQ82952.1| ribosome recycling factor [Lactobacillus reuteri DSM 20016];>	9	9	7	8	7	7	57.8	20.783	187
>gi|148531724|gb|ABQ83723.1| SSU ribosomal protein S3P [Lactobacillus reuteri DSM 20016];>	10	9	9	7	9	9	58.8	24.696	221
>gi|148531543|gb|ABQ83542.1| ribokinase [Lactobacillus reuteri DSM 20016];>	14	11	11	12	9	11	66.8	32.041	307
>gi|133930433|gb|ABO43788.1| ribosomal protein L15 [Lactobacillus reuteri	12	10	9	9	8	8	66.7	15.516	144
>gi|148531716|gb|ABQ83715.1| SSU ribosomal protein S8P [Lactobacillus reuteri DSM 20016];>	11	8	9	8	8	9	75.8	14.536	132

>gi|148530580|gb|ABQ82579.1| LSU ribosomal protein L1P [Lactobacillus reuteri DSM 20016];>	11	9	7	6	7	8	47.4	27.362	253
>gi|183225401|dbj|BAG25918.1| DNA-directed RNA polymerase beta subunit [Lactobacillus reuteri JCM 1112];>	28	22	18	20	19	22	33.3	135.36	1205
>gi|148530865|gb|ABQ82864.1| DivIVA family protein [Lactobacillus reuteri DSM 20016];>	12	9	11	10	11	11	44.3	27.591	246
>gi|148530679|gb|ABQ82678.1| phosphoglucosamine mutase [Lactobacillus reuteri DSM 20016];>	13	12	11	9	9	9	37.9	48.902	451
>gi|148530736|gb|ABQ82735.1| ATP synthase F1 subcomplex beta subunit [Lactobacillus reuteri DSM 20016];>	14	9	9	10	7	4	48.4	51.631	475
>gi|148530962|gb|ABQ82961.1| NusA antitermination factor [Lactobacillus reuteri DSM 20016];>	11	7	9	8	10	9	45.3	44.525	395
>gi|148530642|gb|ABQ82641.1| UDP-glucose pyrophosphorylase [Lactobacillus reuteri DSM 20016];>	14	10	8	10	11	8	65.5	34.101	304
>gi|148531722|gb|ABQ83721.1| LSU ribosomal protein L29P [Lactobacillus reuteri DSM 20016];>	5	4	2	4	3	3	58.8	8.0041	68
>gi|337728880|emb|CCC04000.1| hypothetical protein LRATCC53608_1247 [Lactobacillus reuteri ATCC 53608]	10	7	6	9	8	7	79.7	14.126	123
>gi|148531553|gb|ABQ83552.1| leucyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	17	9	10	12	11	7	37.6	92.918	806
>gi|148530989|gb|ABQ82988.1| aspartyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>;	13	9	6	7	6	5	27.5	68.43	600
>gi|148530903|gb|ABQ82902.1| GTP-binding protein TypA [Lactobacillus reuteri DSM 20016];>	25	22	21	21	21	19	48	68.827	614
>gi|148531725|gb|ABQ83724.1| LSU ribosomal protein L22P [Lactobacillus reuteri DSM 20016];>	6	6	6	6	6	6	52.2	12.394	115
>gi|148531009|gb|ABQ83008.1| aminotransferase [Lactobacillus reuteri DSM 20016];>	17	14	11	14	11	12	52.3	43.107	394
>gi|148530950|gb|ABQ82949.1| SSU ribosomal protein S2P [Lactobacillus reuteri DSM 20016];>	18	15	13	15	13	15	80.2	29.657	262
>gi|148530959|gb|ABQ82958.1| prolyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	20	17	15	16	15	14	52.3	64.256	577
>gi|148531738|gb|ABQ83737.1| DNA-directed RNA polymerase subunit beta [Lactobacillus reuteri DSM 20016	29	19	18	19	17	20	32.7	135.42	1211
>gi|183225377|dbj|BAG25894.1| 50S ribosomal protein L18 [Lactobacillus reuteri JCM 1112];>	9	9	7	7	8	8	69.4	13.342	121
>gi|148531730|gb|ABQ83729.1| LSU ribosomal protein L3P [Lactobacillus reuteri DSM 20016];>	11	9	8	7	7	7	57.5	23.757	219
>gi|227186160|gb|EEI66231.1| possible peptidoglycan-binding protein [Lactobacillus reuteri CF48-3A];>	5	3	3	2	5	5	23.3	27.744	266
>gi|183225744|dbj|BAG26261.1| threonyl-tRNA synthase [Lactobacillus reuteri JCM 1112];>	18	11	10	13	10	11	33.5	71.776	627
>gi|148530419|gb|ABQ82418.1| phosphoglycerate mutase [Lactobacillus reuteri DSM 20016];>	13	10	11	9	10	11	53.5	26.121	228
>gi|227071019|gb|EEI09341.1| bifunctional GMP synthase/glutamine amidotransferase protein [Lactobacillus reuteri MM2-3];>	26	18	20	18	20	15	61.3	59.912	538
>gi|130893166|gb|ABO32596.1| ATP-dependent Clp protease ATP-binding subunit ClpX [Lactobacillus reuteri];>	15	11	4	6	5	8	52.6	45.969	416
>gi|337727880|emb|CCC02968.1| amino acid aminotransferase [Lactobacillus reuteri ATCC 53608]	14	12	10	14	11	10	41.4	43.078	394
>gi|148530413|gb|ABQ82412.1| phosphoribosylformylglycinamidine synthase subunit II	17	7	14	14	12	6	40	80.944	742

[Lactobacillus reuteri DSM 20016									
>gi|148530404|gb|ABQ82403.1| Formate-tetrahydrofolate ligase [Lactobacillus reuteri DSM 20016	19	7	16	15	16	10	41.4	60.251	553
>gi|148531727|gb|ABQ83726.1| LSU ribosomal protein L2P [Lactobacillus reuteri DSM 20016];>	18	17	17	13	13	13	65.1	30.47	281
>gi|148532119|gb|ABQ84118.1| Bleomycin hydrolase [Lactobacillus reuteri DSM 20016];>	14	12	9	8	5	4	38.2	50.851	440
>gi|148530623|gb|ABQ82622.1| chaperonin Cpn10 [Lactobacillus reuteri DSM 20016];>	8	7	8	7	5	5	61.7	10.149	94
>gi|194454505|gb|EDX43402.1| 2,5-didehydrogluconate reductase [Lactobacillus reuteri 100-23];>	11	9	8	6	6	4	46.2	32.469	288
>gi|148532124|gb|ABQ84123.1| asparaginyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	18	14	12	11	9	11	60.2	50.092	432
>gi|148531709|gb|ABQ83708.1| Adenylate kinase [Lactobacillus reuteri DSM 20016];>	12	9	9	9	11	9	68.5	24.609	219
>gi|183225183|dbj|BAG25700.1| ligase [Lactobacillus reuteri JCM 1112];>	18	11	10	12	11	13	59.2	49.294	441
>gi|183225853|dbj|BAG26369.1| ATP-dependent Clp protease ATP-binding subunit [Lactobacillus fermentum IFO 3956];>	7	6	4	4	6	6	10.8	76.678	697
>gi|148531554|gb|ABQ83553.1| methionine adenosyltransferase [Lactobacillus reuteri DSM 20016];>	12	7	8	8	7	6	40.8	43.195	395
>gi|227071143|gb|EEI09459.1| ribosomal protein S9 [Lactobacillus reuteri MM2-3];>	6	5	6	6	6	6	55.6	14.494	133
>gi|148530769|gb|ABQ82768.1| SSU ribosomal protein S4P [Lactobacillus reuteri DSM 20016	18	16	13	14	12	14	83.6	22.943	201
>gi|227071324|gb|EEI09633.1| heat shock protein Hsp33 [Lactobacillus reuteri MM2-3];>	11	8	9	7	5	5	48.3	35.232	323
>gi|148531687|gb|ABQ83686.1| aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B [Lactobacillus reuteri DSM 20016];>	15	11	7	8	5	8	44.7	53.424	474
>gi|194453944|gb|EDX42841.1| glutamyl-tRNA(Gln) amidotransferase, B subunit [Lactobacillus reuteri 100-23];>/	15	11	6	8	5	8	44.7	53.408	474
>gi|148531171|gb|ABQ83170.1| L-lactate dehydrogenase [Lactobacillus reuteri DSM 20016];>	14	10	13	11	11	11	55.9	35.005	324
>gi|148531688|gb|ABQ83687.1| aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit A [Lactobacillus reuteri DSM 20016];>	13	10	9	8	8	9	45.1	53.009	490
>gi|148532166|gb|ABQ84165.1| aldo/keto reductase [Lactobacillus reuteri DSM 20016];>	11	10	7	5	5	2	47.7	32.371	287
>gi|183224883|dbj|BAG25400.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112	6	5	5	4	5	4	50.3	20.834	181
>gi|148531824|gb|ABQ83823.1| translation elongation factor P (EF-P) [Lactobacillus reuteri DSM 20016];>	12	9	9	9	7	7	72.4	20.518	185
>gi|148531706|gb|ABQ83705.1| SSU ribosomal protein S13P [Lactobacillus reuteri DSM 20016];>	11	11	9	9	9	10	70.2	13.687	121
>gi|148530668|gb|ABQ82667.1| phosphotransacetylase [Lactobacillus reuteri DSM 20016];>	13	7	8	7	8	3	61.7	34.699	324
>gi|194453968|gb|EDX42865.1| Adenylate kinase [Lactobacillus reuteri 100-23];>	12	9	8	8	10	8	68.5	24.61	219
>gi|148530585|gb|ABQ82584.1| LSU ribosomal protein L12P [Lactobacillus reuteri DSM 20016	11	8	8	7	9	8	90.1	12.356	121
>gi|337727893|emb|CCC02982.1| conjugated bile salt hydrolase [Lactobacillus reuteri ATCC 53608	9	7	0	4	2	0	48.3	36.06	325
>gi|148530857|gb|ABQ82856.1| UDP-N-acetylmuramoylalanine--D-glutamate ligase [Lactobacillus	4	2	3	4	3	3	17.8	50.332	456

reuteri DSM 20016];>									
>gi|148531728|gb|ABQ83727.1| LSU ribosomal protein L23P [Lactobacillus reuteri DSM 20016];>	7	6	4	7	5	4	60.2	11.25	98
>gi|148531734|gb|ABQ83733.1| SSU ribosomal protein S7P [Lactobacillus reuteri DSM 20016];>	13	12	10	11	6	7	74.4	17.985	156
>gi|148532158|gb|ABQ84157.1| hypoxanthine phosphoribosyltransferase [Lactobacillus reuteri DSM 20016];>	4	2	3	4	3	3	27	20.407	178
>gi|148530771|gb|ABQ82770.1| aminotransferase, class V [Lactobacillus reuteri DSM 20016];>	11	7	8	7	6	8	43.2	42.097	382
>gi|148530744|gb|ABQ82743.1| glycine cleavage H-protein [Lactobacillus reuteri DSM 20016];>	5	3	4	2	3	4	79.4	10.641	97
>gi|183224141|dbj|BAG24658.1| tyrosyl-tRNA synthase [Lactobacillus reuteri JCM 1112];>	13	7	9	9	10	9	35.7	47.69	420
>gi|148530610|gb|ABQ82609.1| UDP-galactose 4-epimerase [Lactobacillus reuteri DSM 20016];>	12	8	6	9	8	7	62.5	36.598	331
>gi|148531721|gb|ABQ83720.1| SSU ribosomal protein S17P [Lactobacillus reuteri DSM 20016];> r	7	6	5	4	3	4	65.9	10.168	88
>gi|227070899|gb|EEI09222.1| D-lactate dehydrogenase [Lactobacillus reuteri MM2-3];> g	11	9	11	8	8	7	53.1	37.311	337
>gi|148530564|gb|ABQ82563.1| cystathionine gamma-lyase [Lactobacillus reuteri DSM 20016	10	6	6	5	5	5	47.4	41.498	380
>gi|148531054|gb|ABQ83053.1| protein of unknown function DUF322 [Lactobacillus reuteri DSM 20016];>	7	6	4	5	5	6	59.8	13.85	127
>gi|148530646|gb|ABQ82645.1| thioredoxin reductase [Lactobacillus reuteri DSM 20016];>	7	6	4	4	4	3	33.2	33.305	310
>gi|148530685|gb|ABQ82684.1| Peptidoglycan-binding LysM [Lactobacillus reuteri DSM 20016];>	7	4	1	5	4	6	40.9	21.661	203
>gi|183227337|dbj|BAG27853.1| elongation factor G [Lactobacillus fermentum IFO 3956];>	11	6	10	9	9	9	29.1	76.351	694
>gi|148530861|gb|ABQ82860.1| cell division protein FtsZ [Lactobacillus reuteri DSM 20016	14	7	10	9	5	9	51.8	44.45	415
>gi|148530828|gb|ABQ82827.1| Adenine-specific DNA methylase-like protein [Lactobacillus reuteri DSM 20016];>	4	2	3	3	3	3	22.4	31.155	277
>gi|148530755|gb|ABQ82754.1| UspA domain protein [Lactobacillus reuteri DSM 20016];>	11	9	7	10	8	8	74.7	18.017	162
>gi|148530284|gb|ABQ82283.1| SSU ribosomal protein S6P [Lactobacillus reuteri DSM 20016];>	7	6	5	7	5	6	84.7	11.378	98
>gi|148530503|gb|ABQ82502.1| UDP-N-acetylglucosamine 1-carboxyvinyltransferase [Lactobacillus reuteri DSM 20016	10	5	5	7	6	5	34.1	45.676	425
>gi|141553038|gb|ABD13939.2| ornithine carbamoyltransferase [Lactobacillus reuteri];>	13	9	4	8	8	5	50.1	37.56	335
>gi|148531703|gb|ABQ83702.1| LSU ribosomal protein L17P [Lactobacillus reuteri DSM 20016];>	6	5	5	6	5	4	52	14.201	127
>gi|148530504|gb|ABQ82503.1| LSU ribosomal protein L31P [Lactobacillus reuteri DSM 20016];>	9	7	6	8	7	7	95.1	9.123	81
>gi|148531412|gb|ABQ83411.1| SSU ribosomal protein S16P [Lactobacillus reuteri DSM 20016];>	7	5	6	6	4	4	68.1	10.449	91
>gi|148531704|gb|ABQ83703.1| DNA-directed RNA polymerase subunit alpha [Lactobacillus reuteri DSM 20016];>	16	8	12	10	8	12	71	34.901	314
>gi|148531459|gb|ABQ83458.1| glutamyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>;	12	5	5	7	6	5	32.7	58.349	501
>gi|148530870|gb|ABQ82869.1| methylthioadenosine nucleosidase [Lactobacillus reuteri DSM 20016];>	7	3	5	6	4	4	51.1	24.687	231
>gi|148531720|gb|ABQ83719.1| LSU ribosomal protein L14P [Lactobacillus reuteri DSM 20016];>	6	6	6	6	6	6	43.4	13.144	122
>gi|148530773|gb|ABQ82772.1| valyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	23	16	13	16	15	10	31.7	102.29	884

>gi|227070649|gb|EEI08979.1| 1,3-propanediol dehydrogenase [Lactobacillus reuteri MM2-3];>	15	11	12	11	10	7	55.8	44.01	405
>gi|227184503|gb|EEI64574.1| amidophosphoribosyltransferase [Lactobacillus reuteri CF48-3A];>	6	5	5	5	5	1	20	53.593	490
>gi|148531751|gb|ABQ83750.1| cold-shock DNA-binding protein family [Lactobacillus reuteri DSM 20016];>	4	4	3	4	4	4	74.2	7.3269	66
>gi|194452927|gb|EDX41825.1| Cystathionine gamma-synthase [Lactobacillus reuteri 100-23];>	9	5	4	5	5	4	41.8	41.471	380
>gi|337729137|emb|CCC04260.1| valyl-tRNA synthase [Lactobacillus reuteri ATCC 53608];>	22	16	11	15	14	8	30.4	102.21	884
>gi|337729037|emb|CCC04160.1| aminotransferase [Lactobacillus reuteri ATCC 53608];>	16	14	9	10	8	5	55.7	43.766	397
>gi|148530808|gb|ABQ82807.1| thioredoxin [Lactobacillus reuteri DSM 20016];>	4	4	3	4	3	3	42.3	11.889	104
>gi|148532037|gb|ABQ84036.1| Endothelin-converting protein 1 [Lactobacillus reuteri DSM 20016];>	12	6	5	6	2	4	25.2	72.39	634
>gi|227071240|gb|EEI09554.1| possible glutathione-disulfide reductase [Lactobacillus reuteri MM2-3];>	6	2	3	3	4	2	19.9	48.997	443
>gi|148531145|gb|ABQ83144.1| 6,7-dimethyl-8-ribityllumazine synthase [Lactobacillus reuteri DSM 20016];>	6	3	4	6	3	5	78.3	16.637	152
>gi|148530382|gb|ABQ82381.1| GTP-binding protein YchF [Lactobacillus reuteri DSM 20016];> L	8	5	4	2	2	3	38.4	39.844	365
>gi|148530410|gb|ABQ82409.1| phosphoribosylaminoimidazole-succinocarboxamide synthase [Lactobacillus reuteri DSM 20016];>	11	5	9	3	7	5	47.3	27.308	239
>gi|148531006|gb|ABQ83005.1| glycyl-tRNA synthetase beta chain [Lactobacillus reuteri DSM 20016];>	17	12	11	13	13	9	34.4	78.536	691
>gi|148532032|gb|ABQ84031.1| cysteine synthase [Lactobacillus reuteri DSM 20016];>	7	7	3	0	0	0	34.9	32.241	307
>gi|148531464|gb|ABQ83463.1| glucokinase [Lactobacillus reuteri DSM 20016];>	8	7	6	5	4	6	34.1	34.42	323
>gi|148530343|gb|ABQ82342.1| FAD-dependent pyridine nucleotide-disulfide oxidoreductase [Lactobacillus reuteri DSM 20016];>	8	7	6	6	6	6	30.1	49.604	449
>gi|148532080|gb|ABQ84079.1| glycerol 2-dehydrogenase (NAD+) [Lactobacillus reuteri DSM 20016];>	8	6	7	5	6	6	39.1	40.698	373
>gi|148531715|gb|ABQ83714.1| LSU ribosomal protein L6P [Lactobacillus reuteri DSM 20016];>gi|148544681|ref|YP_001272051.1| 50S ribosomal protein L6 [Lactobacillus reuteri DSM 20016];>gi|166990974|sp|A5VLJ0.1|RL6_LACRD RecName: Full=50S ribosomal protein L6	9	8	7	8	7	8	44.4	19.635	178
>gi|148532070|gb|ABQ84069.1| histidyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	12	8	8	8	7	7	33.7	49.318	433
>gi|148531549|gb|ABQ83548.1| peptidase V, Metallo peptidase, MEROPS family M20A [Lactobacillus reuteri DSM 20016	9	9	2	5	3	2	25.7	51.22	467
>gi|148532120|gb|ABQ84119.1| NAD-dependent epimerase/dehydratase [Lactobacillus reuteri DSM 20016];>	10	7	6	4	4	5	61.5	23.187	213
>gi|183225184|dbj|BAG25701.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	8	4	5	4	6	5	62	23.595	216
>gi|148531583|gb|ABQ83582.1| Phosphotransferase system, phosphocarrier protein HPr [Lactobacillus reuteri DSM 20016];>	3	2	2	2	1	2	55.7	9.4076	88

>gi|148530367|gb|ABQ82366.1| NADPH-dependent FMN reductase [Lactobacillus reuteri DSM 20016];>	12	6	9	9	4	8	47.6	45.674	416
>gi|148530579|gb|ABQ82578.1| LSU ribosomal protein L11P [Lactobacillus reuteri DSM 20016];>	6	5	6	6	4	4	40.4	14.869	141
>gi|148530781|gb|ABQ82780.1| cell shape determining protein, MreB/Mrl family [Lactobacillus reuteri DSM 20016];>	10	7	6	7	4	5	46.2	34.966	333
>gi|148530402|gb|ABQ82401.1| cyclopropane-fatty-acyl-phospholipid synthase [Lactobacillus reuteri DSM 20016];>	4	2	3	4	3	2	14.1	46.488	403
>gi|148530721|gb|ABQ82720.1| bacterial peptide chain release factor 1 (bRF-1) [Lactobacillus reuteri DSM 20016];>	6	4	4	3	4	4	26.5	41.287	362
>gi|148531108|gb|ABQ83107.1| hypothetical protein Lreu_0844 [Lactobacillus reuteri DSM 20016];>	5	2	1	2	0	5	41.4	20.426	181
>gi|148530491|gb|ABQ82490.1| UDP-N-acetylglucosamine pyrophosphorylase [Lactobacillus reuteri DSM 20016];>	15	11	12	7	5	4	42.4	50.01	455
>gi|148530418|gb|ABQ82417.1| phosphoribosylamine--glycine ligase [Lactobacillus reuteri DSM 20016	11	5	6	7	5	2	37.5	45.76	419
>gi|148531723|gb|ABQ83722.1| LSU ribosomal protein L16P [Lactobacillus reuteri DSM 20016];>	7	6	6	6	6	5	54.9	16.006	144
>gi|148530555|gb|ABQ82554.1| putative nicotinate phosphoribosyltransferase [Lactobacillus reuteri DSM 20016];>	9	6	4	3	3	4	25.8	55.609	488
>gi|148530811|gb|ABQ82810.1| non-canonical purine NTP pyrophosphatase, rdgB/HAM1 family [Lactobacillus reuteri DSM 20016	8	5	4	7	4	5	67.2	21.286	195
>gi|148531499|gb|ABQ83498.1| bacterial translation initiation factor 3 (bIF-3) [Lactobacillus reuteri DSM 20016];>	6	3	3	3	3	4	46.5	19.487	170
>gi|146142505|gb|ABQ01722.1| fumarase [Lactobacillus reuteri];>	15	7	11	8	11	8	42	50.302	462
>gi|148531491|gb|ABQ83490.1| iojap-like protein [Lactobacillus reuteri DSM 20016	4	3	4	4	3	3	47.5	13.309	118
>gi|227071376|gb|EEI09682.1| malate dehydrogenase (NAD) [Lactobacillus reuteri MM2-3];>	5	3	3	3	3	3	27.4	35.211	321
>gi|148531450|gb|ABQ83449.1| LSU ribosomal protein L21P [Lactobacillus reuteri DSM 20016];>	5	5	3	2	3	3	54.9	11.256	102
>gi|148531929|gb|ABQ83928.1| dipeptidase A, Cysteine peptidase, MEROPS family C69 [Lactobacillus reuteri DSM 20016];>	10	9	7	6	6	6	29.5	54.294	478
>gi|148530366|gb|ABQ82365.1| NADPH-dependent FMN reductase [Lactobacillus reuteri DSM 20016];>	7	5	6	7	7	6	56.2	23.02	203
>gi|148530739|gb|ABQ82738.1| UDP-N-acetylglucosamine 1-carboxyvinyltransferase [Lactobacillus reuteri DSM 20016	4	4	2	3	1	1	15.8	47.242	438
>gi|148530965|gb|ABQ82964.1| bacterial translation initiation factor 2 (bIF-2) [Lactobacillus reuteri DSM 20016];>	8	3	5	5	4	8	17.2	83.537	752
>gi|227071794|gb|EEI10083.1| glycerone kinase [Lactobacillus reuteri MM2-3];>	9	1	5	4	3	6	25.2	62.132	575
>gi|148530288|gb|ABQ82287.1| LSU ribosomal protein L9P [Lactobacillus reuteri DSM 20016];>	11	8	9	7	8	7	61.3	16.692	150
>gi|148530542|gb|ABQ82541.1| lysyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	15	11	11	10	9	4	33.7	58.296	508

>gi|148532090|gb|ABQ84089.1| hypothetical protein Lreu_1851 [Lactobacillus reuteri DSM 20016];> L	2	1	2	2	2	1	52.5	7.0627	61
>gi|148531050|gb|ABQ83049.1| Inorganic diphosphatase [Lactobacillus reuteri DSM 20016];>	9	4	4	7	6	7	36.3	34.194	311
>gi|148531705|gb|ABQ83704.1| SSU ribosomal protein S11P [Lactobacillus reuteri DSM 20016];>	5	3	5	4	5	3	58.1	13.759	129
>gi|183225766|dbj|BAG26283.1| aspartate-ammonia ligase [Lactobacillus reuteri JCM 1112];>	7	6	2	5	3	4	25.4	39.72	339
>gi|183224315|dbj|BAG24832.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	3	2	2	2	2	2	55.7	11.883	106
>gi|148530407|gb|ABQ82406.1| 5-(carboxyamino)imidazole ribonucleotide mutase [Lactobacillus reuteri DSM 20016];>	6	3	4	5	4	1	62.1	16.958	161
>gi|148531889|gb|ABQ83888.1| prolinase, Serine peptidase, MEROPS family S33 [Lactobacillus reuteri DSM 20016];>	4	2	3	3	2	2	19.1	34.841	303
>gi|148531413|gb|ABQ83412.1| signal recognition particle subunit FFH/SRP54 (srp54) [Lactobacillus reuteri DSM 20016	7	4	4	3	3	2	16.6	54.114	481
>gi|227071709|gb|EEI10000.1| ATP-dependent RNA helicase [Lactobacillus reuteri MM2-3];>	6	5	1	0	0	1	17.8	53.802	467
>gi|148530526|gb|ABQ82525.1| alanine racemase [Lactobacillus reuteri DSM 20016	4	2	3	2	3	1	14.7	41.162	375
>gi|148530594|gb|ABQ82593.1| ribonucleoside-diphosphate reductase class Ib alpha subunit [Lactobacillus reuteri DSM 20016|	17	15	12	10	9	10	34.7	82.738	723
>gi|148530370|gb|ABQ82369.1| seryl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	9	6	7	5	8	7	32	49.29	435
>gi|148531225|gb|ABQ83224.1| hypothetical protein Lreu_0962 [Lactobacillus reuteri DSM 20016];>	4	1	1	1	4	2	14.1	88.735	774
>gi|148531486|gb|ABQ83485.1| two component transcriptional regulator, winged helix family [Lactobacillus reuteri DSM 20016];>	3	2	1	2	2	2	20.6	26.33	228
>gi|183224607|dbj|BAG25124.1| pyruvate dehydrogenase complex E1 component alpha subunit [Lactobacillus reuteri JCM 1112	7	5	4	5	3	5	28.6	41.361	371
>gi|148530556|gb|ABQ82555.1| NH(3)-dependent NAD(+) synthetase [Lactobacillus reuteri DSM 20016];>	7	6	2	4	2	2	42.9	30.54	275
>gi|227070039|gb|EEI08417.1| peptidase T [Lactobacillus reuteri MM2-3];>	6	4	3	3	5	2	24.1	46.484	419
>gi|148530942|gb|ABQ82941.1| hydroxymethylglutaryl-CoA synthase [Lactobacillus reuteri DSM 20016];>	4	4	3	3	3	1	16.4	42.757	385
>gi|148531575|gb|ABQ83574.1| ribonucleoside-triphosphate reductase class III catalytic subunit / ribonucleoside-triphosphate reductase [Lactobacillus reuteri DSM 20016];>	4	1	2	1	2	1	5	85.159	744
>gi|296313256|gb|ACZ97544.2| D-alanine-D-alanine ligase [Lactobacillus reuteri]	3	3	2	2	2	2	17.9	33.684	301
>gi|183227099|dbj|BAG27615.1| glucose kinase [Lactobacillus fermentum IFO 3956];>	4	4	4	2	1	2	17.2	33.926	320
>gi|148530994|gb|ABQ82993.1| GatB/Yqey domain protein [Lactobacillus reuteri DSM 20016];>	3	2	2	2	3	1	25.7	16.725	148
>gi|148531735|gb|ABQ83734.1| SSU ribosomal protein S12P [Lactobacillus reuteri DSM 20016];>	8	6	5	5	5	4	56.8	15.503	139
>gi|148531417|gb|ABQ83416.1| RNAse III [Lactobacillus reuteri DSM 20016];>	5	2	3	4	3	3	39.9	26.606	233
>gi|148531286|gb|ABQ83285.1| Phosphoglycerate mutase [Lactobacillus reuteri DSM 20016];>	10	9	6	7	7	4	38.1	30.347	278

>gi|133930489|gb|ABO43816.1| conserved hypothetical protein [Lactobacillus reuteri];>	3	1	2	2	2	3	54.8	8.2855	73
>gi|148530501|gb|ABQ82500.1| CTP synthase [Lactobacillus reuteri DSM 20016];>	11	6	9	6	6	3	30.7	59.6	534
>gi|148531497|gb|ABQ83496.1| LSU ribosomal protein L20P [Lactobacillus reuteri DSM 20016];>	8	8	7	6	5	5	46.2	13.515	117
>gi|148530553|gb|ABQ82552.1| aspartate racemase [Lactobacillus reuteri DSM 20016];> 1112];>	7	6	4	5	4	4	39	26.763	236
>gi|194453683|gb|EDX42580.1| translation initiation factor IF-3 [Lactobacillus reuteri 100-23];>	6	3	4	4	2	3	46.5	19.488	170
>gi|183225693|dbj|BAG26210.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	4	3	2	2	3	2	16.2	41.737	364
>gi|148530484|gb|ABQ82483.1| dimethyladenosine transferase [Lactobacillus reuteri DSM 20016];>	7	4	2	2	2	4	26.9	33.039	297
>gi|148530409|gb|ABQ82408.1| Adenylosuccinate lyase [Lactobacillus reuteri DSM 20016];>	7	0	5	5	4	3	23.2	49.454	431
>gi|227186201|gb|EEI66272.1| response regulator [Lactobacillus reuteri CF48-3A];>	6	4	4	4	5	4	41.4	26.602	237
>gi|148530993|gb|ABQ82992.1| SSU ribosomal protein S21P [Lactobacillus reuteri DSM 20016];>	5	3	4	4	4	3	44.4	7.7178	63
>gi|148531037|gb|ABQ83036.1| degV family protein [Lactobacillus reuteri DSM 20016];>	8	7	4	5	5	4	35.4	30.742	280
>gi|183225222|dbj|BAG25739.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	5	1	3	3	3	1	46.5	18.557	170
>gi|148531899|gb|ABQ83898.1| short-chain dehydrogenase/reductase SDR [Lactobacillus reuteri DSM 20016	6	4	2	1	2	2	37.5	26.924	251
>gi|148530952|gb|ABQ82951.1| uridylate kinase [Lactobacillus reuteri DSM 20016];>	9	7	6	5	5	4	42.5	25.876	240
>gi|148531003|gb|ABQ83002.1| GTP-binding protein Era [Lactobacillus reuteri DSM 20016];>	3	2	2	3	1	1	13.6	33.79	301
>gi|148530492|gb|ABQ82491.1| ribose-phosphate pyrophosphokinase [Lactobacillus reuteri DSM 20016	7	6	4	3	3	1	24.9	36.16	329
>gi|148531468|gb|ABQ83467.1| LSU ribosomal protein L33P [Lactobacillus reuteri DSM 20016];>	3	3	3	3	3	3	49	5.9998	49
>gi|148530899|gb|ABQ82898.1| Dihydrolipoyllysine-residue succinyltransferase [Lactobacillus reuteri DSM 20016];>	3	1	1	1	1	2	10.4	48.372	444
>gi|148530412|gb|ABQ82411.1| phosphoribosylformylglycinamidine synthase subunit I [Lactobacillus reuteri DSM 20016];>	5	2	5	3	3	2	33.6	24.732	226
>gi|183227331|dbj|BAG27847.1| 30S ribosomal protein S19 [Lactobacillus fermentum IFO 3956];>	7	5	6	4	4	6	37.6	10.489	93
>gi|148531529|gb|ABQ83528.1| phosphomethylpyrimidine kinase [Lactobacillus reuteri DSM 20016L	3	1	1	1	0	1	22.9	29.317	271
>gi|148530995|gb|ABQ82994.1| Choloylglycine hydrolase [Lactobacillus reuteri DSM 20016];>	8	6	0	3	1	0	42.2	36.104	325
>gi|148531731|gb|ABQ83730.1| SSU ribosomal protein S10P [Lactobacillus reuteri DSM 20016];>	10	8	8	8	9	7	73.5	11.777	102
>gi|148532002|gb|ABQ84001.1| NrdI family protein [Lactobacillus reuteri DSM 20016];>	4	4	3	3	3	3	27.1	17.501	155
>gi|112943421|gb|ABI26308.1| carbamate kinase [Lactobacillus reuteri];>	7	2	2	1	4	2	35.8	33.007	310
>gi|133930543|gb|ABO43842.1| tryptophanyl-tRNA synthetase [Lactobacillus reuteri];>	5	4	3	3	4	3	22.4	38.079	340
>gi|148530877|gb|ABQ82876.1| ribose-phosphate pyrophosphokinase [Lactobacillus reuteri DSM 20016];>	9	7	5	5	0	3	33.3	36.06	324

>gi|148530673|gb|ABQ82672.1| UDP-N-acetylmuramate dehydrogenase [Lactobacillus reuteri DSM 20016	4	4	3	2	2	2	19.1	32.389	298
>gi|227071578|gb|EEI09875.1| ribokinase [Lactobacillus reuteri MM2-3];>	3	3	2	2	2	3	11.7	34.703	326
>gi|227184829|gb|EEI64900.1| cysteinyl-tRNA synthetase [Lactobacillus reuteri CF48-3A];>	4	1	2	1	2	1	10.3	54.866	478
>gi|148530914|gb|ABQ82913.1| SSU ribosomal protein S15P [Lactobacillus reuteri DSM 20016	5	4	3	3	3	3	52.8	10.416	89
>gi|148531141|gb|ABQ83140.1| Purine nucleosidase [Lactobacillus reuteri DSM 20016	4	1	2	2	0	0	24.8	34.734	314
>gi|148530515|gb|ABQ82514.1| DEAD/DEAH box helicase domain protein [Lactobacillus reuteri DSM 20016];>	8	7	2	1	1	1	22.5	56.522	498
>gi|337728387|emb|CCC03488.1| GTPases [Lactobacillus reuteri ATCC 53608]	6	3	2	3	1	4	16.9	47.753	425
>gi|183225282|dbj|BAG25799.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	3	1	1	1	1	1	12.6	49.015	438
>gi|148530772|gb|ABQ82771.1| thiamine biosynthesis/tRNA modification protein ThiI [Lactobacillus reuteri DSM 20016];>	8	7	0	2	1	1	30.5	45.726	406
>gi|112943728|gb|ABI26320.1| fructose-2,6-bisphosphatase [Lactobacillus reuteri];>	5	4	4	3	2	2	33.2	24.287	217
>gi|148531523|gb|ABQ83522.1| PpiC-type peptidyl-prolyl cis-trans isomerase [Lactobacillus reuteri DSM 20016];>	3	3	1	0	3	2	11.2	34.663	312
>gi|148530411|gb|ABQ82410.1| phosphoribosylformylglycinamidine synthase, purS [Lactobacillus reuteri DSM 20016];>	3	0	2	2	3	1	54.9	9.3323	82
>gi|148531415|gb|ABQ83414.1| signal recognition particle-docking protein FtsY [Lactobacillus reuteri DSM 20016];>	4	4	4	4	4	4	11.2	55.604	508
>gi|183227650|dbj|BAG28166.1| threonyl-tRNA synthase [Lactobacillus fermentum IFO 3956	5	4	2	3	2	3	8	72.259	634
>gi|148530821|gb|ABQ82820.1| protein of unknown function DUF28 [Lactobacillus reuteri DSM 20016];> p	5	3	3	3	5	3	32.3	27.161	248
>gi|148531458|gb|ABQ83457.1| aminotransferase [Lactobacillus reuteri DSM 20016	2	1	1	2	2	1	7.1	43.367	393
>gi|183224869|dbj|BAG25386.1| cell division protein [Lactobacillus reuteri JCM 1112];>	4	2	1	2	4	2	43.1	14.251	123
>gi|148531448|gb|ABQ83447.1| LSU ribosomal protein L27P [Lactobacillus reuteri DSM 20016];>	4	4	3	3	3	4	48.4	9.9211	93
>gi|148531400|gb|ABQ83399.1| phage antirepressor protein [Lactobacillus reuteri DSM 20016];>	2	1	0	0	1	2	12.7	30.203	267
>gi|227071437|gb|EEI09740.1| transcriptional antiterminator NusG [Lactobacillus reuteri MM2-3];>	3	3	3	3	2	1	28.5	20.875	186
>gi|148531005|gb|ABQ83004.1| glycyl-tRNA synthetase alpha chain [Lactobacillus reuteri DSM 20016];>	5	5	4	4	4	3	19.2	37.843	328
>gi|148530913|gb|ABQ82912.1| SSU ribosomal protein S20P [Lactobacillus reuteri DSM 20016];>	3	2	3	3	3	2	34.5	9.2436	84
>gi|148530415|gb|ABQ82414.1| phosphoribosylformylglycinamidine cyclo-ligase [Lactobacillus reuteri DSM 20016];>	10	8	6	7	6	3	49	36.975	345
>gi|148531712|gb|ABQ83711.1| LSU ribosomal protein L30P [Lactobacillus reuteri DSM 20016];>	3	3	3	3	3	2	58.3	6.5566	60
>gi|148531102|gb|ABQ83101.1| hypothetical protein Lreu_0838 [Lactobacillus reuteri DSM 20016];>	12	11	6	8	5	8	37.4	39.346	358

>gi|148530311|gb|ABQ82310.1| aldehyde dehydrogenase [Lactobacillus reuteri DSM 20016];>	3	2	1	0	1	1	9.6	50.505	457
>gi|148531407|gb|ABQ83406.1| LSU ribosomal protein L19P [Lactobacillus reuteri DSM 20016	10	10	9	8	8	8	49.2	14.824	128
>gi|183224788|dbj|BAG25305.1| hypothetical phage protein [Lactobacillus reuteri JCM 1112	5	2	3	3	2	3	28.8	25.075	219
>gi|183225283|dbj|BAG25800.1| putative autolysin [Lactobacillus reuteri JCM 1112];>	2	0	1	1	2	1	7.9	60.393	532
>gi|148530734|gb|ABQ82733.1| ATP synthase F1 subcomplex alpha subunit [Lactobacillus reuteri DSM 20016	12	8	5	9	6	3	25.7	55.226	509
>gi|148530873|gb|ABQ82872.1| tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase [Lactobacillus reuteri DSM 20016	5	3	2	1	1	4	21	42.769	377
>gi|148531869|gb|ABQ83868.1| GTP-binding protein, HSR1-related [Lactobacillus reuteri DSM 20016];>	7	4	3	4	2	5	19.3	47.758	425
>gi|227071698|gb|EEI09989.1| phosphoesterase [Lactobacillus reuteri MM2-3];>	1	1	1	0	0	0	8.5	19.857	176
>gi|148531433|gb|ABQ83432.1| DNA-directed RNA polymerase subunit omega [Lactobacillus reuteri DSM 20016];>	4	3	3	2	3	3	75	7.9219	72
>gi|148531769|gb|ABQ83768.1| DJ-1 family protein [Lactobacillus reuteri DSM 20016];>	4	1	3	4	1	1	31.1	20.995	190
>gi|148531430|gb|ABQ83429.1| methionyl-tRNA formyltransferase [Lactobacillus reuteri DSM 20016];>	3	2	1	1	1	1	15.5	34.729	317
>gi|148530792|gb|ABQ82791.1| RecA protein [Lactobacillus reuteri DSM 20016];>	5	2	1	2	1	4	19.1	39.063	362
>gi|148531196|gb|ABQ83195.1| degV family protein [Lactobacillus reuteri DSM 20016];>	4	0	2	3	1	2	27.4	32.228	292
>gi|148530459|gb|ABQ82458.1| methionine-R-sulfoxide reductase [Lactobacillus reuteri DSM 20016	4	3	1	2	2	1	36.6	16.101	142
>gi|148531776|gb|ABQ83775.1| flavocytochrome c [Lactobacillus reuteri DSM 20016];>	9	7	7	7	6	6	27.8	50.13	464
>gi|112943339|gb|ABI26305.1| sphingosine kinase [Lactobacillus reuteri];>	2	2	0	0	1	0	8.9	37.14	337
>gi|227071203|gb|EEI09517.1| UDP-galactopyranose mutase [Lactobacillus reuteri MM2-3];>	6	4	5	5	5	2	21.1	44.975	384
>gi|227185166|gb|EEI65237.1| aspartate semialdehyde dehydrogenase [Lactobacillus reuteri CF48-3A];>	6	2	3	4	5	3	29.3	38.935	358
>gi|148531329|gb|ABQ83328.1| FAD-dependent pyridine nucleotide-disulfide oxidoreductase [Lactobacillus reuteri DSM 20016];>	4	2	1	2	2	2	7.6	60.056	555
>gi|148530569|gb|ABQ82568.1| ribose-5-phosphate isomerase [Lactobacillus reuteri DSM 20016];>	5	5	4	4	4	3	26	25.036	227
>gi|133930447|gb|ABO43795.1| E1 component beta subunit [Lactobacillus reuteri];>	3	1	2	2	1	2	18.8	35.243	325
>gi|148530634|gb|ABQ82633.1| SSU ribosomal protein S30P [Lactobacillus reuteri DSM 20016];>	5	3	3	1	3	3	46.2	21.144	182
>gi|227070968|gb|EEI09291.1| glutamate-1-semialdehyde 2,1-aminomutase [Lactobacillus reuteri MM2-3	5	3	3	4	4	2	18.1	48.026	443
>gi|148531476|gb|ABQ83475.1| phenylalanyl-tRNA synthetase, alpha subunit [Lactobacillus reuteri DSM 20016];>	3	1	1	0	2	1	11.5	39.026	348
>gi|148531008|gb|ABQ83007.1| RNA polymerase, sigma 70 subunit, RpoD [Lactobacillus reuteri DSM 20016];>	5	4	2	4	2	2	17.6	43.385	380

>gi|148531221|gb|ABQ83220.1| 1-deoxy-D-xylulose-5-phosphate synthase [Lactobacillus reuteri DSM 20016];>	6	3	2	4	4	3	18.3	65.608	591
>gi|148530819|gb|ABQ82818.1| transcriptional regulator, LacI family [Lactobacillus reuteri DSM 20016];>	7	5	5	4	4	3	27.4	36.722	336
>gi|148530319|gb|ABQ82318.1| OsmC family protein [Lactobacillus reuteri DSM 20016];>	3	3	2	2	2	1	22.5	15.724	142
>gi|337729366|emb|CCC04496.1| conserved hypothetical protein [Lactobacillus reuteri ATCC 53608]	3	3	0	0	0	0	8.2	44.72	402
>gi|227070870|gb|EEI09194.1| iron-containing alcohol dehydrogenase [Lactobacillus reuteri MM2-3];>	3	0	2	1	2	1	12.9	40.929	379
>gi|148531487|gb|ABQ83486.1| LSU ribosomal protein L32P [Lactobacillus reuteri DSM 20016];>	2	2	2	2	2	1	25.4	6.5727	59
>gi|148530523|gb|ABQ82522.1| D-alanine-activating enzyme [Lactobacillus reuteri DSM 20016];>	9	4	5	8	3	1	28.3	55.989	508
>gi|148530389|gb|ABQ82388.1| inosine-5-monophosphate dehydrogenase [Lactobacillus reuteri DSM 20016	5	4	4	4	5	3	23.7	39.373	380
>gi|148531780|gb|ABQ83779.1| Peptidylprolyl isomerase [Lactobacillus reuteri DSM 20016	4	3	3	3	4	2	35.5	22.199	197
>gi|183225758|dbj|BAG26275.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112 [	3	2	3	3	2	2	20.7	22.577	203
>gi|183225128|dbj|BAG25645.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112	3	1	0	2	0	0	12.1	35.441	297
>gi|148530538|gb|ABQ82537.1| hypoxanthine phosphoribosyltransferase [Lactobacillus reuteri DSM 20016];>	5	3	3	2	2	2	31.1	20.464	180
>gi|148531027|gb|ABQ83026.1| cytidylate kinase [Lactobacillus reuteri DSM 20016	4	2	1	1	3	3	24.1	25.015	228
>gi|148531233|gb|ABQ83232.1| nucleotide deoxyribosyltransferase [Lactobacillus reuteri DSM 20016];>	1	1	0	0	0	1	11.9	18.201	160
>gi|148531479|gb|ABQ83478.1| tRNA/rRNA methyltransferase (SpoU) [Lactobacillus reuteri DSM 20016];>	3	3	0	0	1	2	16.7	28.31	258
>gi|148530337|gb|ABQ82336.1| glutamate-cysteine ligase [Lactobacillus reuteri DSM 20016	3	1	1	0	1	2	10.8	51.01	446
>gi|183225058|dbj|BAG25575.1| phage major head protein [Lactobacillus reuteri JCM 1112];>	1	1	1	0	1	1	3.8	43.887	394
>gi|227186252|gb|EEI66323.1| possible aspartate transaminase [Lactobacillus reuteri CF48-3A];>	6	6	0	2	0	0	24.3	42.745	395
>gi|148531241|gb|ABQ83240.1| DSBA oxidoreductase [Lactobacillus reuteri DSM 20016];>J	2	2	0	1	0	0	16.3	24.566	215
>gi|148530690|gb|ABQ82689.1| glucose-6-phosphate isomerase [Lactobacillus reuteri DSM 20016];>	3	3	0	0	0	0	10.6	50.35	452
>gi|148530481|gb|ABQ82480.1| methionyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	7	6	5	4	4	2	12.3	76.638	675
>gi|148530860|gb|ABQ82859.1| cell division protein FtsA [Lactobacillus reuteri DSM 20016];>	2	1	2	1	1	1	5.3	50.485	457
>gi|148531330|gb|ABQ83329.1| Peroxiredoxin [Lactobacillus reuteri DSM 20016];>	6	3	1	2	2	1	34.2	21.041	187
>gi|148531472|gb|ABQ83471.1| transcription elongation factor GreA [Lactobacillus reuteri DSM 20016];>	7	5	5	5	3	4	60.1	18.019	158
>gi|148531067|gb|ABQ83066.1| prophage antirepressor [Lactobacillus reuteri DSM 20016];>	2	1	0	0	1	2	12.1	28.734	257
>gi|148531094|gb|ABQ83093.1| hypothetical protein Lreu_0830 [Lactobacillus reuteri DSM	3	1	3	2	3	0	22.7	16.079	141

20016];>									
>gi|183225267|dbj|BAG25784.1| dextransucrase [Lactobacillus reuteri JCM 1112	1	1	1	1	1	1	1.1	167.91	1488
>gi|148531941|gb|ABQ83940.1| porphobilinogen synthase [Lactobacillus reuteri DSM 20016	1	0	1	1	0	0	4.6	35.84	323
>gi|148531613|gb|ABQ83612.1| hypothetical protein Lreu_1363 [Lactobacillus reuteri DSM 20016];>	2	1	1	1	1	2	13.8	19.693	189
>gi|148530657|gb|ABQ82656.1| protein of unknown function UPF0052 and CofD [Lactobacillus reuteri DSM 20016];>	4	3	2	2	0	0	17.7	36.198	328
>gi|148531444|gb|ABQ83443.1| protein of unknown function DUF322 [Lactobacillus reuteri DSM 20016];>	5	4	4	2	1	2	40.7	15.835	145
>gi|148531785|gb|ABQ83784.1| hypothetical protein Lreu_1539 [Lactobacillus reuteri DSM 20016];>	4	3	2	3	1	0	39	16.827	146
>gi|148531708|gb|ABQ83707.1| bacterial translation initiation factor 1 (bIF-1) [Lactobacillus reuteri DSM 20016];>	5	1	2	4	4	3	75	8.1615	72
>gi|183226401|dbj|BAG26917.1| cell division initiation protein [Lactobacillus fermentum IFO 3956];>	4	3	4	3	4	4	11.4	25.101	228
>gi|148531846|gb|ABQ83845.1| tRNA/rRNA methyltransferase (SpoU) [Lactobacillus reuteri DSM 20016];>	4	2	1	2	1	1	22.4	31.804	290
>gi|148530684|gb|ABQ82683.1| Cof-like hydrolase [Lactobacillus reuteri DSM 20016];>	3	2	2	1	2	1	16.8	30.206	268
>gi|227070209|gb|EEI08583.1| diaminopimelate decarboxylase [Lactobacillus reuteri MM2-3];>	5	5	0	1	1	1	13.6	48.355	441
>gi|148531975|gb|ABQ83974.1| LSU ribosomal protein L29P [Lactobacillus reuteri DSM 20016];>	5	0	1	3	2	2	26.8	51.127	477
>gi|148530611|gb|ABQ82610.1| peptidase M22, glycoprotease [Lactobacillus reuteri DSM 20016];>	3	1	2	2	1	1	17	26.806	241
>gi|148530405|gb|ABQ82404.1| acetolactate synthase, catabolic [Lactobacillus reuteri DSM 20016	10	6	4	6	4	2	32.6	60.994	559
>gi|148532050|gb|ABQ84049.1| hypothetical protein Lreu_1810 [Lactobacillus reuteri DSM 20016];>	8	4	4	4	3	5	20.4	46.453	411
>gi|148531719|gb|ABQ83718.1| LSU ribosomal protein L24P [Lactobacillus reuteri DSM 20016];>	2	2	1	1	1	1	10.8	10.975	102
>gi|148530791|gb|ABQ82790.1| competence/damage-inducible protein cinA [Lactobacillus reuteri DSM 20016];>	1	0	0	0	1	1	4.8	45.373	415
>gi|148530881|gb|ABQ82880.1| Tetrahydrodipicolinate succinyltransferase N-terminal domain protein [Lactobacillus reuteri DSM 20016];>	2	1	1	1	1	1	27.1	24.876	236
>gi|148530680|gb|ABQ82679.1| glutamine--fructose-6-phosphate transaminase [Lactobacillus reuteri DSM 20016];>	10	4	3	4	6	1	30.7	66.611	606
>gi|148530883|gb|ABQ82882.1| dihydrodipicolinate synthase [Lactobacillus reuteri DSM 20016];>	6	4	2	2	2	4	32.6	33.823	307
>gi|148532020|gb|ABQ84019.1| PEBP family protein [Lactobacillus reuteri DSM 20016];> J	1	1	0	1	1	0	10.7	18.294	168
>gi|148530788|gb|ABQ82787.1| peptidase M16 domain protein [Lactobacillus reuteri DSM 20016];>	4	3	1	1	1	1	15.5	49.689	432

>gi|194453256|gb|EDX42154.1| Tetrahydrodipicolinate succinyltransferase domain protein [Lactobacillus reuteri 100-23];>	2	1	0	1	0	0	27.1	24.89	236
>gi|148531434|gb|ABQ83433.1| guanylate kinase [Lactobacillus reuteri DSM 20016];>	2	2	2	2	2	2	10.7	23.546	206
>gi|148531057|gb|ABQ83056.1| gluconate kinase, FGGY family [Lactobacillus reuteri DSM 20016];>	8	3	5	7	5	3	30.8	55.417	509
>gi|183224052|dbj|BAG24569.1| beta-phosphoglucomutase [Lactobacillus reuteri JCM 1112];>	7	1	5	1	2	2	44.4	24.587	225
>gi|148530659|gb|ABQ82658.1| ATP-dependent Clp protease proteolytic subunit ClpP [Lactobacillus reuteri DSM 20016];>	4	2	3	3	4	2	44.7	21.433	197
>gi|227185294|gb|EEI65365.1| HAD superfamily hydrolase [Lactobacillus reuteri CF48-3A];>	5	3	4	4	3	4	22.7	30.425	273
>gi|148530442|gb|ABQ82441.1| triosephosphate isomerase [Lactobacillus reuteri DSM 20016];>	4	1	3	4	2	1	35.5	28.377	256
>gi|148531423|gb|ABQ83422.1| LSU ribosomal protein L28P [Lactobacillus reuteri DSM 20016];>	3	3	3	3	3	3	31.1	6.9271	61
>gi|227070541|gb|EEI08874.1| phosphoribosylaminoimidazole carboxylase [Lactobacillus reuteri MM2-3];>	7	4	6	2	2	2	32.8	42.428	384
>gi|148532170|gb|ABQ84169.1| UDP-N-acetylmuramyl-tripeptide synthetase [Lactobacillus reuteri DSM 20016];>	5	3	2	4	1	1	14.5	57.457	516
>gi|148530622|gb|ABQ82621.1| CoA-binding domain protein [Lactobacillus reuteri DSM 20016];>	4	2	0	1	2	3	29.9	23.939	214
>gi|148531276|gb|ABQ83275.1| thiamine-phosphate diphosphorylase [Lactobacillus reuteri DSM 20016];>	2	1	2	2	1	0	18.1	23.849	215
>gi|337728189|emb|CCC03281.1| UDP-galactopyranose mutase [Lactobacillus reuteri ATCC 53608]	5	5	3	3	3	2	16.7	43.523	372
>gi|130893190|gb|ABO32597.1| ATP-dependent Clp protease ATP-binding subunit ClpC [Lactobacillus reuteri];>	7	3	5	4	6	6	12.5	92.89	830
>gi|337728167|emb|CCC03258.1| putative cold shock protein [Lactobacillus reuteri ATCC 53608]	4	4	1	1	0	0	71.2	7.2839	66
>gi|148530641|gb|ABQ82640.1| glycerol 3-phosphate dehydrogenase (NAD(P)+) [Lactobacillus reuteri DSM 20016	7	4	1	3	5	3	26	36.905	338
>gi|148531418|gb|ABQ83417.1| acyl carrier protein [Lactobacillus reuteri DSM 20016];>	3	2	2	3	3	2	45.2	9.4942	84
>gi|227070893|gb|EEI09216.1| DNA helicase [Lactobacillus reuteri MM2-3];>	6	5	3	3	2	3	10.2	90.014	775
>gi|133930439|gb|ABO43791.1| 2-keto-4-pentenoate hydratase [Lactobacillus reuteri];>	2	2	0	1	1	1	9.5	29.068	263
>gi|148530846|gb|ABQ82845.1| 6-phosphogluconolactonase [Lactobacillus reuteri DSM 20016	5	4	3	0	2	0	21.6	37.901	342
>gi|227070638|gb|EEI08968.1| DNA-binding response regulator [Lactobacillus reuteri MM2-3];>	2	1	2	2	2	2	11.6	27.524	241
>gi|148530493|gb|ABQ82492.1| hypothetical protein Lreu_0222 [Lactobacillus reuteri DSM 20016	3	2	2	3	3	3	24.1	25.919	245
>gi|148530511|gb|ABQ82510.1| protein tyrosine phosphatase [Lactobacillus reuteri DSM 20016];>	2	0	2	0	0	0	26.6	17.398	154
>gi|148530787|gb|ABQ82786.1| peptidase M16 domain protein [Lactobacillus reuteri DSM 20016	3	1	2	2	0	2	13.3	47.131	415
>gi|148531897|gb|ABQ83896.1| FAD-dependent pyridine nucleotide-disulfide oxidoreductase [Lactobacillus reuteri DSM 20016];>	3	1	3	3	3	1	15.7	35.791	332

>gi|148530924|gb|ABQ82923.1| GTP-binding protein Obg/CgtA [Lactobacillus reuteri DSM 20016];>	10	5	5	6	3	2	28.8	47.975	438
>gi|148530514|gb|ABQ82513.1| UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase [Lactobacillus reuteri DSM 20016];>	4	2	2	2	1	1	12.2	50.527	459
>gi|148530344|gb|ABQ82343.1| guanosine monophosphate reductase [Lactobacillus reuteri DSM 20016	3	2	2	1	2	2	15.1	35.96	324
>gi|148531928|gb|ABQ83927.1| ybaK/ebsC protein [Lactobacillus reuteri DSM 20016	1	0	1	1	1	1	11.9	18.647	168
>gi|148530723|gb|ABQ82722.1| translation factor SUA5 [Lactobacillus reuteri DSM 20016	3	1	0	2	2	1	12.6	37.261	342
>gi|148531462|gb|ABQ83461.1| hypothetical protein Lreu_1204 [Lactobacillus reuteri DSM 20016];>	3	2	2	2	2	3	69	6.3153	58
>gi|148530724|gb|ABQ82723.1| serine hydroxymethyltransferase [Lactobacillus reuteri DSM 20016	7	4	5	4	3	4	24.8	44.95	411
>gi|148530416|gb|ABQ82415.1| formyltetrahydrofolate-dependent phosphoribosylglycinamide formyltransferase [Lactobacillus reuteri DSM 20016	2	0	2	1	2	0	35.3	21.163	190
>gi|148531989|gb|ABQ83988.1| microcompartments protein [Lactobacillus reuteri DSM 20016];> reuteri DSM 20016];>gi|183225636|dbj|BAG26153.1| propanediol utilization protein PduA [Lacto	1	0	1	1	1	1	33.3	9.5708	93
>gi|300379511|gb|ADK08388.1| OsmC family protein [Lactobacillus reuteri]	3	2	2	1	2	2	19.7	15.723	142
>gi|148531530|gb|ABQ83529.1| malate dehydrogenase (NAD) [Lactobacillus reuteri DSM 20016];>	6	5	3	4	4	4	29.5	33.353	312
>gi|227071420|gb|EEI09725.1| FMN reductase [Lactobacillus reuteri MM2-3];>	3	3	3	3	3	3	13	26.152	238
>gi|148530971|gb|ABQ82970.1| GrpE protein [Lactobacillus reuteri DSM 20016];>	2	1	1	2	2	2	11.6	21.43	190
>gi|148531795|gb|ABQ83794.1| Esterase/lipase-like protein [Lactobacillus reuteri DSM 20016];>	4	2	4	1	2	2	29.8	26.628	238
>gi|183225127|dbj|BAG25644.1| hypothetical protein [Lactobacillus reuteri JCM 1112];>	5	4	1	4	3	2	16.5	40.161	352
>gi|148530605|gb|ABQ82604.1| Uroporphyrin-III C/tetrapyrrole (Corrin/Porphyrin) methyltransferase [Lactobacillus reuteri DSM 20016];>	2	1	0	0	1	0	14.6	32.603	288
>gi|148531075|gb|ABQ83074.1| RecT protein [Lactobacillus reuteri DSM 20016	2	1	2	2	2	1	7.4	34.762	309
>gi|148530868|gb|ABQ82867.1| NUDIX hydrolase [Lactobacillus reuteri DSM 20016];>	3	2	0	1	0	0	22.4	20.827	183
>gi|148532082|gb|ABQ84081.1| D-Ala-D-Ala carboxypeptidase A, Serine peptidase, MEROPS family S11 [Lactobacillus reuteri DSM 20016];>	2	0	1	1	0	0	9.7	44.354	403
>gi|148530602|gb|ABQ82601.1| protein of unknown function DUF970 [Lactobacillus reuteri DSM 20016];>	1	0	1	1	1	0	15.9	11.874	107
>gi|148530286|gb|ABQ82285.1| SSU ribosomal protein S18P [Lactobacillus reuteri DSM 20016];>	4	3	3	3	2	1	51.3	9.1536	78
>gi|148530885|gb|ABQ82884.1| aminotransferase [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	4.6	42.991	394
>gi|148531640|gb|ABQ83639.1| DEAD/DEAH box helicase domain protein [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	3	48.772	433
>gi|148530810|gb|ABQ82809.1| glutamate racemase [Lactobacillus reuteri DSM 20016];>	2	2	1	1	0	1	15.7	28.92	267
>gi|148530966|gb|ABQ82965.1| ribosome-binding factor A [Lactobacillus reuteri DSM 20016];>	3	2	2	1	2	2	16.8	13.412	119

>gi|148530664|gb|ABQ82663.1| RNAse R [Lactobacillus reuteri DSM 20016];>	6	5	4	4	5	4	9.4	91.867	801
>gi|148532172|gb|ABQ84171.1| glucose inhibited division protein A [Lactobacillus reuteri DSM 20016];>	4	3	4	3	4	3	7.9	71.918	647
>gi|148531131|gb|ABQ83130.1| hypothetical protein Lreu_0867 [Lactobacillus reuteri DSM 20016];>	5	3	3	2	5	2	69.2	10.576	91
>gi|148530760|gb|ABQ82759.1| Glyoxalase/bleomycin resistance protein/dioxygenase [Lactobacillus reuteri DSM 20016	4	3	2	0	2	1	45.2	15.166	135
>gi|148530279|gb|ABQ82278.1| DNA polymerase III, beta subunit [Lactobacillus reuteri DSM 20016];>	7	5	4	5	3	4	26.3	41.831	380
>gi|148531622|gb|ABQ83621.1| lipopolysaccharide biosynthesis protein [Lactobacillus reuteri DSM 20016];>r	2	2	1	1	1	1	15.3	22.766	209
>gi|148530920|gb|ABQ82919.1| small GTP-binding protein [Lactobacillus reuteri DSM 20016];>	2	1	1	2	0	0	19.4	22.641	196
>gi|148531473|gb|ABQ83472.1| uridine kinase [Lactobacillus reuteri DSM 20016	4	1	4	3	2	2	38.5	25.134	218
>gi|148531188|gb|ABQ83187.1| putative RNA methylase [Lactobacillus reuteri DSM 20016	1	0	1	1	1	0	5	44.845	398
>gi|148531409|gb|ABQ83408.1| tRNA (Guanine37-N(1)-) methyltransferase [Lactobacillus reuteri DSM 20016	2	2	0	1	0	0	7.9	28.476	252
>gi|148530539|gb|ABQ82538.1| membrane protease FtsH catalytic subunit [Lactobacillus reuteri DSM 20016];>gi|148543505|ref|YP_001270875.1| ATP-dependent metalloprotease FtsH [Lactobacillus reuteri DSM 20016];>gi|194454716|gb|EDX43613.1| ATP-dependent metall	4	0	2	4	2	1	8.4	77.238	702
>gi|148531585|gb|ABQ83584.1| ATPase AAA-2 domain protein [Lactobacillus reuteri DSM 20016];>	7	2	2	1	2	4	15.8	81.867	734
>gi|227070055|gb|EEI08433.1| GTP-binding protein EngA [Lactobacillus reuteri MM2-3];>	5	3	2	2	2	4	13.2	49.465	440
>gi|227071710|gb|EEI10001.1| phosphoesterase [Lactobacillus reuteri MM2-3];>	5	4	3	2	2	3	12.6	37.97	342
>gi|194453106|gb|EDX42004.1| Glycine hydroxymethyltransferase [Lactobacillus reuteri 100-23];>	6	3	2	4	1	1	20.7	44.929	411
>gi|148531144|gb|ABQ83143.1| GTP cyclohydrolase II [Lactobacillus reuteri DSM 20016	2	2	0	0	0	1	6.9	43.927	393
>gi|148531658|gb|ABQ83657.1| nitroreductase [Lactobacillus reuteri DSM 20016];>	3	2	2	2	3	1	12.9	24.752	217
>gi|300379499|gb|ADK08380.1| D-alanine-D-alanyl carrier protein ligase [Lactobacillus reuteri]	3	1	2	3	2	1	10.3	29.719	271
>gi|227070071|gb|EEI08449.1| aldose 1-epimerase [Lactobacillus reuteri MM2-3	3	2	2	2	3	2	12.2	34.581	304
>gi|148530714|gb|ABQ82713.1| arginine deiminase [Lactobacillus reuteri DSM 20016];>	6	2	5	4	4	2	22	46.242	410
>gi|227070613|gb|EEI08943.1| possible asparagine synthase (glutamine-hydrolyzing) [Lactobacillus reuteri MM2-3	5	3	3	1	1	3	8.1	76.34	652
>gi|148531438|gb|ABQ83437.1| hemolysin A [Lactobacillus reuteri DSM 20016];>	2	1	1	0	0	1	8.8	30.273	273
>gi|227070057|gb|EEI08435.1| tetratricopeptide repeat family protein [Lactobacillus reuteri MM2-3];>	3	3	0	1	2	1	10.8	50.211	434
>gi|183224279|dbj|BAG24796.1| putative lipase/esterase [Lactobacillus reuteri JCM 1112];>	4	3	1	1	2	1	15.4	33.174	292
>gi|227070559|gb|EEI08890.1| possible gamma-glutamyl-gamma-aminobutyrate hydrolase	4	1	4	4	0	0	30.8	27.281	247

[Lactobacillus reuteri MM2-3									
>gi|148531143|gb|ABQ83142.1| riboflavin synthase, alpha subunit [Lactobacillus reuteri DSM 20016];>	2	1	2	1	1	1	12.5	21.593	200
>gi|148531778|gb|ABQ83777.1| Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP(+)) [Lactobacillus reuteri DSM 20016];>	2	1	0	0	0	1	5.4	59.597	542
>gi|148531335|gb|ABQ83334.1| SSU ribosomal protein S14P [Lactobacillus reuteri DSM 20016];>	5	5	3	4	3	3	32.6	10.399	89
>gi|68160862|gb|AAY86877.1| unknown extracellular protein lr1434 [Lactobacillus reuteri	1	0	0	1	0	1	4.1	22.351	193
>gi|148531828|gb|ABQ83827.1| quorum-sensing autoinducer 2 (AI-2), LuxS [Lactobacillus reuteri DSM 20016];>	4	2	3	2	2	2	33.5	17.695	158
>gi|148532110|gb|ABQ84109.1| cytochrome b5 [Lactobacillus reuteri DSM 20016	1	0	0	1	1	1	34.7	8.1163	72
>gi|148530983|gb|ABQ82982.1| [LSU ribosomal protein L11P]-lysine N-methyltransferase [Lactobacillus reuteri DSM 20016];>	2	1	0	0	0	1	12.5	34.966	319
>gi|148531752|gb|ABQ83751.1| iron dependent repressor [Lactobacillus reuteri DSM 20016];>	1	1	0	1	1	1	5.4	25.242	224
>gi|148531126|gb|ABQ83125.1| GCN5-related N-acetyltransferase [Lactobacillus reuteri DSM 20016	1	1	1	1	0	1	6.8	20.109	177
>gi|337729100|emb|CCC04223.1| glutamate racemase [Lactobacillus reuteri ATCC 53608]	2	2	0	0	0	0	15.7	28.972	267
>gi|183225285|dbj|BAG25802.1| muramidase [Lactobacillus reuteri JCM 1112];>	2	1	0	2	0	1	9.8	55.89	492
>gi|227071772|gb|EEI10061.1| transcription antitermination protein NusB [Lactobacillus reuteri MM2-3];>	4	2	2	3	1	2	39	16.148	141
>gi|148531439|gb|ABQ83438.1| farnesyl-diphosphate synthase [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	4.5	31.64	290
>gi|148531452|gb|ABQ83451.1| hypothetical protein Lreu_1194 [Lactobacillus reuteri DSM 20016];>	3	3	1	1	0	0	23.2	19.53	168
>gi|148530896|gb|ABQ82895.1| peptide deformylase [Lactobacillus reuteri DSM 20016	1	0	1	1	0	1	7	20.898	186
>gi|148531404|gb|ABQ83403.1| protein of unknown function DUF1535 [Lactobacillus reuteri DSM 20016];>	2	0	0	0	1	1	16.6	18.631	169
>gi|148532093|gb|ABQ84092.1| transcriptional regulator, ArsR family [Lactobacillus reuteri DSM 20016];>	2	0	2	2	2	2	29.8	12.182	104
>gi|227070037|gb|EEI08415.1| S-adenosyl-L-methionine-dependent methyltransferase [Lactobacillus reuteri MM2-3];>	3	1	3	2	1	3	24.8	26.69	238
>gi|183227291|dbj|BAG27807.1| glutamyl-tRNA amidotransferase subunit B [Lactobacillus fermentum IFO 3956];>	3	3	0	0	0	0	5.9	52.972	474
>gi|183226941|dbj|BAG27457.1| glycerol kinase [Lactobacillus fermentum IFO 3956];>	2	2	2	2	2	2	2.4	55.633	503
>gi|148531804|gb|ABQ83803.1| nitroreductase [Lactobacillus reuteri DSM 20016];>	2	2	1	1	0	0	13.4	28.863	253
>gi|183224586|dbj|BAG25103.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112	3	0	0	2	1	2	20	26.479	230
>gi|227071080|gb|EEI09398.1| metallo-beta-lactamase [Lactobacillus reuteri MM2-3];>	2	1	1	0	0	0	7.3	70.828	628
>gi|148531689|gb|ABQ83688.1| aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit C	1	1	0	1	0	0	13.3	11.572	105

[Lactobacillus reuteri DSM 20016];>									
>gi|148531635|gb|ABQ83634.1| methionine aminopeptidase, type I [Lactobacillus reuteri DSM 20016];>	3	2	0	0	1	0	7.4	31.665	285
>gi|148531871|gb|ABQ83870.1| CsbD family protein [Lactobacillus reuteri DSM 20016];>	1	0	1	1	1	1	18.5	7.1913	65
>gi|148530789|gb|ABQ82788.1| Uncharacterized protein [Lactobacillus reuteri DSM 20016];>	2	1	1	0	0	0	7	38.296	345
>gi|148530740|gb|ABQ82739.1| cell shape determining protein, MreB/Mrl family [Lactobacillus reuteri DSM 20016];>	4	2	1	1	1	3	10.9	35.48	330
>gi|148530879|gb|ABQ82878.1| aspartate kinase [Lactobacillus reuteri DSM 20016];>	2	2	1	1	1	0	7.1	50.12	452
>gi|227069946|gb|EEI08331.1| conserved hypothetical protein [Lactobacillus reuteri MM2-3];>	3	2	1	1	1	0	16.6	19.78	175
>gi|148530385|gb|ABQ82384.1| phosphopentomutase [Lactobacillus reuteri DSM 20016];>	1	1	0	0	1	1	4.8	44.002	397
>gi|148531693|gb|ABQ83692.1| 5-(carboxyamino)imidazole ribonucleotide synthase [Lactobacillus reuteri DSM 20016];>	5	1	3	5	2	2	21.5	42.001	377
>gi|183224803|dbj|BAG25320.1| hypothetical protein [Lactobacillus reuteri JCM 1112	2	1	2	0	0	0	11.6	40.672	371
>gi|148531891|gb|ABQ83890.1| Ferritin, Dps family protein [Lactobacillus reuteri DSM 20016];>	2	1	0	2	0	0	20	18.223	155
>gi|227071130|gb|EEI09446.1| TrmA family tRNA (uracil-5-)-methyltransferase [Lactobacillus reuteri MM2-3	3	3	1	1	1	1	7.5	53.815	480
>gi|148532085|gb|ABQ84084.1| carbohydrate kinase, YjeF related protein [Lactobacillus reuteri DSM 20016];>	2	1	1	2	2	0	14.2	22.43	212
>gi|148530310|gb|ABQ82309.1| pyrroline-5-carboxylate reductase [Lactobacillus reuteri DSM 20016];>	3	2	1	0	2	0	18.1	29.38	270
>gi|148530742|gb|ABQ82741.1| hypothetical protein Lreu_0473 [Lactobacillus reuteri DSM 20016];>	3	2	2	2	2	2	56.8	8.7648	74
>gi|148531987|gb|ABQ83986.1| Glycerol dehydratase [Lactobacillus reuteri DSM 20016];>	4	0	2	0	3	0	9.3	62.092	558
>gi|227070920|gb|EEI09243.1| possible phosphatase [Lactobacillus reuteri MM2-3];>	1	1	0	1	1	1	6.9	24.08	218
>gi|148530818|gb|ABQ82817.1| Xaa-Pro aminopeptidase, Metallo peptidase, MEROPS family M24B [Lactobacillus reuteri DSM 20016];>	4	2	1	1	2	0	19.8	40.615	369
>gi|148531942|gb|ABQ83941.1| hydroxymethylbilane synthase [Lactobacillus reuteri DSM 20016];>	2	0	2	1	1	1	8.9	33.658	305
>gi|148530901|gb|ABQ82900.1| protein of unknown function UPF0223 [Lactobacillus reuteri DSM 20016];>	2	0	0	0	2	1	28.4	11.867	102
>gi|148530732|gb|ABQ82731.1| ATP synthase F0 subcomplex B subunit [Lactobacillus reuteri DSM 20016];>	3	2	1	0	0	1	20.3	19.254	172
>gi|148530839|gb|ABQ82838.1| dipeptidase A, Cysteine peptidase, MEROPS family C69 [Lactobacillus reuteri DSM 20016];>	3	0	0	1	2	0	7.4	51.591	458
>gi|227070615|gb|EEI08945.1| tRNA modification GTPase TrmE [Lactobacillus reuteri MM2-3];>	1	0	0	1	1	1	3.6	52.527	477
>gi|148531114|gb|ABQ83113.1| hypothetical protein Lreu_0850 [Lactobacillus reuteri DSM 20016];>	2	0	0	1	0	1	22.2	10.169	90

>gi|148530807|gb|ABQ82806.1| MutS2 family protein [Lactobacillus reuteri DSM 20016];>	1	0	1	1	0	1	3.7	88.897	791
>gi|148530576|gb|ABQ82575.1| RNA methyltransferase, TrmH family, group 3 [Lactobacillus reuteri DSM 20016];>	3	2	1	1	1	2	12.9	27.28	249
>gi|148530520|gb|ABQ82519.1| DltD, C-terminal domain protein [Lactobacillus reuteri DSM 20016];>	2	1	0	1	0	1	4.4	49.455	429
>gi|148531488|gb|ABQ83487.1| protein of unknown function DUF177 [Lactobacillus reuteri DSM 20016];>	2	1	1	1	1	1	18.9	20.961	185
>gi|183224273|dbj|BAG24790.1| transcription accessory protein [Lactobacillus reuteri JCM 1112	3	0	1	0	2	2	8.1	82.072	727
>gi|148530907|gb|ABQ82906.1| Phosphopantetheine adenylyltransferase [Lactobacillus reuteri DSM 20016];>	3	1	1	1	1	1	43.9	19.361	173
>gi|148531951|gb|ABQ83950.1| anaerobic cobaltochelatase [Lactobacillus reuteri DSM 20016];>	2	0	2	1	0	0	18.5	29.176	259
>gi|15667450|dbj|BAB68226.1| putative autoaggregation-mediating protein [Enterococcus faecium]	2	1	1	0	1	1	12.4	23.402	210
>gi|148531410|gb|ABQ83409.1| 16S rRNA processing protein RimM [Lactobacillus reuteri DSM 20016];>	1	1	1	0	1	1	7.1	19.219	168
>gi|148532087|gb|ABQ84086.1| histidyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	5	4	2	2	2	1	15.3	48.236	425
>gi|148530614|gb|ABQ82613.1| pyrroline-5-carboxylate reductase [Lactobacillus reuteri DSM 20016];>	4	3	2	1	1	1	21	26.78	257
>gi|148530963|gb|ABQ82962.1| protein of unknown function DUF448 [Lactobacillus reuteri DSM 20016	2	1	2	1	2	1	21	11.662	100
>gi|148531021|gb|ABQ83020.1| condensin subunit ScpB [Lactobacillus reuteri DSM 20016];>	2	1	0	1	0	0	12.4	22.54	201
>gi|148531915|gb|ABQ83914.1| hypothetical protein Lreu_1675 [Lactobacillus reuteri DSM 20016];>	1	0	1	1	0	0	15.1	17.238	152
>gi|194453122|gb|EDX42020.1| conserved hypothetical protein [Lactobacillus reuteri 100-23];>	3	2	1	1	2	2	55.4	8.7849	74
>gi|148532109|gb|ABQ84108.1| cytochrome b5 [Lactobacillus reuteri DSM 20016];>	1	1	0	0	1	0	23.5	8.9459	81
>gi|183224377|dbj|BAG24894.1| hypothetical protein [Lactobacillus reuteri JCM 1112];>	1	1	1	1	1	1	20.9	5.106	43
>gi|148531981|gb|ABQ83980.1| microcompartments protein [Lactobacillus reuteri DSM 20016];>	2	2	2	2	2	2	43.8	9.7479	96
>gi|227071654|gb|EEI09947.1| oxalyl-CoA decarboxylase [Lactobacillus reuteri MM2-3];>]	2	2	2	2	1	1	3.9	63.052	583
>gi|148532091|gb|ABQ84090.1| transcriptional regulator, LacI family [Lactobacillus reuteri DSM 20016];>	1	1	0	1	0	0	3.5	35.062	314
>gi|148530327|gb|ABQ82326.1| Recombination helicase AddA [Lactobacillus reuteri DSM 20016];>	2	0	0	0	0	2	2	159.71	1392
>gi|148530541|gb|ABQ82540.1| tRNA-U20-dihydrouridine synthase [Lactobacillus reuteri DSM 20016];>	2	1	1	1	0	0	8.4	37.061	333
>gi|227070201|gb|EEI08575.1| conserved hypothetical protein [Lactobacillus reuteri MM2-3];>	3	1	1	1	1	3	37.6	13.567	117
>gi|148531740|gb|ABQ83739.1| transcriptional regulator, TetR family [Lactobacillus reuteri DSM 20016];>	1	0	1	1	1	1	15	22.79	200

>gi|273067912|gb|ACZ97545.1| methionyl-tRNA synthetase [Lactobacillus reuteri]	3	3	1	0	1	0	12.2	28.262	254
>gi|148531115|gb|ABQ83114.1| hypothetical protein Lreu_0851 [Lactobacillus reuteri DSM 20016	2	0	0	2	0	2	21.1	10.116	90
>gi|194454750|gb|EDX43647.1| hypothetical protein Lreu23DRAFT_5175 [Lactobacillus reuteri 100-23];>	2	1	0	0	0	2	10.9	26.491	230
>gi|148531041|gb|ABQ83040.1| Ras superfamily GTP-binding protein YlqF [Lactobacillus reuteri DSM 20016	1	0	0	0	1	1	4.1	32.562	290
>gi|148531985|gb|ABQ83984.1| dehydratase, small subunit [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	9.3	19.319	172
>gi|183224263|dbj|BAG24780.1| hypothetical protein [Lactobacillus reuteri JCM 1112	2	1	1	0	1	1	9.7	25.982	237
>gi|148531756|gb|ABQ83755.1| UspA domain protein [Lactobacillus reuteri DSM 20016];>	1	1	0	1	1	1	7	17.57	158
>gi|183224837|dbj|BAG25354.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	2	1	0	0	0	1	5.3	48.212	430
>gi|183224293|dbj|BAG24810.1| 50S ribosomal protein L33 [Lactobacillus reuteri JCM 1112];>	1	0	0	1	1	0	20.4	5.8137	49
>gi|148530735|gb|ABQ82734.1| ATP synthase F1 subcomplex gamma subunit [Lactobacillus reuteri DSM 20016];>	2	2	0	0	0	0	9.2	34.796	314
>gi|148530858|gb|ABQ82857.1| UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase [Lactobacillus reuteri DSM 20016];>	1	1	0	0	1	0	8.4	40.606	370
>gi|148532176|gb|ABQ84175.1| ribonuclease P protein component [Lactobacillus reuteri DSM 20016];>	1	1	0	1	0	0	10.3	13.651	117
>gi|183226906|dbj|BAG27422.1| NADH dehydrogenase [Lactobacillus fermentum IFO 3956];>	1	1	1	1	1	1	2.9	43.987	407
>gi|148531170|gb|ABQ83169.1| LrgB family protein [Lactobacillus reuteri DSM 20016];>	2	2	1	0	2	0	15.9	26.306	246
>gi|148531561|gb|ABQ83560.1| hypothetical protein Lreu_1303 [Lactobacillus reuteri DSM 20016];>	2	2	0	1	1	1	49.1	6.3843	55
>gi|148530753|gb|ABQ82752.1| aldo/keto reductase [Lactobacillus reuteri DSM 20016];>	2	2	0	0	0	0	8.2	32.082	280
>gi|148531976|gb|ABQ83975.1| ATP:cob(I)alamin adenosyltransferase [Lactobacillus reuteri DSM 20016];>	3	1	3	1	2	3	21	16.903	157
>gi|148531694|gb|ABQ83693.1| xanthine phosphoribosyltransferase [Lactobacillus reuteri DSM 20016];>	2	2	0	0	0	0	16.8	21.113	191
>gi|183227494|dbj|BAG28010.1| ribonucleoside-diphosphate reductase alpha subunit [Lactobacillus fermentum IFO 3956];>	1	0	1	1	1	1	1.1	81.572	721
>gi|227071855|gb|EEI10143.1| penicillin-binding protein [Lactobacillus reuteri MM2-3];>	1	0	0	0	1	1	1.7	76.108	699
>gi|148531033|gb|ABQ83032.1| ABC transporter related [Lactobacillus reuteri DSM 20016];>	2	1	2	0	1	1	4.2	72.792	636
>gi|148531690|gb|ABQ83689.1| CamS sex pheromone cAM373 family protein [Lactobacillus reuteri DSM 20016];>	3	2	0	1	0	1	10.8	40.775	371
>gi|146749579|gb|ABQ44375.1| inosine-uridine nucleoside N-ribohydrolase [Lactobacillus reuteri];>	3	3	0	0	0	0	14.9	32.558	302
>gi|194453331|gb|EDX42228.1| cell envelope-related transcriptional attenuator [Lactobacillus	1	1	1	1	1	1	4.2	38.126	334

reuteri 100-23];>									
>gi|194452845|gb|EDX41743.1| Cof-like hydrolase [Lactobacillus reuteri 100-23];>	1	1	1	1	1	0	5.1	30.945	272
>gi|148531531|gb|ABQ83530.1| hypothetical protein Lreu_1273 [Lactobacillus reuteri DSM 20016];>	2	1	1	1	2	1	25.6	10.018	90
>gi|148531691|gb|ABQ83690.1| DNA ligase, NAD-dependent [Lactobacillus reuteri DSM 20016	1	1	0	0	1	1	1.6	76.002	680
>gi|148531361|gb|ABQ83360.1| hypothetical protein Lreu_1103 [Lactobacillus reuteri DSM 20016];>	2	1	0	1	1	1	28.8	9.0878	80
>gi|227185710|gb|EEI65781.1| hypothetical protein HMPREF0534_0888 [Lactobacillus reuteri CF48-3A];>	1	0	1	1	1	1	15	6.9798	60
>gi|148530613|gb|ABQ82612.1| O-sialoglycoprotein endopeptidase [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	4.4	37.453	343
>gi|183224190|dbj|BAG24707.1| DNA helicase [Lactobacillus reuteri JCM 1112];>	2	1	0	1	0	0	2.8	88.988	773
>gi|148530894|gb|ABQ82893.1| protein of unknown function DUF1447 [Lactobacillus reuteri DSM 20016];>	1	0	1	1	1	1	10	8.3122	70
>gi|148531717|gb|ABQ83716.1| SSU ribosomal protein S14P [Lactobacillus reuteri DSM 20016	3	3	3	1	1	1	27.9	7.1065	61
>gi|227185217|gb|EEI65288.1| cytochrome b5 [Lactobacillus reuteri CF48-3A];>	2	0	0	0	1	1	35.4	9.2174	82
>gi|148531692|gb|ABQ83691.1| ATP-dependent DNA helicase PcrA [Lactobacillus reuteri DSM 20016];>	2	2	2	2	1	2	2.9	86.29	757
>gi|148530733|gb|ABQ82732.1| ATP synthase F1 subcomplex delta subunit [Lactobacillus reuteri DSM 20016];>	2	1	0	1	0	0	15.6	20.468	180
>gi|148530347|gb|ABQ82346.1| Uracil phosphoribosyltransferase [Lactobacillus reuteri DSM 20016];>	1	0	0	1	1	0	6.7	20.252	179
>gi|227071504|gb|EEI09804.1| glutamate-5-semialdehyde dehydrogenase [Lactobacillus reuteri MM2-3	4	4	0	1	0	0	9.9	45.499	416
>gi|148531876|gb|ABQ83875.1| hypothetical protein Lreu_1635 [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	2.9	31.711	279
>gi|148531498|gb|ABQ83497.1| LSU ribosomal protein L35P [Lactobacillus reuteri DSM 20016	2	2	0	1	0	0	20	7.615	65

Cell wall proteome of *L. reuteri* MM4KO

Appendix 2 - CWP Proteins Identified	Peptides	Peptides sample 1	Peptides sample 2	Peptides sample 3	Peptides sample 4	Peptides sample 5	Sequence Coverage [%]	Mol. Weight [kDa]	Sequence Length
>gi|296313256|gb|ACZ97544.2| D-alanine-D-alanine ligase [Lactobacillus reuteri]	5	4	3	3	4	3	20.9	33.684	301
>gi|183227647|dbj|BAG28163.1| dehydrogenase [Lactobacillus fermentum IFO 3956];>]	10	9	8	8	8	9	30.1	35.835	336
>gi|227070018|gb|EEI08398.1| conserved hypothetical protein [Lactobacillus reuteri MM2-3];>	10	8	8	9	9	8	68.7	15.313	134
>gi|183226184|dbj|BAG26700.1| phosphoglycerate kinase [Lactobacillus fermentum IFO 3956];>	11	8	8	10	7	7	21.2	42.696	401
>gi|183225951|dbj|BAG26467.1| phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase [Lactobacillus fermentum IFO 3956	12	10	10	9	10	9	20.3	57.132	517
>gi|183226186|dbj|BAG26702.1| enolase [Lactobacillus fermentum IFO 3956];>	12	11	11	11	12	10	38.2	47.79	440
>gi|148531658|gb|ABQ83657.1| nitroreductase [Lactobacillus reuteri DSM 20016];>	12	7	9	9	12	10	68.2	24.752	217
>gi|148530868|gb|ABQ82867.1| NUDIX hydrolase [Lactobacillus reuteri DSM 20016	12	9	8	9	9	12	76	20.827	183
>gi|337728880|emb|CCC04000.1| hypothetical protein LRATCC53608_1247 [Lactobacillus reuteri ATCC 53608]	12	10	8	11	11	10	76.4	14.126	123
>gi|183224883|dbj|BAG25400.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	13	9	10	11	12	13	65.2	20.834	181
>gi|148530835|gb|ABQ82834.1| hypothetical protein Lreu_0566 [Lactobacillus reuteri DSM 20016];>	14	12	12	11	9	10	77.9	18.628	163
>gi|148531898|gb|ABQ83897.1| Inosine/uridine-preferring nucleoside hydrolase [Lactobacillus reuteri DSM 20016];>	14	11	11	10	12	13	81.9	34.905	320
>gi|148530811|gb|ABQ82810.1| non-canonical purine NTP pyrophosphatase, rdgB/HAM1 family [Lactobacillus reuteri DSM 20016];>	14	12	11	12	10	12	83.6	21.286	195
>gi|148531526|gb|ABQ83525.1| protein of unknown function DUF964 [Lactobacillus reuteri DSM 20016];>	14	13	10	13	12	13	89.4	14.096	123
>gi|148530453|gb|ABQ82452.1| 3-beta hydroxysteroid dehydrogenase/isomerase [Lactobacillus reuteri DSM 20016];>	15	14	15	15	15	14	69	31.632	284
>gi|183225729|dbj|BAG26246.1| hypothetical protein [Lactobacillus reuteri JCM 1112	15	11	10	7	12	13	80.5	19.891	169
>gi|148531716|gb|ABQ83715.1| SSU ribosomal protein S8P [Lactobacillus reuteri DSM 20016];>	15	11	13	12	12	13	87.1	14.536	132
>gi|227071142|gb|EEI09458.1| acetoin dehydrogenase [Lactobacillus reuteri MM2-3];>	15	12	11	12	11	14	89.9	28.157	267
>gi|337728896|emb|CCC04016.1| ribokinase [Lactobacillus reuteri ATCC 53608]	16	14	13	15	14	15	75.6	32.044	307
>gi|148530952|gb|ABQ82951.1| uridylate kinase [Lactobacillus reuteri DSM 20016	16	11	13	12	15	13	76.7	25.876	240
>gi|227071324|gb|EEI09633.1| heat shock protein Hsp33 [Lactobacillus reuteri MM2-3];>	17	13	13	11	14	11	64.1	35.232	323
>gi|148532120|gb|ABQ84119.1| NAD-dependent epimerase/dehydratase [Lactobacillus reuteri DSM 20016];>	17	10	10	10	16	10	95.3	23.187	213

>gi|183227337|dbj|BAG27853.1| elongation factor G [Lactobacillus fermentum IFO 3956];>	18	14	15	14	15	14	34.3	76.351	694
>gi|148532119|gb|ABQ84118.1| Bleomycin hydrolase [Lactobacillus reuteri DSM 20016	18	14	14	14	14	13	55.5	50.851	440
>gi|227070209|gb|EEI08583.1| diaminopimelate decarboxylase [Lactobacillus reuteri MM2-3];>	18	9	5	11	12	14	59.4	48.355	441
>gi|194453648|gb|EDX42545.1| glucokinase, ROK family [Lactobacillus reuteri 100-23	18	16	14	14	14	15	63.2	34.493	323
>gi|148530668|gb|ABQ82667.1| phosphotransacetylase [Lactobacillus reuteri DSM 20016	18	17	17	16	14	13	75.9	34.699	324
>gi|148530340|gb|ABQ82339.1| 2,5-didehydrogluconate reductase [Lactobacillus reuteri DSM 20016];>	18	14	15	11	11	14	79.2	32.62	288
>gi|227186201|gb|EEI66272.1| response regulator [Lactobacillus reuteri CF48-3A];>	18	15	14	11	14	11	79.3	26.602	237
>gi|148531543|gb|ABQ83542.1| ribokinase [Lactobacillus reuteri DSM 20016	18	15	14	15	15	17	92.5	32.041	307
>gi|148532092|gb|ABQ84091.1| Mannosyl-glycoprotein endo-beta-N-acetylglucosamidase [Lactobacillus reuteri DSM 20016];>	19	11	13	14	17	10	46.3	60.446	568
>gi|148531464|gb|ABQ83463.1| glucokinase [Lactobacillus reuteri DSM 20016];>	19	18	15	14	16	17	63.2	34.42	323
>gi|148530523|gb|ABQ82522.1| D-alanine-activating enzyme [Lactobacillus reuteri DSM 20016];>	19	15	17	15	16	16	66.7	55.989	508
>gi|148530646|gb|ABQ82645.1| thioredoxin reductase [Lactobacillus reuteri DSM 20016];>	19	16	15	13	12	11	81	33.305	310
>gi|148530593|gb|ABQ82592.1| ribonucleoside-diphosphate reductase class Ib beta subunit [Lactobacillus reuteri DSM 20016];>	20	16	15	16	16	20	81.1	39.248	339
>gi|324978533|gb|EGC15482.1| cysteine--tRNA ligase [Lactobacillus reuteri MM4-1A];>	21	16	15	17	14	14	56.9	54.852	478
>gi|183226438|dbj|BAG26954.1| elongation factor Tu [Lactobacillus fermentum IFO 3956];>	21	21	20	20	20	19	57.1	43.474	396
>gi|148532080|gb|ABQ84079.1| glycerol 2-dehydrogenase (NAD+) [Lactobacillus reuteri DSM 20016];>	21	19	19	18	17	18	69.4	40.698	373
>gi|148530778|gb|ABQ82777.1| FAD-dependent pyridine nucleotide-disulfide oxidoreductase [Lactobacillus reuteri DSM 20016];>	21	17	18	15	20	17	74.8	44.031	404
>gi|183225128|dbj|BAG25645.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	21	19	16	18	14	17	81.8	35.441	297
>gi|68161011|gb|AAY86937.1| lr0280 [Lactobacillus reuteri]	22	14	10	18	10	18	70.7	36.729	331
>gi|148530857|gb|ABQ82856.1| UDP-N-acetylmuramoylalanine--D-glutamate ligase [Lactobacillus reuteri DSM 20016];>.	22	16	14	15	15	18	74.6	50.332	456
>gi|148531776|gb|ABQ83775.1| flavocytochrome c [Lactobacillus reuteri DSM 20016	22	18	15	14	19	19	76.9	50.13	464
>gi|194452797|gb|EDX41695.1| Inorganic diphosphatase [Lactobacillus reuteri 100-23];>	22	21	17	19	17	18	77.2	34.18	311
>gi|227071240|gb|EEI09554.1| possible glutathione-disulfide reductase [Lactobacillus reuteri MM2-3];> r	22	19	10	16	14	18	77.7	48.997	443
>gi|148530995|gb|ABQ82994.1| Choloylglycine hydrolase [Lactobacillus reuteri DSM 20016];>	22	20	17	15	19	18	83.1	36.104	325
>gi|148530419|gb|ABQ82418.1| phosphoglycerate mutase [Lactobacillus reuteri DSM 20016];>	22	17	21	22	21	21	87.3	26.121	228
>gi|148530846|gb|ABQ82845.1| 6-phosphogluconolactonase [Lactobacillus reuteri DSM 20016];>	22	17	18	20	20	16	90.4	37.901	342
>gi|148530724|gb|ABQ82723.1| serine hydroxymethyltransferase [Lactobacillus reuteri DSM 20016];>	23	20	18	21	21	19	67.2	44.95	411

>gi|148530414|gb|ABQ82413.1| amidophosphoribosyltransferase [Lactobacillus reuteri DSM 20016	23	16	22	21	19	9	67.4	52.984	484
>gi|148530679|gb|ABQ82678.1| phosphoglucosamine mutase [Lactobacillus reuteri DSM 20016];>	23	21	17	20	19	20	76.5	48.902	451
>gi|148531050|gb|ABQ83049.1| Inorganic diphosphatase [Lactobacillus reuteri DSM 20016	23	21	18	20	19	19	77.8	34.194	311
>gi|148531899|gb|ABQ83898.1| short-chain dehydrogenase/reductase SDR [Lactobacillus reuteri DSM 20016];>	23	17	20	18	15	17	78.5	26.924	251
>gi|148530491|gb|ABQ82490.1| UDP-N-acetylglucosamine pyrophosphorylase [Lactobacillus reuteri DSM 20016];>	24	18	20	19	18	20	61.8	50.01	455
>gi|148531554|gb|ABQ83553.1| methionine adenosyltransferase [Lactobacillus reuteri DSM 20016];>	24	22	17	20	17	19	76.5	43.195	395
>gi|148532032|gb|ABQ84031.1| cysteine synthase [Lactobacillus reuteri DSM 20016];>	24	20	18	19	16	18	87	32.241	307
>gi|148530641|gb|ABQ82640.1| glycerol 3-phosphate dehydrogenase (NAD(P)+) [Lactobacillus reuteri DSM 20016];> G	24	18	20	17	17	18	88.8	36.905	338
>gi|148530389|gb|ABQ82388.1| inosine-5-monophosphate dehydrogenase [Lactobacillus reuteri DSM 20016];>	24	21	19	22	21	23	92.6	39.373	380
>gi|227185848|gb|EEI65919.1| acetate kinase [Lactobacillus reuteri CF48-3A];>	25	19	20	22	21	20	72.6	43.61	398
>gi|148531549|gb|ABQ83548.1| peptidase V, Metallo peptidase, MEROPS family M20A [Lactobacillus reuteri DSM 20016];>	26	22	23	22	22	19	67.7	51.22	467
>gi|183225183|dbj|BAG25700.1| ligase [Lactobacillus reuteri JCM 1112];>	26	21	16	21	23	23	69.2	49.294	441
>gi|148532099|gb|ABQ84098.1| Alcohol dehydrogenase GroES domain protein [Lactobacillus reuteri DSM 20016];>	26	22	21	21	24	22	81.5	35.918	336
>gi|148530695|gb|ABQ82694.1| ornithine carbamoyltransferase [Lactobacillus reuteri DSM 20016];> L	26	20	19	18	23	21	83.3	37.559	335
>gi|227185071|gb|EEI65142.1| elongation factor EF1B [Lactobacillus reuteri CF48-3A	26	22	20	20	22	20	85.2	31.961	291
>gi|148530410|gb|ABQ82409.1| phosphoribosylaminoimidazole-succinocarboxamide synthase [Lactobacillus reuteri DSM 20016	26	22	20	17	18	14	86.2	27.308	239
>gi|148531171|gb|ABQ83170.1| L-lactate dehydrogenase [Lactobacillus reuteri DSM 20016];>	26	21	20	20	23	22	87	35.005	324
>gi|148530972|gb|ABQ82971.1| chaperone protein DnaK [Lactobacillus reuteri DSM 20016];>	27	18	18	14	18	23	61	67.211	621
>gi|148530555|gb|ABQ82554.1| putative nicotinate phosphoribosyltransferase [Lactobacillus reuteri DSM 20016];>	27	21	20	21	21	22	66.6	55.609	488
>gi|148531641|gb|ABQ83640.1| oxidoreductase domain protein [Lactobacillus reuteri DSM 20016];>	27	25	20	18	20	21	70.8	38.272	339
>gi|148531932|gb|ABQ83931.1| (R)-2-hydroxyisocaproate dehydrogenase [Lactobacillus reuteri DSM 20016];>	27	26	24	24	25	24	88	37.358	334
>gi|148531688|gb|ABQ83687.1| aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit A [Lactobacillus reuteri DSM 20016];>gi|148544654|ref|YP_001272024.1| aspartyl/glutamyl-tRNA amidotransferase subunit A [Lactobacillus reuteri DSM 20016];>gi|166989678|sp	28	19	18	23	23	22	77.8	53.009	490

>gi|148530951|gb|ABQ82950.1| translation elongation factor Ts (EF-Ts) [Lactobacillus reuteri DSM 20016];>	29	26	24	23	26	23	85.2	31.962	291
>gi|337728297|emb|CCC03392.1| alcohol dehydrogenase [Lactobacillus reuteri ATCC 53608	29	25	26	24	28	26	92.7	36.158	342
>gi|227185611|gb|EEI65682.1| alcohol dehydrogenase [Lactobacillus reuteri CF48-3A];>	29	25	26	24	28	26	92.7	36.184	342
>gi|148530918|gb|ABQ82917.1| trigger factor [Lactobacillus reuteri DSM 20016	30	21	15	26	17	25	69.5	48.747	436
>gi|227186481|gb|EEI66552.1| D-alanine--D-alanine ligase [Lactobacillus reuteri CF48-3A	30	27	27	26	28	25	75.1	42.569	378
>gi|183224464|dbj|BAG24981.1| D-alanine--D-alanine ligase [Lactobacillus reuteri JCM 1112];>	30	27	28	26	28	26	76.2	43.001	382
>gi|148531929|gb|ABQ83928.1| dipeptidase A, Cysteine peptidase, MEROPS family C69 [Lactobacillus reuteri DSM 20016	30	21	23	25	21	28	78.9	54.294	478
>gi|337728095|emb|CCC03185.1| methionyl-tRNA synthase [Lactobacillus reuteri ATCC 53608]	31	22	18	26	17	28	56.1	76.572	675
>gi|148531687|gb|ABQ83686.1| aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B [Lactobacillus reuteri DSM 20016	31	17	22	24	25	27	75.7	53.424	474
>gi|148530370|gb|ABQ82369.1| seryl-tRNA synthetase [Lactobacillus reuteri DSM 20016	31	24	20	25	24	27	79.1	49.29	435
>gi|148530447|gb|ABQ82446.1| pyridine nucleotide-disulfide oxidoreductase dimerization region [Lactobacillus reuteri DSM 20016];>	31	28	26	28	27	30	79.8	49.944	451
>gi|148531743|gb|ABQ83742.1| Alcohol dehydrogenase GroES domain protein [Lactobacillus reuteri DSM 20016	31	27	29	27	30	29	92.7	36.124	342
>gi|227184598|gb|EEI64669.1| phosphoketolase [Lactobacillus reuteri CF48-3A];>	33	27	27	27	30	24	47.6	91.435	803
>gi|148530959|gb|ABQ82958.1| prolyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	33	16	4	19	12	25	73.7	64.256	577
>gi|183224383|dbj|BAG24900.1| enolase [Lactobacillus reuteri JCM 1112];>	33	29	28	29	31	29	75.7	49.951	457
>gi|148530409|gb|ABQ82408.1| Adenylosuccinate lyase [Lactobacillus reuteri DSM 20016];>	33	27	28	27	28	28	82.8	49.454	431
>gi|148530829|gb|ABQ82828.1| acetate kinase [Lactobacillus reuteri DSM 20016];>	33	24	26	28	28	26	88.9	43.595	398
>gi|148531460|gb|ABQ83459.1| L-glutamine synthetase [Lactobacillus reuteri DSM 20016];>	33	31	28	30	32	29	89	50.835	447
>gi|148531290|gb|ABQ83289.1| LPXTG-motif cell wall anchor domain [Lactobacillus reuteri DSM 20016];>	34	24	29	24	28	28	62.4	80.889	752
>gi|148530680|gb|ABQ82679.1| glutamine--fructose-6-phosphate transaminase [Lactobacillus reuteri DSM 20016];>	34	23	27	24	27	28	72.3	66.611	606
>gi|148530917|gb|ABQ82916.1| translation elongation factor 1A (EF-1A/EF-Tu) [Lactobacillus reuteri DSM 20016];>	34	33	31	32	33	31	81.3	43.432	396
>gi|148532037|gb|ABQ84036.1| Endothelin-converting protein 1 [Lactobacillus reuteri DSM 20016];>	35	19	19	25	24	26	62.8	72.39	634
>gi|227071557|gb|EEI09855.1| glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) [Lactobacillus reuteri MM2-3];>	35	34	34	33	35	33	91.6	37.062	345
>gi|148531028|gb|ABQ83027.1| SSU ribosomal protein S1P [Lactobacillus reuteri DSM 20016];>gi|148543994|ref|YP_001271364.1| 30S ribosomal protein S1 [Lactobacillus reuteri DSM 20016];>gi|183224732|dbj|BAG25249.1| 30S ribosomal protein S1 [Lactobacillus reut	35	32	22	31	19	27	92.5	45.995	416
>gi|148530903|gb|ABQ82902.1| GTP-binding protein TypA [Lactobacillus reuteri DSM 20016];>	36	29	29	32	33	31	63	68.827	614

>gi|183225744|dbj|BAG26261.1| threonyl-tRNA synthase [Lactobacillus reuteri JCM 1112];>	36	19	20	25	19	33	64.8	71.776	627
>gi|337727906|emb|CCC02995.1| L-lactate dehydrogenase [Lactobacillus reuteri ATCC 53608]	37	30	30	31	30	30	90	34.069	319
>gi|148530404|gb|ABQ82403.1| Formate-tetrahydrofolate ligase [Lactobacillus reuteri DSM 20016];>	38	23	29	29	35	24	74.1	60.251	553
>gi|227070122|gb|EEI08498.1| malate dehydrogenase (NAD) [Lactobacillus reuteri MM2-3];>	38	31	31	32	31	31	88.7	34.901	326
>gi|148531006|gb|ABQ83005.1| glycyl-tRNA synthetase beta chain [Lactobacillus reuteri DSM 20016	39	31	31	35	33	32	66.9	78.536	691
>gi|148530481|gb|ABQ82480.1| methionyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	39	28	26	31	24	36	69.6	76.638	675
>gi|227185690|gb|EEI65761.1| GMP synthase (glutamine-hydrolyzing) [Lactobacillus reuteri CF48-3A];>	39	33	29	30	32	31	77.1	59.976	538
>gi|148531553|gb|ABQ83552.1| leucyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	43	26	26	32	26	26	59.1	92.918	806
>gi|148530331|gb|ABQ82330.1| maltose phosphorylase [Lactobacillus reuteri DSM 20016];>	43	27	31	31	37	30	62.3	86.744	750
>gi|148531528|gb|ABQ83527.1| arginyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	43	32	28	36	40	38	80.1	63.878	562
>gi|148530345|gb|ABQ82344.1| Adenylosuccinate synthetase [Lactobacillus reuteri DSM 20016];> s	43	39	41	39	38	37	83.1	47.725	432
>gi|227071019|gb|EEI09341.1| bifunctional GMP synthase/glutamine amidotransferase protein [Lactobacillus reuteri MM2-3];>	43	35	31	33	35	35	85.7	59.912	538
>gi|337729265|emb|CCC04392.1| phosphoglucomutase [Lactobacillus reuteri ATCC 53608]	44	35	37	35	36	32	82.8	63.747	574
>gi|148530650|gb|ABQ82649.1| alpha-phosphoglucomutase [Lactobacillus reuteri DSM 20016];>	44	36	38	36	36	32	82.8	63.706	574
>gi|227185787|gb|EEI65858.1| valyl-tRNA synthetase [Lactobacillus reuteri CF48-3A];>	45	36	34	33	32	30	53.9	104.65	907
>gi|148530417|gb|ABQ82416.1| IMP cyclohydrolase [Lactobacillus reuteri DSM 20016];>	45	39	43	40	39	32	83.4	57.032	512
>gi|194453154|gb|EDX42052.1| valyl-tRNA synthetase [Lactobacillus reuteri 100-23];>	47	39	35	34	32	31	56.8	102.19	884
>gi|337729137|emb|CCC04260.1| valyl-tRNA synthase [Lactobacillus reuteri ATCC 53608]	47	38	35	34	33	31	57.2	102.21	884
>gi|143798699|gb|ABF06644.2| sucrose phosphorylase [Lactobacillus reuteri];>	47	40	38	41	36	37	84.1	55.969	485
>gi|148531459|gb|ABQ83458.1| glutamyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	47	38	36	39	37	42	84.2	58.349	501
>gi|148530624|gb|ABQ82623.1| chaperonin GroEL [Lactobacillus reuteri DSM 20016];>	47	41	38	41	35	37	94.5	57.123	542
>gi|337728521|emb|CCC03625.1| glucose-6-phosphate 1-dehydrogenase [Lactobacillus reuteri ATCC 53608]	48	43	45	44	44	43	87.2	56.355	493
>gi|227070837|gb|EEI09162.1| glucose-6-phosphate 1-dehydrogenase [Lactobacillus reuteri MM2-3];>	49	46	47	46	46	46	86.7	56.713	496
>gi|194454244|gb|EDX43141.1| glucose-6-phosphate 1-dehydrogenase [Lactobacillus reuteri 100-23];>	49	45	47	45	45	45	87.2	56.374	493
>gi|148531016|gb|ABQ83015.1| pyruvate kinase [Lactobacillus reuteri DSM 20016];>	50	45	46	46	44	45	78.9	51.825	473
>gi|148532121|gb|ABQ84120.1| lysyl aminopeptidase, Metallo peptidase, MEROPS family M01 [Lactobacillus reuteri DSM 20016];>	51	39	42	39	34	40	75.8	95.323	843
>gi|183224518|dbj|BAG25035.1| alanyl-tRNA synthase [Lactobacillus reuteri JCM 1112];>	52	38	33	37	35	43	72.9	98.648	885

>gi|148530773|gb|ABQ82772.1| valyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	53	43	39	38	37	38	64.1	102.29	884
>gi|148530866|gb|ABQ82865.1| Isoleucyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	56	36	30	42	45	47	73.5	109.95	960
>gi|194453034|gb|EDX41932.1| Phosphoglycerate kinase [Lactobacillus reuteri 100-23];>	56	48	47	47	48	46	96	42.961	401
>gi|337728440|emb|CCC03541.1| xylulose 5-phosphate phosphoketolase [Lactobacillus reuteri ATCC 53608]	57	48	43	47	51	42	78.2	91.372	803
>gi|194454221|gb|EDX43118.1| Phosphoketolase [Lactobacillus reuteri 100-23];>	57	47	43	47	51	42	78.2	91.374	803
>gi|183224381|dbj|BAG24898.1| phosphoglycerate kinase [Lactobacillus reuteri JCM 1112];>	57	49	48	48	50	48	96	42.96	401
>gi|148531926|gb|ABQ83925.1| Phosphoketolase [Lactobacillus reuteri DSM 20016];>	58	48	45	47	51	45	79.2	91.403	803
>gi|148531733|gb|ABQ83732.1| translation elongation factor 2 (EF-2/EF-G) [Lactobacillus reuteri DSM 20016];>	60	49	48	51	50	50	85.2	76.77	695
>gi|337729321|emb|CCC04450.1| aldehyde-alcohol dehydrogenase [Lactobacillus reuteri ATCC 53608];>	61	49	55	49	51	36	71	97.181	878
>gi|337728522|emb|CCC03626.1| 6-phosphogluconate dehydrogenase [Lactobacillus reuteri ATCC 53608];>	62	60	59	56	59	58	96.7	53.444	478
>gi|183225653|dbj|BAG26170.1| 6-phosphogluconate dehydrogenase [Lactobacillus reuteri JCM 1112];>	64	62	61	58	61	60	96.7	53.417	478
>gi|148531571|gb|ABQ83570.1| hypothetical protein Lreu_1313 [Lactobacillus reuteri DSM 20016];>	65	59	48	56	54	51	89.8	64.749	566
>gi|148530592|gb|ABQ82591.1| alcohol dehydrogenase AdhE / acetaldehyde dehydrogenase [Lactobacillus reuteri DSM 20016	75	57	71	66	68	51	83.6	97.187	878
>gi|148530322|gb|ABQ82321.1| ATPase AAA-2 domain protein [Lactobacillus reuteri DSM 20016];>	86	80	68	51	71	63	85.1	82.305	745
>gi|227070899|gb|EEI09222.1| D-lactate dehydrogenase [Lactobacillus reuteri MM2-3];> g	17	12	13	12	14	12	65.6	37.311	337
>gi|148531037|gb|ABQ83036.1| degV family protein [Lactobacillus reuteri DSM 20016];> J	17	12	11	12	12	14	78.2	30.742	280
>gi|227070649|gb|EEI08979.1| 1,3-propanediol dehydrogenase [Lactobacillus reuteri MM2-3];>	25	16	21	15	22	20	73.6	44.01	405
>gi|148531009|gb|ABQ83008.1| aminotransferase [Lactobacillus reuteri DSM 20016];>	26	10	12	15	24	17	78.9	43.107	394
>gi|130893244|gb|ABO32599.1| D-lactate dehydrogenase [Lactobacillus reuteri	13	12	12	10	11	10	37.8	40.533	362
>gi|148530455|gb|ABQ82454.1| Substrate-binding region of ABC-type glycine betaine transport system [Lactobacillus reuteri DSM 20016];>	19	13	14	9	14	15	59.9	32.953	299
>gi|269930626|gb|ACZ53583.1| phosphoketolase [Lactobacillus reuteri]	16	13	8	13	12	9	83.1	21.656	189
>gi|148531057|gb|ABQ83056.1| gluconate kinase, FGGY family [Lactobacillus reuteri DSM 20016];>	21	17	16	16	12	16	63.9	55.417	509
>gi|148530556|gb|ABQ82555.1| NH(3)-dependent NAD(+) synthetase [Lactobacillus reuteri DSM 20016];>	20	14	14	12	15	16	86.9	30.54	275
>gi|183227581|dbj|BAG28097.1| 6-phosphogluconate dehydrogenase [Lactobacillus fermentum IFO 3956];>	12	12	11	11	11	10	22.8	53.488	479
>gi|148530989|gb|ABQ82988.1| aspartyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>;	11	5	2	5	7	7	21.2	68.43	600

>gi|148531795|gb|ABQ83794.1| Esterase/lipase-like protein [Lactobacillus reuteri DSM 20016];>	9	6	8	6	7	9	71.4	26.628	238
>gi|183225853|dbj|BAG26369.1| ATP-dependent Clp protease ATP-binding subunit [Lactobacillus fermentum IFO 3956];>	9	9	6	5	9	6	10.9	76.678	697
>gi|148530828|gb|ABQ82827.1| Adenine-specific DNA methylase-like protein [Lactobacillus reuteri DSM 20016];>	14	12	13	9	12	13	75.1	31.155	277
>gi|146142505|gb|ABQ01722.1| fumarase [Lactobacillus reuteri];>	21	10	13	12	20	16	61.3	50.302	462
>gi|148531824|gb|ABQ83823.1| translation elongation factor P (EF-P) [Lactobacillus reuteri DSM 20016];>	11	8	9	8	10	8	55.7	20.518	185
>gi|148530870|gb|ABQ82869.1| methylthioadenosine nucleosidase [Lactobacillus reuteri DSM 20016];>	10	8	8	9	8	9	64.9	24.687	231
>gi|227070039|gb|EEI08417.1| peptidase T [Lactobacillus reuteri MM2-3];>	18	15	9	12	11	11	52.5	46.484	419
>gi|183226063|dbj|BAG26579.1| nicotinate phosphoribosyltransferase [Lactobacillus fermentum IFO 3956];>	6	6	4	6	5	5	14.6	55.469	486
>gi|148530771|gb|ABQ82770.1| aminotransferase, class V [Lactobacillus reuteri DSM 20016];>	16	9	14	11	12	13	66.8	42.097	382
>gi|148530760|gb|ABQ82759.1| Glyoxalase/bleomycin resistance protein/dioxygenase [Lactobacillus reuteri DSM 20016];>	7	5	7	5	5	4	51.1	15.166	135
>gi|183224279|dbj|BAG24796.1| putative lipase/esterase [Lactobacillus reuteri JCM 1112];>	13	10	10	12	10	9	65.1	33.174	292
>gi|183225901|dbj|BAG26417.1| inosine-5-monophosphate dehydrogenase [Lactobacillus fermentum IFO 3956];>	9	8	8	8	8	9	28.2	39.708	380
>gi|148532158|gb|ABQ84157.1| hypoxanthine phosphoribosyltransferase [Lactobacillus reuteri DSM 20016];>	11	10	11	11	11	10	53.4	20.407	178
>gi|148531690|gb|ABQ83689.1| CamS sex pheromone cAM373 family protein [Lactobacillus reuteri DSM 20016];>	5	3	3	3	3	5	19.9	40.775	371
>gi|148530387|gb|ABQ82386.1| purine nucleoside phosphorylase [Lactobacillus reuteri DSM 20016];>	13	9	12	11	12	11	66.5	25.751	236
>gi|148531709|gb|ABQ83708.1| Adenylate kinase [Lactobacillus reuteri DSM 20016];> r	15	12	11	12	11	10	85.4	24.609	219
>gi|148530564|gb|ABQ82563.1| cystathionine gamma-lyase [Lactobacillus reuteri DSM 20016];>	10	9	7	9	8	9	46.1	41.498	380
>gi|148530578|gb|ABQ82577.1| transcription antitermination protein nusG [Lactobacillus reuteri DSM 20016	14	12	11	11	12	12	72.8	20.289	180
>gi|148531828|gb|ABQ83827.1| quorum-sensing autoinducer 2 (AI-2), LuxS [Lactobacillus reuteri DSM 20016];>	16	11	13	12	11	13	91.1	17.695	158
>gi|148530539|gb|ABQ82538.1| membrane protease FtsH catalytic subunit [Lactobacillus reuteri DSM 20016];>	22	17	17	15	13	16	37	77.238	702
>gi|148530343|gb|ABQ82342.1| FAD-dependent pyridine nucleotide-disulfide oxidoreductase [Lactobacillus reuteri DSM 20016];>	14	10	10	13	12	12	64.1	49.604	449
>gi|194453968|gb|EDX42865.1| Adenylate kinase [Lactobacillus reuteri 100-23];>	15	12	10	11	10	9	85.4	24.61	219
>gi|148531330|gb|ABQ83329.1| Peroxiredoxin [Lactobacillus reuteri DSM 20016];>	14	10	8	8	10	9	92	21.041	187

>gi|337727893|emb|CCC02982.1| conjugated bile salt hydrolase [Lactobacillus reuteri ATCC 53608]	15	13	11	12	15	12	60.3	36.06	325
>gi|183226183|dbj|BAG26699.1| glyceraldehyde 3-phosphate dehydrogenase [Lactobacillus fermentum IFO 3956	10	8	7	7	9	8	34.1	36.208	337
>gi|148530953|gb|ABQ82952.1| ribosome recycling factor [Lactobacillus reuteri DSM 20016];>	16	12	10	8	6	12	85.6	20.783	187
>gi|148531713|gb|ABQ83712.1| SSU ribosomal protein S5P [Lactobacillus reuteri DSM 20016];>	16	13	15	14	13	12	81.1	17.646	169
>gi|148532132|gb|ABQ84131.1| hypothetical protein Lreu_1894 [Lactobacillus reuteri DSM 20016];>	6	5	3	3	4	2	53.3	13.741	120
>gi|148531221|gb|ABQ83220.1| 1-deoxy-D-xylulose-5-phosphate synthase [Lactobacillus reuteri DSM 20016	19	10	11	10	11	10	41.6	65.608	591
>gi|227071790|gb|EEI10079.1| ribulose-5-phosphate 3-epimerase [Lactobacillus reuteri MM2-3];>	14	8	10	11	13	11	55	25.102	231
>gi|1667472|gb|AAB53259.1| chloramphenicol acetyltransferase-TC [Lactobacillus reuteri]	13	11	6	8	7	7	54.6	27.351	238
>gi|148531415|gb|ABQ83414.1| signal recognition particle-docking protein FtsY [Lactobacillus reuteri DSM 20016];>	21	15	15	14	12	13	40.7	55.604	508
>gi|148531196|gb|ABQ83195.1| degV family protein [Lactobacillus reuteri DSM 20016J	10	8	3	6	6	7	53.4	32.228	292
>gi|148530696|gb|ABQ82695.1| carbamate kinase [Lactobacillus reuteri DSM 20016];>	16	13	13	10	11	9	76.5	33.008	310
>gi|148531869|gb|ABQ83868.1| GTP-binding protein, HSR1-related [Lactobacillus reuteri DSM 20016];>	18	15	12	12	12	14	52.2	47.758	425
>gi|194454118|gb|EDX43015.1| quorum-sensing autoinducer 2 (AI-2), LuxS [Lactobacillus reuteri 100-23];>	13	9	10	10	9	10	82.9	17.727	158
>gi|227071698|gb|EEI09989.1| phosphoesterase [Lactobacillus reuteri MM2-3];>	8	7	6	7	8	7	55.1	19.857	176
>gi|38018477|gb|AAR08285.1| GroEL [Lactobacillus reuteri DSM 20016]	8	7	8	8	6	6	59.5	18.192	168
>gi|148530310|gb|ABQ82309.1| pyrroline-5-carboxylate reductase [Lactobacillus reuteri DSM 20016];>	7	6	4	3	2	4	37.4	29.38	270
>gi|112943316|gb|ABI26304.1| predicted flavoprotein [Lactobacillus reuteri];>	11	10	11	10	10	10	64.2	21.504	190
>gi|148531889|gb|ABQ83888.1| prolinase, Serine peptidase, MEROPS family S33 [Lactobacillus reuteri DSM 20016];>	9	3	3	7	9	8	58.7	34.841	303
>gi|148530865|gb|ABQ82864.1| DivIVA family protein [Lactobacillus reuteri DSM 20016];>	17	10	7	13	6	13	41.5	27.591	246
>gi|148530569|gb|ABQ82568.1| ribose-5-phosphate isomerase [Lactobacillus reuteri DSM 20016];>	15	12	13	12	11	12	80.2	25.036	227
>gi|148532002|gb|ABQ84001.1| NrdI family protein [Lactobacillus reuteri DSM 20016];>	6	5	5	6	6	6	41.9	17.501	155
>gi|148532070|gb|ABQ84069.1| histidyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	24	15	17	21	18	22	78.5	49.318	433
>gi|148530878|gb|ABQ82877.1| diaminopimelate epimerase [Lactobacillus reuteri DSM 20016];>	11	8	6	4	9	7	59.5	36.381	333
>gi|183225766|dbj|BAG26283.1| aspartate-ammonia ligase [Lactobacillus reuteri JCM 1112];>	18	10	6	13	15	15	62.2	39.72	339
>gi|148531417|gb|ABQ83416.1| RNAse III [Lactobacillus reuteri DSM 20016];>.	16	12	7	11	13	13	87.6	26.606	233
>gi|112943099|gb|ABI26296.1| single-stranded DNA-binding protein [Lactobacillus reuteri];>	7	6	5	7	6	6	38.5	20.605	187

>gi|227070727|gb|EEI09055.1| asparaginyl-tRNA synthetase [Lactobacillus reuteri MM2-3];>	5	1	1	2	2	5	14.7	52.076	448
>gi|148530690|gb|ABQ82689.1| glucose-6-phosphate isomerase [Lactobacillus reuteri DSM 20016];>	23	17	16	17	17	16	62.4	50.35	452
>gi|194452927|gb|EDX41825.1| Cystathionine gamma-synthase [Lactobacillus reuteri 100-23];>	9	8	7	8	7	8	40.5	41.471	380
>gi|194453139|gb|EDX42037.1| Glyoxalase/bleomycin resistance protein/dioxygenase [Lactobacillus reuteri 100-23];>	6	3	5	4	3	3	51.1	15.183	135
>gi|148531521|gb|ABQ83520.1| histidine triad (HIT) protein [Lactobacillus reuteri DSM 20016];>	8	3	3	3	5	4	61.8	16.333	144
>gi|148531583|gb|ABQ83582.1| Phosphotransferase system, phosphocarrier protein HPr [Lactobacillus reuteri DSM 20016	7	7	5	5	4	5	62.5	9.4076	88
>gi|148530721|gb|ABQ82720.1| bacterial peptide chain release factor 1 (bRF-1) [Lactobacillus reuteri DSM 20016];>	7	5	4	3	3	7	27.6	41.287	362
>gi|148531973|gb|ABQ83972.1| acetate kinase [Lactobacillus reuteri DSM 20016];>	9	3	3	5	7	6	36.8	43.802	394
>gi|148530413|gb|ABQ82412.1| phosphoribosylformylglycinamidine synthase subunit II [Lactobacillus reuteri DSM 20016];>	17	5	9	11	13	8	37.2	80.944	742
>gi|148531241|gb|ABQ83240.1| DSBA oxidoreductase [Lactobacillus reuteri DSM 20016];> J	7	7	1	4	4	5	41.9	24.566	215
>gi|148530514|gb|ABQ82513.1| UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase [Lactobacillus reuteri DSM 20016];>	15	10	9	6	9	11	47.7	50.527	459
>gi|148530755|gb|ABQ82754.1| UspA domain protein [Lactobacillus reuteri DSM 20016];>	9	7	4	9	7	7	48.1	18.017	162
>gi|148530792|gb|ABQ82791.1| RecA protein [Lactobacillus reuteri DSM 20016];>	8	5	6	5	5	7	26.8	39.063	362
>gi|148530415|gb|ABQ82414.1| phosphoribosylformylglycinamidine cyclo-ligase [Lactobacillus reuteri DSM 20016 R	19	15	16	12	17	6	81.7	36.975	345
>gi|130893166|gb|ABO32596.1| ATP-dependent Clp protease ATP-binding subunit ClpX [Lactobacillus reuteri];>	16	7	4	9	8	14	52.6	45.969	416
>gi|148530580|gb|ABQ82579.1| LSU ribosomal protein L1P [Lactobacillus reuteri DSM 20016];>	8	7	7	4	4	6	37.2	27.362	253
>gi|227070541|gb|EEI08874.1| phosphoribosylaminoimidazole carboxylase [Lactobacillus reuteri MM2-3];>	18	10	15	10	12	12	72.9	42.428	384
>gi|148531276|gb|ABQ83275.1| thiamine-phosphate diphosphorylase [Lactobacillus reuteri DSM 20016];>	14	9	12	6	11	12	86.5	23.849	215
>gi|148530601|gb|ABQ82600.1| thymidylate kinase [Lactobacillus reuteri DSM 20016];>	8	7	7	6	5	7	34.7	24.198	213
>gi|148530538|gb|ABQ82537.1| hypoxanthine phosphoribosyltransferase [Lactobacillus reuteri DSM 20016];>	14	8	12	12	11	14	58.9	20.464	180
>gi|148530877|gb|ABQ82876.1| ribose-phosphate pyrophosphokinase [Lactobacillus reuteri DSM 20016];>	17	15	11	11	10	12	69.1	36.06	324
>gi|148530808|gb|ABQ82807.1| thioredoxin [Lactobacillus reuteri DSM 20016];>	7	7	7	7	7	7	55.8	11.889	104
>gi|148530568|gb|ABQ82567.1| aminopeptidase C, Cysteine peptidase, MEROPS family C01B [Lactobacillus reuteri DSM 20016];>	17	8	10	11	9	6	44.6	51.985	446
>gi|148530279|gb|ABQ82278.1| DNA polymerase III, beta subunit [Lactobacillus reuteri DSM	14	13	14	11	12	12	49.7	41.831	380

20016];>									
>gi|148530965|gb|ABQ82964.1| bacterial translation initiation factor 2 (bIF-2) [Lactobacillus reuteri DSM 20016];>	9	7	6	5	8	5	19.5	83.537	752
>gi|148531054|gb|ABQ83053.1| protein of unknown function DUF322 [Lactobacillus reuteri DSM 20016];>	11	8	7	7	10	11	74	13.85	127
>gi|227071794|gb|EEI10083.1| glycerone kinase [Lactobacillus reuteri MM2-3];>	12	5	7	6	6	7	32.2	62.132	575
>gi|148531975|gb|ABQ83974.1| LSU ribosomal protein L29P [Lactobacillus reuteri DSM 20016];>	14	5	10	10	8	10	46.1	51.127	477
>gi|183225457|dbj|BAG25974.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	6	5	6	5	5	3	40.2	20.101	179
>gi|227070201|gb|EEI08575.1| conserved hypothetical protein [Lactobacillus reuteri MM2-3];>	14	11	6	4	7	10	85.5	13.567	117
>gi|337728945|emb|CCC04065.1| phosphoribosylformylglycinamidine synthase II [Lactobacillus reuteri ATCC 53608]	14	3	6	7	9	7	30.9	80.963	742
>gi|148531012|gb|ABQ83011.1| protein of unknown function DUF34 [Lactobacillus reuteri DSM 20016];>	6	4	5	5	3	5	23.4	30.534	274
>gi|148531233|gb|ABQ83232.1| nucleotide deoxyribosyltransferase [Lactobacillus reuteri DSM 20016];>	10	5	7	5	6	9	60.6	18.201	160
>gi|148530418|gb|ABQ82417.1| phosphoribosylamine--glycine ligase [Lactobacillus reuteri DSM 20016];>	13	3	9	11	11	1	46.5	45.76	419
>gi|148530375|gb|ABQ82374.1| Inosine/uridine-preferring nucleoside hydrolase [Lactobacillus reuteri DSM 20016];>	12	6	8	8	8	10	62.3	32.573	302
>gi|148531734|gb|ABQ83733.1| SSU ribosomal protein S7P [Lactobacillus reuteri DSM 20016];>	6	5	5	6	5	5	38.5	17.985	156
>gi|183224027|dbj|BAG24544.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112	10	7	9	6	7	5	45	20.228	180
>gi|148531034|gb|ABQ83033.1| thymidylate synthase [Lactobacillus reuteri DSM 20016];>	9	6	8	8	6	9	27.2	36.982	320
>gi|148530494|gb|ABQ82493.1| Cof-like hydrolase [Lactobacillus reuteri DSM 20016	9	8	7	6	3	5	32.2	30.465	273
>gi|183225222|dbj|BAG25739.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	9	6	6	6	6	5	56.5	18.557	170
>gi|148530832|gb|ABQ82831.1| HAD-superfamily subfamily IIA hydrolase like protein [Lactobacillus reuteri DSM 20016];>	9	6	3	4	6	7	42.6	28.505	256
>gi|148530871|gb|ABQ82870.1| aminotransferase, class V [Lactobacillus reuteri DSM 20016];>	6	2	1	4	2	5	27.9	42.481	384
>gi|148530787|gb|ABQ82786.1| peptidase M16 domain protein [Lactobacillus reuteri DSM 20016];>	10	7	7	6	7	9	33	47.131	415
>gi|148530492|gb|ABQ82491.1| ribose-phosphate pyrophosphokinase [Lactobacillus reuteri DSM 20016];>	11	9	10	10	10	8	38.9	36.16	329
>gi|148530623|gb|ABQ82622.1| chaperonin Cpn10 [Lactobacillus reuteri DSM 20016];>	8	6	5	6	8	6	71.3	10.149	94
>gi|148531694|gb|ABQ83693.1| xanthine phosphoribosyltransferase [Lactobacillus reuteri DSM 20016];>	10	7	7	7	7	8	52.4	21.113	191
>gi|112943728|gb|ABI26320.1| fructose-2,6-bisphosphatase [Lactobacillus reuteri];>	8	5	6	6	8	7	48.8	24.287	217

>gi|148530585|gb|ABQ82584.1| LSU ribosomal protein L12P [Lactobacillus reuteri DSM 20016];>	10	7	5	6	4	6	95	12.356	121
>gi|148531033|gb|ABQ83032.1| ABC transporter related [Lactobacillus reuteri DSM 20016];>	16	6	10	10	10	6	32.2	72.792	636
>gi|148531131|gb|ABQ83130.1| hypothetical protein Lreu_0867 [Lactobacillus reuteri DSM 20016];>	11	11	9	8	10	9	63.7	10.576	91
>gi|148530873|gb|ABQ82872.1| tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase [Lactobacillus reuteri DSM 20016];>	12	5	0	7	8	11	50.7	42.769	377
>gi|148531941|gb|ABQ83940.1| porphobilinogen synthase [Lactobacillus reuteri DSM 20016];>	9	7	7	6	5	8	44	35.84	323
>gi|148531141|gb|ABQ83140.1| Purine nucleosidase [Lactobacillus reuteri DSM 20016];>	10	8	5	6	7	5	67.8	34.734	314
>gi|148530769|gb|ABQ82768.1| SSU ribosomal protein S4P [Lactobacillus reuteri DSM 20016];>	15	15	15	13	13	14	57.2	22.943	201
>gi|183225185|dbj|BAG25702.1| thioredoxin [Lactobacillus reuteri JCM 1112];>	5	4	4	4	3	5	28.4	12.828	109
>gi|148531891|gb|ABQ83890.1| Ferritin, Dps family protein [Lactobacillus reuteri DSM 20016];>	10	4	2	3	5	7	89	18.223	155
>gi|148530571|gb|ABQ82570.1| deoxyuridine 5-triphosphate nucleotidohydrolase [Lactobacillus reuteri DSM 20016	8	4	6	6	7	7	44.9	20.3	178
>gi|148531730|gb|ABQ83729.1| LSU ribosomal protein L3P [Lactobacillus reuteri DSM 20016];>	10	9	9	6	5	8	45.2	23.757	219
>gi|148530714|gb|ABQ82713.1| arginine deiminase [Lactobacillus reuteri DSM 20016];>	19	14	9	11	15	18	64.9	46.242	410
>gi|148531477|gb|ABQ83476.1| transcriptional regulator, HxlR family [Lactobacillus reuteri DSM 20016];>	10	8	7	9	8	9	67.9	13.039	112
>gi|148531718|gb|ABQ83717.1| LSU ribosomal protein L5P [Lactobacillus reuteri DSM 20016];>	10	5	8	8	5	10	60	20.171	180
>gi|133930435|gb|ABO43789.1| DNA-binding protein [Lactobacillus reuteri];>	6	4	5	5	4	2	48.4	9.5238	91
>gi|183225377|dbj|BAG25894.1| 50S ribosomal protein L18 [Lactobacillus reuteri JCM 1112];>	6	5	5	6	5	4	55.4	13.342	121
>gi|183226422|dbj|BAG26938.1| GTP-binding protein [Lactobacillus fermentum IFO 3956];>	14	13	13	12	14	11	21.3	68.695	615
>gi|148531329|gb|ABQ83328.1| FAD-dependent pyridine nucleotide-disulfide oxidoreductase [Lactobacillus reuteri DSM 20016];>	9	2	4	3	6	7	23.6	60.056	555
>gi|148530659|gb|ABQ82658.1| ATP-dependent Clp protease proteolytic subunit ClpP [Lactobacillus reuteri DSM 20016];>	11	7	9	8	8	10	78.7	21.433	197
>gi|148531780|gb|ABQ83779.1| Peptidylprolyl isomerase [Lactobacillus reuteri DSM 20016];>	9	6	5	6	7	8	58.9	22.199	197
>gi|148531452|gb|ABQ83451.1| hypothetical protein Lreu_1194 [Lactobacillus reuteri DSM 20016];>	8	7	8	5	6	6	51.8	19.53	168
>gi|148530412|gb|ABQ82411.1| phosphoribosylformylglycinamidine synthase subunit I [Lactobacillus reuteri DSM 20016];>	9	6	7	8	8	5	73.9	24.732	226
>gi|148530884|gb|ABQ82883.1| dihydrodipicolinate reductase [Lactobacillus reuteri DSM 20016];>	8	4	2	5	4	3	50.6	27.932	259
>gi|148531434|gb|ABQ83433.1| guanylate kinase [Lactobacillus reuteri DSM 20016	10	6	7	7	6	8	49	23.546	206
>gi|148530584|gb|ABQ82583.1| LSU ribosomal protein L10P [Lactobacillus reuteri DSM 20016];>	5	3	3	3	2	3	39.2	18.139	166
>gi|227070613|gb|EEI08943.1| possible asparagine synthase (glutamine-hydrolyzing) [Lactobacillus reuteri MM2-3];>gi|227186280|gb|EEI66351.1| possible asparagine synthase	14	10	11	8	7	4	25.9	76.34	652

(glutamine-hydrolyzing) [Lactobacillus reuteri CF48-3A];>gi|227364300|ref|ZP_03848393.									
>gi|148532020|gb|ABQ84019.1| PEBP family protein [Lactobacillus reuteri DSM 20016];> J	6	3	5	3	5	3	37.5	18.294	168
>gi|227071420|gb|EEI09725.1| FMN reductase [Lactobacillus reuteri MM2-3];>	6	3	4	4	4	2	24.8	26.152	238
>gi|148531323|gb|ABQ83322.1| glycerol kinase [Lactobacillus reuteri DSM 20016	12	7	8	7	10	7	32.4	55.388	500
>gi|227070129|gb|EEI08505.1| D-tyrosyl-tRNA(Tyr) deacylase [Lactobacillus reuteri MM2-3];>	5	4	4	4	3	4	53.5	17.733	159
>gi|133930437|gb|ABO43790.1| cytosine deaminase [Lactobacillus reuteri];>	11	9	10	9	8	10	37.9	46.441	412
>gi|148530405|gb|ABQ82404.1| acetolactate synthase, catabolic [Lactobacillus reuteri DSM 20016];>	11	0	5	0	11	2	31.5	60.994	559
>gi|148530924|gb|ABQ82923.1| GTP-binding protein Obg/CgtA [Lactobacillus reuteri DSM 20016];>	17	13	10	8	9	13	48.2	47.975	438
>gi|148530579|gb|ABQ82578.1| LSU ribosomal protein L11P [Lactobacillus reuteri DSM 20016];>	9	6	5	8	6	8	53.9	14.869	141
>gi|148531486|gb|ABQ83485.1| two component transcriptional regulator, winged helix family [Lactobacillus reuteri DSM 20016];>	5	5	3	3	4	4	28.9	26.33	228
>gi|183224837|dbj|BAG25354.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	7	4	7	3	4	3	25.3	48.212	430
>gi|227070517|gb|EEI08850.1| deoxyribose-phosphate aldolase [Lactobacillus reuteri MM2-3];>	10	7	6	5	4	5	51.1	25.76	237
>gi|148530821|gb|ABQ82820.1| protein of unknown function DUF28 [Lactobacillus reuteri DSM 20016];>	11	9	8	7	6	7	63.3	27.161	248
>gi|227071111|gb|EEI09429.1| Dyp family peroxidase family protein [Lactobacillus reuteri MM2-3];>	11	7	7	5	10	6	40.1	36.463	317
>gi|148531334|gb|ABQ83333.1| hypothetical protein Lreu_1076 [Lactobacillus reuteri DSM 20016];>	1	1	1	0	0	0	9.7	17.798	154
>gi|148531061|gb|ABQ83060.1| hypothetical protein Lreu_0797 [Lactobacillus reuteri DSM 20016];>	4	3	1	1	0	3	27.4	31.07	285
>gi|148531725|gb|ABQ83724.1| LSU ribosomal protein L22P [Lactobacillus reuteri DSM 20016];>	7	5	5	3	4	4	53	12.394	115
>gi|148531458|gb|ABQ83457.1| aminotransferase [Lactobacillus reuteri DSM 20016];>	11	5	6	7	8	6	41	43.367	393
>gi|148530344|gb|ABQ82343.1| guanosine monophosphate reductase [Lactobacillus reuteri DSM 20016	11	6	5	7	7	9	48.5	35.96	324
>gi|148530883|gb|ABQ82882.1| dihydrodipicolinate synthase [Lactobacillus reuteri DSM 20016];>	8	7	3	7	5	7	46.9	33.823	307
>gi|148531980|gb|ABQ83979.1| Propanediol utilization protein [Lactobacillus reuteri DSM 20016];>	7	2	4	3	3	6	33.6	23.962	214
>gi|183225130|dbj|BAG25647.1| peptide methionine sulfoxide reductase [Lactobacillus reuteri JCM 1112];>	5	4	4	5	3	3	45.4	21.132	185
>gi|148531027|gb|ABQ83026.1| cytidylate kinase [Lactobacillus reuteri DSM 20016];>	9	6	6	5	4	7	46.9	25.015	228
>gi|133930513|gb|ABO43828.1| triosephosphate isomerase [Lactobacillus reuteri];>	12	10	7	9	7	8	64.4	29.214	264
>gi|148530610|gb|ABQ82609.1| UDP-galactose 4-epimerase [Lactobacillus reuteri DSM 20016];>	16	11	12	7	12	4	81.3	36.598	331

>gi|227070968|gb|EEI09291.1| glutamate-1-semialdehyde 2,1-aminomutase [Lactobacillus reuteri MM2-3];>	12	10	7	5	5	8	53.7	48.026	443
>gi|148531721|gb|ABQ83720.1| SSU ribosomal protein S17P [Lactobacillus reuteri DSM 20016];>	8	8	7	6	6	5	68.2	10.168	88
>gi|183226317|dbj|BAG26833.1| valyl-tRNA synthase [Lactobacillus fermentum IFO 3956	13	12	9	9	7	5	15.8	101.85	884
>gi|227070651|gb|EEI08981.1| rhodanese domain protein [Lactobacillus reuteri MM2-3];>	10	7	6	6	8	8	57.1	29.725	261
>gi|148531143|gb|ABQ83142.1| riboflavin synthase, alpha subunit [Lactobacillus reuteri DSM 20016];>	6	4	5	4	6	5	33	21.593	200
>gi|183224298|dbj|BAG24815.1| aminotransferase [Lactobacillus reuteri JCM 1112	12	6	5	9	10	9	50.5	44.088	400
>gi|148531444|gb|ABQ83443.1| protein of unknown function DUF322 [Lactobacillus reuteri DSM 20016];>	6	2	4	3	4	3	48.3	15.835	145
>gi|148531723|gb|ABQ83722.1| LSU ribosomal protein L16P [Lactobacillus reuteri DSM 20016];>	9	6	7	6	6	6	61.8	16.006	144
>gi|148531005|gb|ABQ83004.1| glycyl-tRNA synthetase alpha chain [Lactobacillus reuteri DSM 20016];>	14	12	7	7	10	10	56.7	37.843	328
>gi|183224803|dbj|BAG25320.1| hypothetical protein [Lactobacillus reuteri JCM 1112];>	12	4	9	9	6	6	48	40.672	371
>gi|148530530|gb|ABQ82529.1| Hydratase/decarboxylase [Lactobacillus reuteri DSM 20016];>	4	2	0	4	2	2	41.4	29.033	263
>gi|148531715|gb|ABQ83714.1| LSU ribosomal protein L6P [Lactobacillus reuteri DSM 20016];>	13	7	6	11	6	10	44.4	19.635	178
>gi|148530501|gb|ABQ82500.1| CTP synthase [Lactobacillus reuteri DSM 20016];>	7	4	3	3	5	4	20.6	59.6	534
>gi|148530321|gb|ABQ82320.1| aromatic amino acid aminotransferase apoenzyme [Lactobacillus reuteri DSM 20016];>	11	9	4	8	6	9	42.5	42.52	395
>gi|148531442|gb|ABQ83441.1| methenyltetrahydrofolate cyclohydrolase / 5,10-methylenetetrahydrofolate dehydrogenase (NADP+) [Lactobacillus reuteri DSM 20016];>	10	6	7	6	6	3	45.5	30.424	286
>gi|148530435|gb|ABQ82434.1| short-chain dehydrogenase/reductase SDR [Lactobacillus reuteri DSM 20016];>	11	8	4	3	4	9	61.8	26.39	246
>gi|337728953|emb|CCC04073.1| acetolactate synthase [Lactobacillus reuteri ATCC 53608]	10	0	5	1	10	2	24.9	61.077	559
>gi|227070793|gb|EEI09119.1| NADPH dehydrogenase [Lactobacillus reuteri MM2-3];>	4	1	2	0	3	1	26.1	26.711	230
>gi|148530861|gb|ABQ82860.1| cell division protein FtsZ [Lactobacillus reuteri DSM 20016];>	12	8	7	8	9	10	42.2	44.45	415
>gi|227184822|gb|EEI64893.1| possible 2-aminoadipate transaminase [Lactobacillus reuteri CF48-3A];>	10	6	5	7	9	7	40.6	43.84	397
>gi|148531693|gb|ABQ83692.1| 5-(carboxyamino)imidazole ribonucleotide synthase [Lactobacillus reuteri DSM 20016];>	13	8	8	8	10	11	57.6	42.001	377
>gi|227185166|gb|EEI65237.1| aspartate semialdehyde dehydrogenase [Lactobacillus reuteri CF48-3A	6	0	2	1	6	2	29.3	38.935	358
>gi|148531473|gb|ABQ83472.1| uridine kinase [Lactobacillus reuteri DSM 20016];>	7	3	7	5	6	7	40.4	25.134	218
>gi|112943339|gb|ABI26305.1| sphingosine kinase [Lactobacillus reuteri	8	7	4	2	5	5	27.3	37.14	337
>gi|227071141|gb|EEI09457.1| mannosyl-glycoprotein endo-beta-N-acetylglucosamidase [Lactobacillus reuteri MM2-3];>	4	2	1	4	2	2	22.1	25.877	222

>gi|183225693|dbj|BAG26210.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	4	2	1	2	3	4	19.8	41.737	364
>gi|148530734|gb|ABQ82733.1| ATP synthase F1 subcomplex alpha subunit [Lactobacillus reuteri DSM 20016];>	16	14	10	9	9	6	35.4	55.226	509
>gi|148530542|gb|ABQ82541.1| lysyl-tRNA synthetase [Lactobacillus reuteri DSM 20016];>	14	7	1	9	8	13	31.7	58.296	508
>gi|148531976|gb|ABQ83975.1| ATP:cob(I)alamin adenosyltransferase [Lactobacillus reuteri DSM 20016];>	5	1	4	4	4	4	33.1	16.903	157
>gi|148530651|gb|ABQ82650.1| hydrolase of HD superfamily-like protein [Lactobacillus reuteri DSM 20016];>	9	6	7	8	7	6	42.9	24.304	212
>gi|148530744|gb|ABQ82743.1| glycine cleavage H-protein [Lactobacillus reuteri DSM 20016];>	6	3	2	4	3	6	75.3	10.641	97
>gi|183225184|dbj|BAG25701.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	5	1	2	2	1	2	40.3	23.595	216
>gi|148531499|gb|ABQ83498.1| bacterial translation initiation factor 3 (bIF-3) [Lactobacillus reuteri DSM 20016 i	4	3	3	2	3	4	31.2	19.487	170
>gi|183225758|dbj|BAG26275.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	6	4	3	2	5	5	33	22.577	203
>gi|148530319|gb|ABQ82318.1| OsmC family protein [Lactobacillus reuteri DSM 20016];>	8	6	8	5	6	6	45.1	15.724	142
>gi|148531724|gb|ABQ83723.1| SSU ribosomal protein S3P [Lactobacillus reuteri DSM 20016];>	9	8	9	8	8	7	40.3	24.696	221
>gi|148531729|gb|ABQ83728.1| LSU ribosomal protein L4P [Lactobacillus reuteri DSM 20016];>	6	6	6	4	4	5	41.1	22.29	207
>gi|183224273|dbj|BAG24790.1| transcription accessory protein [Lactobacillus reuteri JCM 1112];>	10	4	5	4	4	6	23.1	82.072	727
>gi|148530614|gb|ABQ82613.1| pyrroline-5-carboxylate reductase [Lactobacillus reuteri DSM 20016];>	8	6	5	3	5	4	46.7	26.78	257
>gi|148530311|gb|ABQ82310.1| aldehyde dehydrogenase [Lactobacillus reuteri DSM 20016];>	7	3	6	3	3	0	22.3	50.505	457
>gi|148531769|gb|ABQ83768.1| DJ-1 family protein [Lactobacillus reuteri DSM 20016];>	5	2	3	4	5	2	35.3	20.995	190
>gi|148530379|gb|ABQ82378.1| chromosome segregation ATPase [Lactobacillus reuteri DSM 20016];>	6	4	3	2	2	4	30.1	28.007	256
>gi|227184876|gb|EEI64947.1| pyrroline-5-carboxylate reductase [Lactobacillus reuteri CF48-3A];>	7	5	6	2	5	3	33.9	26.871	257
>gi|148531565|gb|ABQ83564.1| heat shock protein Hsp20 [Lactobacillus reuteri DSM 20016	3	2	1	1	3	2	25.2	16.747	143
>gi|148530950|gb|ABQ82949.1| SSU ribosomal protein S2P [Lactobacillus reuteri DSM 20016];>	10	7	6	9	4	9	36.3	29.657	262
>gi|148531756|gb|ABQ83755.1| UspA domain protein [Lactobacillus reuteri DSM 20016];>	6	2	2	4	4	6	38	17.57	158
>gi|227071504|gb|EEI09804.1| glutamate-5-semialdehyde dehydrogenase [Lactobacillus reuteri MM2-3	12	8	8	8	10	9	38.5	45.499	416
>gi|148531915|gb|ABQ83914.1| hypothetical protein Lreu_1675 [Lactobacillus reuteri DSM 20016];>	1	0	1	1	1	0	8.6	17.238	152
>gi|183225283|dbj|BAG25800.1| putative autolysin [Lactobacillus reuteri JCM 1112	2	0	0	0	1	1	7.7	60.393	532

>gi|148530881|gb|ABQ82880.1| Tetrahydrodipicolinate succinyltransferase N-terminal domain protein [Lactobacillus reuteri DSM 20016];>	5	3	2	2	3	5	38.6	24.876	236
>gi|148531412|gb|ABQ83411.1| SSU ribosomal protein S16P [Lactobacillus reuteri DSM 20016];>	4	4	4	3	2	3	45.1	10.449	91
>gi|194454489|gb|EDX43386.1| aminotransferase class I and II [Lactobacillus reuteri 100-23	8	6	2	5	3	6	32.4	42.531	395
>gi|148530962|gb|ABQ82961.1| NusA antitermination factor [Lactobacillus reuteri DSM 20016];>	5	4	1	0	3	2	22.3	44.525	395
>gi|148530371|gb|ABQ82370.1| amino acid ABC transporter membrane protein, PAAT family / amino acid ABC transporter substrate-binding protein, PAAT family [Lactobacillus reuteri DSM 20016];>gi|148543337|ref|YP_001270707.1| polar amino acid ABC transporter i	2	1	0	1	0	1	6.6	53.65	487
>gi|148531728|gb|ABQ83727.1| LSU ribosomal protein L23P [Lactobacillus reuteri DSM 20016];>	7	7	5	5	2	4	66.3	11.25	98
>gi|148530842|gb|ABQ82841.1| NAD(+) kinase [Lactobacillus reuteri DSM 20016	6	4	6	3	5	6	24.8	30.666	270
>gi|148530804|gb|ABQ82803.1| Holliday junction resolvase YqgF [Lactobacillus reuteri DSM 20016];>	6	3	4	6	4	5	40.8	16.525	147
>gi|148532168|gb|ABQ84167.1| signal peptidase I [Lactobacillus reuteri DSM 20016];>	3	1	2	2	2	2	18.4	22.703	201
>gi|148532131|gb|ABQ84130.1| glutamine amidotransferase class-I [Lactobacillus reuteri DSM 20016	4	3	3	3	4	3	31.8	25.353	223
>gi|148530718|gb|ABQ82717.1| CobB/CobQ domain protein glutamine amidotransferase [Lactobacillus reuteri DSM 20016];>	3	3	1	0	0	2	21.3	26.134	235
>gi|148530475|gb|ABQ82474.1| tryptophanyl-tRNA synthetase [Lactobacillus reuteri DSM 20016	10	4	2	5	9	8	47.9	38.062	340
>gi|227071501|gb|EEI09801.1| LL-diaminopimelate aminotransferase [Lactobacillus reuteri MM2-3];>	3	0	1	2	2	1	10.4	43.448	395
>gi|148531738|gb|ABQ83737.1| DNA-directed RNA polymerase subunit beta [Lactobacillus reuteri DSM 20016];>	14	5	3	7	7	11	20.6	135.42	1211
>gi|148531215|gb|ABQ83214.1| Glyoxalase/bleomycin resistance protein/dioxygenase [Lactobacillus reuteri DSM 20016];>	4	3	3	4	4	4	28.5	14.777	130
>gi|148530964|gb|ABQ82963.1| LSU ribosomal protein L7AE [Lactobacillus reuteri DSM 20016];>	5	5	3	1	2	4	43.7	11.651	103
>gi|183224121|dbj|BAG24638.1| hypothetical protein [Lactobacillus reuteri JCM 1112];>	9	3	8	6	6	6	54.3	24.277	219
>gi|148530284|gb|ABQ82283.1| SSU ribosomal protein S6P [Lactobacillus reuteri DSM 20016];>	7	6	5	5	5	6	63.3	11.378	98
>gi|148530288|gb|ABQ82287.1| LSU ribosomal protein L9P [Lactobacillus reuteri DSM 20016];>	7	3	5	2	4	3	37.3	16.692	150
>gi|148531488|gb|ABQ83487.1| protein of unknown function DUF177 [Lactobacillus reuteri DSM 20016];>	4	4	3	3	2	3	25.9	20.961	185
>gi|183227420|dbj|BAG27936.1| autoinducer-2 production protein [Lactobacillus fermentum IFO 3956];>	8	5	6	6	6	6	50	17.719	158
>gi|148531987|gb|ABQ83986.1| Glycerol dehydratase [Lactobacillus reuteri DSM 20016];>	13	1	10	2	6	6	29	62.092	558
>gi|148531286|gb|ABQ83285.1| Phosphoglycerate mutase [Lactobacillus reuteri DSM 20016];>	6	4	5	3	4	5	21.9	30.347	278
>gi|148531703|gb|ABQ83702.1| LSU ribosomal protein L17P [Lactobacillus reuteri DSM 20016];>	4	4	2	2	2	3	32.3	14.201	127
>gi|148530459|gb|ABQ82458.1| methionine-R-sulfoxide reductase [Lactobacillus reuteri DSM 20016	7	4	5	4	5	4	54.9	16.101	142

>gi|148530385|gb|ABQ82384.1| phosphopentomutase [Lactobacillus reuteri DSM 20016];>	13	8	7	10	10	8	51.4	44.002	397
>gi|183226446|dbj|BAG26962.1| GTP-binding protein [Lactobacillus fermentum IFO 3956];>	9	9	8	6	8	8	24.4	47.427	435
>gi|148531540|gb|ABQ83539.1| hypothetical protein Lreu_1282 [Lactobacillus reuteri DSM 20016];>	3	3	3	3	3	3	38	10.138	92
>gi|148532166|gb|ABQ84165.1| aldo/keto reductase [Lactobacillus reuteri DSM 20016];>	7	3	3	4	6	3	29.6	32.371	287
>gi|148530541|gb|ABQ82540.1| tRNA-U20-dihydrouridine synthase [Lactobacillus reuteri DSM 20016];>	10	4	1	3	4	4	52	37.061	333
>gi|148530761|gb|ABQ82760.1| hypothetical protein Lreu_0492 [Lactobacillus reuteri DSM 20016];>	3	2	2	2	2	3	16.6	27.759	247
>gi|148530631|gb|ABQ82630.1| protein of unknown function UPF0029 [Lactobacillus reuteri DSM 20016];>	5	4	3	2	3	2	29	23.548	210
>gi|148530642|gb|ABQ82641.1| UDP-glucose pyrophosphorylase [Lactobacillus reuteri DSM 20016];>	4	1	3	2	2	2	14.1	34.101	304
>gi|148531708|gb|ABQ83707.1| bacterial translation initiation factor 1 (bIF-1) [Lactobacillus reuteri DSM 20016];>	6	4	3	2	3	5	55.6	8.1615	72
>gi|148532063|gb|ABQ84062.1| thioredoxin [Lactobacillus reuteri DSM 20016	5	4	3	4	3	4	49.5	12.064	105
>gi|148530691|gb|ABQ82690.1| hypothetical protein Lreu_0421 [Lactobacillus reuteri DSM 20016];>	5	3	1	1	3	5	36.4	11.143	99
>gi|183225646|dbj|BAG26163.1| hypothetical protein [Lactobacillus reuteri JCM 1112];>	2	0	0	0	0	2	13.5	19.84	178
>gi|148530896|gb|ABQ82895.1| peptide deformylase [Lactobacillus reuteri DSM 20016];>	5	3	4	4	3	4	28.5	20.898	186
>gi|227071772|gb|EEI10061.1| transcription antitermination protein NusB [Lactobacillus reuteri MM2-3	3	2	2	3	3	3	32.6	16.148	141
>gi|148530837|gb|ABQ82836.1| Negative regulator of genetic competence [Lactobacillus reuteri DSM 20016];>	4	2	0	3	4	4	24.7	25.701	223
>gi|148530983|gb|ABQ82982.1| [LSU ribosomal protein L11P]-lysine N-methyltransferase [Lactobacillus reuteri DSM 20016	11	6	7	7	7	9	73	34.966	319
>gi|227070539|gb|EEI08872.1| acetolactate decarboxylase [Lactobacillus reuteri MM2-3];>	6	4	6	6	6	5	42.7	27.238	246
>gi|148531705|gb|ABQ83704.1| SSU ribosomal protein S11P [Lactobacillus reuteri DSM 20016];>	3	3	2	2	2	2	22.5	13.759	129
>gi|227070644|gb|EEI08974.1| phosphatidylglycerophosphatase [Lactobacillus reuteri MM2-3];>	9	6	8	5	7	5	56.2	18.978	169
>gi|148531731|gb|ABQ83730.1| SSU ribosomal protein S10P [Lactobacillus reuteri DSM 20016];>	5	4	4	3	4	5	46.1	11.777	102
>gi|148530997|gb|ABQ82996.1| argininosuccinate lyase [Lactobacillus reuteri DSM 20016];>	5	3	2	1	1	0	13	51.92	461
>gi|148530860|gb|ABQ82859.1| cell division protein FtsA [Lactobacillus reuteri DSM 20016];>	4	3	4	3	3	4	9.2	50.485	457
>gi|227071143|gb|EEI09459.1| ribosomal protein S9 [Lactobacillus reuteri MM2-3];>	7	7	6	6	5	7	33.1	14.494	133
>gi|183224278|dbj|BAG24795.1| phosphoglycerate mutase [Lactobacillus reuteri JCM 1112	7	6	4	1	3	3	36.1	29.591	255
>gi|148531843|gb|ABQ83842.1| Peptidoglycan-binding LysM [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	0	4.5	37.509	355
>gi|148530634|gb|ABQ82633.1| SSU ribosomal protein S30P [Lactobacillus reuteri DSM 20016];>	4	3	2	3	4	3	35.7	21.144	182

>gi|148530681|gb|ABQ82680.1| hypothetical protein Lreu_0411 [Lactobacillus reuteri DSM 20016];>	4	3	3	3	3	2	44.7	12.29	103
>gi|148531711|gb|ABQ83710.1| LSU ribosomal protein L15P [Lactobacillus reuteri DSM 20016];>	6	6	5	5	4	5	31.9	15.447	144
>gi|227071637|gb|EEI09931.1| uracil phosphoribosyltransferase [Lactobacillus reuteri MM2-3];>	5	3	3	5	4	4	27.1	24.512	221
>gi|148530736|gb|ABQ82735.1| ATP synthase F1 subcomplex beta subunit [Lactobacillus reuteri DSM 20016];>	7	6	4	4	4	4	16.4	51.631	475
>gi|148531735|gb|ABQ83734.1| SSU ribosomal protein S12P [Lactobacillus reuteri DSM 20016];>	3	3	2	2	2	2	19.4	15.503	139
>gi|148531720|gb|ABQ83719.1| LSU ribosomal protein L14P [Lactobacillus reuteri DSM 20016];>	7	5	6	3	3	5	51.6	13.144	122
>gi|148531538|gb|ABQ83537.1| dihydroneopterin aldolase [Lactobacillus reuteri DSM 20016];>	3	1	0	2	3	2	71.2	12.739	111
>gi|148530994|gb|ABQ82993.1| GatB/Yqey domain protein [Lactobacillus reuteri DSM 20016];>	2	1	2	2	2	2	17.6	16.725	148
>gi|148531536|gb|ABQ83535.1| GTP cyclohydrolase I [Lactobacillus reuteri DSM 20016];>	7	5	6	7	5	6	42.7	22.03	192
>gi|148531841|gb|ABQ83840.1| GCN5-related N-acetyltransferase [Lactobacillus reuteri DSM 20016];>	6	2	0	3	0	4	38.2	19.96	173
>gi|148531888|gb|ABQ83887.1| dipeptidyl-peptidase IV, Serine peptidase, MEROPS family S15 [Lactobacillus reuteri DSM 20016	3	0	0	0	3	0	5.8	90.378	800
>gi|148531698|gb|ABQ83697.1| LSU ribosomal protein L13P [Lactobacillus reuteri DSM 20016];>	7	3	5	4	3	3	45.6	16.304	147
>gi|337728903|emb|CCC04023.1| conserved hypothetical protein [Lactobacillus reuteri ATCC 53608];>	7	3	4	4	3	4	28.3	30.777	286
>gi|148531566|gb|ABQ83565.1| aldo/keto reductase [Lactobacillus reuteri DSM 20016];>	8	6	3	6	4	4	45.4	30.851	282
>gi|148531897|gb|ABQ83896.1| FAD-dependent pyridine nucleotide-disulfide oxidoreductase [Lactobacillus reuteri DSM 20016	8	7	3	6	4	6	45.2	35.791	332
>gi|227185170|gb|EEI65241.1| amidohydrolase [Lactobacillus reuteri CF48-3A];>	5	2	2	5	3	3	23.5	42.662	383
>gi|227070071|gb|EEI08449.1| aldose 1-epimerase [Lactobacillus reuteri MM2-3];>	7	4	4	5	4	5	45.7	34.581	304
>gi|148532139|gb|ABQ84138.1| protein of unknown function DUF1002 [Lactobacillus reuteri DSM 20016];>	8	2	4	3	3	6	20.7	35.908	333
>gi|148530739|gb|ABQ82738.1| UDP-N-acetylglucosamine 1-carboxyvinyltransferase [Lactobacillus reuteri DSM 20016];>	5	4	3	4	4	4	16.2	47.242	438
>gi|148530367|gb|ABQ82366.1| NADPH-dependent FMN reductase [Lactobacillus reuteri DSM 20016];>	7	5	1	4	3	6	27.4	45.674	416
>gi|227070907|gb|EEI09230.1| possible Bis(5-nucleosyl)-tetraphosphatase (asymmetrical) [Lactobacillus reuteri MM2-3];>	3	1	3	1	2	2	23.2	19.718	168
>gi|148531449|gb|ABQ83448.1| protein of unknown function DUF464 [Lactobacillus reuteri DSM 20016];>	2	2	1	1	1	1	28	11.705	107
>gi|148530526|gb|ABQ82525.1| alanine racemase [Lactobacillus reuteri DSM 20016];>	3	2	2	2	1	1	11.5	41.162	375
>gi|148531531|gb|ABQ83530.1| hypothetical protein Lreu_1273 [Lactobacillus reuteri DSM 20016];>	5	4	4	5	4	5	52.2	10.018	90
>gi|148531352|gb|ABQ83351.1| N-acetylmuramoyl-L-alanine amidase, family 2 [Lactobacillus	3	0	1	3	3	2	10.8	44.687	399

reuteri DSM 20016];>									
>gi|148530885|gb|ABQ82884.1| aminotransferase [Lactobacillus reuteri DSM 20016	5	1	0	4	2	4	19.8	42.991	394
>gi|148530622|gb|ABQ82621.1| CoA-binding domain protein [Lactobacillus reuteri DSM 20016];>	6	2	3	3	3	5	29.9	23.939	214
>gi|148531448|gb|ABQ83447.1| LSU ribosomal protein L27P [Lactobacillus reuteri DSM 20016];>	5	5	4	4	4	4	50.5	9.9211	93
>gi|148531151|gb|ABQ83150.1| 1,4-Dihydroxy-2-naphthoate synthase [Lactobacillus reuteri DSM 20016];>	7	6	3	3	4	4	33.3	30.019	273
>gi|336448856|gb|AEI57471.1| sensor histidine kinase [Lactobacillus reuteri SD2112];>	4	2	0	0	0	3	10.8	44.299	390
>gi|148531917|gb|ABQ83916.1| lipoate-protein ligase [Lactobacillus reuteri DSM 20016];>	3	2	1	0	1	1	9.8	38.474	337
>gi|148531212|gb|ABQ83211.1| protein of unknown function DUF21 [Lactobacillus reuteri DSM 20016];>	3	2	1	1	3	2	7.1	52.119	453
>gi|148531585|gb|ABQ83584.1| ATPase AAA-2 domain protein [Lactobacillus reuteri DSM 20016];>	7	5	3	2	1	7	12.5	81.867	734
>gi|148530478|gb|ABQ82477.1| hypothetical protein Lreu_0207 [Lactobacillus reuteri DSM 20016];>	5	2	1	2	1	4	21.9	36.908	319
>gi|337728714|emb|CCC03829.1| succinate-semialdehyde dehydrogenase [Lactobacillus reuteri ATCC 53608]	4	1	3	0	0	1	12.3	50.52	457
>gi|148532165|gb|ABQ84164.1| Amidohydrolase 3 [Lactobacillus reuteri DSM 20016	2	1	1	0	1	0	8	57.42	522
>gi|148530913|gb|ABQ82912.1| SSU ribosomal protein S20P [Lactobacillus reuteri DSM 20016];>	2	2	2	2	2	1	25	9.2436	84
>gi|183225401|dbj|BAG25918.1| DNA-directed RNA polymerase beta subunit [Lactobacillus reuteri JCM 1112];>	10	4	0	6	3	9	11	135.36	1205
>gi|148530653|gb|ABQ82652.1| Excinuclease ABC subunit B [Lactobacillus reuteri DSM 20016];>	5	3	2	5	1	0	12.9	79.421	690
>gi|148531404|gb|ABQ83403.1| protein of unknown function DUF1535 [Lactobacillus reuteri DSM 20016];>	2	1	0	1	2	2	7.7	18.631	169
>gi|148530740|gb|ABQ82739.1| cell shape determining protein, MreB/Mrl family [Lactobacillus reuteri DSM 20016];>	6	2	2	3	4	4	25.2	35.48	330
>gi|183225948|dbj|BAG26464.1| amidophosphoribosyltransferase [Lactobacillus fermentum IFO 3956	3	3	3	3	3	2	9.2	53.518	487
>gi|130893136|gb|ABO32595.1| D-lactate dehydrogenase [Lactobacillus reuteri];>	9	5	6	6	5	5	34.2	36.867	330
>gi|148531986|gb|ABQ83985.1| dehydratase, medium subunit [Lactobacillus reuteri DSM 20016];>	5	3	2	2	3	3	27.1	25.808	236
>gi|148530742|gb|ABQ82741.1| hypothetical protein Lreu_0473 [Lactobacillus reuteri DSM 20016];>	4	4	2	2	2	3	58.1	8.7648	74
>gi|148531145|gb|ABQ83144.1| 6,7-dimethyl-8-ribityllumazine synthase [Lactobacillus reuteri DSM 20016	4	0	0	3	1	4	49.3	16.637	152
>gi|183224141|dbj|BAG24658.1| tyrosyl-tRNA synthase [Lactobacillus reuteri JCM 1112];>	8	2	3	5	4	7	28.1	47.69	420
>gi|148530599|gb|ABQ82598.1| DNA replication and repair protein RecR [Lactobacillus reuteri DSM 20016];>	7	3	5	1	6	4	39	22.089	200

>gi|183224612|dbj|BAG25129.1| myo-inositol-1(or 4)-monophosphatase [Lactobacillus reuteri JCM 1112];>	6	3	3	4	4	4	36	28.91	261
>gi|148531752|gb|ABQ83751.1| iron dependent repressor [Lactobacillus reuteri DSM 20016];>	6	5	3	2	2	3	47.8	25.242	224
>gi|148532003|gb|ABQ84002.1| 3-demethylubiquinone-9 3-methyltransferase [Lactobacillus reuteri DSM 20016];>	2	2	0	2	2	2	22.7	15.941	141
>gi|148531643|gb|ABQ83642.1| conserved hypothetical protein 730 [Lactobacillus reuteri DSM 20016	4	3	3	3	3	3	23.1	21.895	195
>gi|148532146|gb|ABQ84145.1| hypothetical protein Lreu_1908 [Lactobacillus reuteri DSM 20016];>	3	2	2	1	1	1	17.4	18.28	161
>gi|148531438|gb|ABQ83437.1| hemolysin A [Lactobacillus reuteri DSM 20016];>	4	3	2	1	4	3	15	30.273	273
>gi|148530817|gb|ABQ82816.1| hypothetical protein Lreu_0548 [Lactobacillus reuteri DSM 20016];>	4	1	1	1	2	4	52.6	16.479	152
>gi|148531846|gb|ABQ83845.1| tRNA/rRNA methyltransferase (SpoU) [Lactobacillus reuteri DSM 20016];>	6	4	2	3	3	1	32.8	31.804	290
>gi|227070920|gb|EEI09243.1| possible phosphatase [Lactobacillus reuteri MM2-3];>	5	5	4	4	4	4	26.6	24.08	218
>gi|148530836|gb|ABQ82835.1| arsenate reductase-like protein [Lactobacillus reuteri DSM 20016];>	4	2	0	1	0	4	29.3	15.539	133
>gi|183227318|dbj|BAG27834.1| 30S ribosomal protein S5 [Lactobacillus fermentum IFO 3956	4	3	3	2	3	2	16.6	17.581	169
>gi|337728855|emb|CCC03974.1| primosomal protein DnaI [Lactobacillus reuteri ATCC 53608	2	2	1	1	2	2	7.3	35.819	313
>gi|183226170|dbj|BAG26686.1| phosphoglucomutase [Lactobacillus fermentum IFO 3956	6	5	5	6	4	4	8.9	63.671	574
>gi|148532108|gb|ABQ84107.1| Methionine synthase, vitamin-B12 independent [Lactobacillus reuteri DSM 20016	7	5	1	1	0	3	28.8	42.352	378
>gi|337728259|emb|CCC03354.1| DNA-directed RNA polymerase alpha subunit [Lactobacillus reuteri ATCC 53608	8	1	1	1	2	5	37.3	34.887	314
>gi|148531689|gb|ABQ83688.1| aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit C [Lactobacillus reuteri DSM 20016	4	3	1	2	2	1	51.4	11.572	105
>gi|148530799|gb|ABQ82798.1| protein translocase subunit yajC [Lactobacillus reuteri DSM 20016];>	3	0	1	1	3	2	22.1	16.384	154
>gi|183227208|dbj|BAG27724.1| ATP-dependent Clp protease ATP-binding subunit [Lactobacillus fermentum IFO 3956	3	2	0	1	1	2	6.4	82.854	748
>gi|148531956|gb|ABQ83955.1| precorrin-4 C11-methyltransferase [Lactobacillus reuteri DSM 20016];>	6	1	3	2	5	2	33.6	27.872	253
>gi|227071446|gb|EEI09749.1| possible acetyltransferase [Lactobacillus reuteri MM2-3];>	5	5	4	2	3	3	53.8	12.089	104
>gi|148530788|gb|ABQ82787.1| peptidase M16 domain protein [Lactobacillus reuteri DSM 20016];>	8	6	4	5	5	5	29.4	49.689	432
>gi|60172697|gb|AAX14531.1| ABC-type cobalt transport system [Lactobacillus reuteri	1	0	0	1	0	1	13.6	12.127	110
>gi|148531160|gb|ABQ83159.1| protein of unknown function DUF59 [Lactobacillus reuteri DSM 20016	3	3	3	2	3	3	28.2	12.081	110

>gi|148530382|gb|ABQ82381.1| GTP-binding protein YchF [Lactobacillus reuteri DSM 20016];>	4	1	2	1	1	4	15.6	39.844	365
>gi|148531350|gb|ABQ83349.1| hypothetical protein Lreu_1092 [Lactobacillus reuteri DSM 20016];>	2	2	0	2	1	2	16.9	9.0102	77
>gi|148530400|gb|ABQ82399.1| orotate phosphoribosyltransferase [Lactobacillus reuteri DSM 20016];>	2	0	0	1	1	2	10.8	23.434	213
>gi|148530503|gb|ABQ82502.1| UDP-N-acetylglucosamine 1-carboxyvinyltransferase [Lactobacillus reuteri DSM 20016];>	12	8	8	5	5	3	45.2	45.676	425
>gi|194453840|gb|EDX42737.1| conserved hypothetical protein [Lactobacillus reuteri 100-23];>	3	3	2	2	3	2	12.7	28.451	245
>gi|148530789|gb|ABQ82788.1| Uncharacterized protein [Lactobacillus reuteri DSM 20016];>	3	1	3	1	1	2	11.6	38.296	345
>gi|148530810|gb|ABQ82809.1| glutamate racemase [Lactobacillus reuteri DSM 20016];>	3	2	2	1	3	2	20.6	28.92	267
>gi|148531475|gb|ABQ83474.1| phenylalanyl-tRNA synthetase beta subunit [Lactobacillus reuteri DSM 20016	4	1	1	1	2	3	7.5	89.003	805
>gi|148532009|gb|ABQ84008.1| UspA domain protein [Lactobacillus reuteri DSM 20016];>	2	1	2	2	1	1	15.6	17.199	154
>gi|148531712|gb|ABQ83711.1| LSU ribosomal protein L30P [Lactobacillus reuteri DSM 20016];>	3	2	2	2	1	2	58.3	6.5566	60
>gi|148531706|gb|ABQ83705.1| SSU ribosomal protein S13P [Lactobacillus reuteri DSM 20016];>	5	4	2	5	2	4	38	13.687	121
>gi|183225952|dbj|BAG26468.1| phosphoribosylamine-glycine ligase [Lactobacillus fermentum IFO 3956];>	2	1	1	1	2	1	4.3	45.159	417
>gi|148530853|gb|ABQ82852.1| S-adenosyl-methyltransferase MraW [Lactobacillus reuteri DSM 20016	1	1	0	0	0	1	6	35.666	318
>gi|148530901|gb|ABQ82900.1| protein of unknown function UPF0223 [Lactobacillus reuteri DSM 20016];>	3	2	2	2	3	3	23.5	11.867	102
>gi|148530553|gb|ABQ82552.1| aspartate racemase [Lactobacillus reuteri DSM 20016];>	8	2	1	0	8	0	53	26.763	236
>gi|148530906|gb|ABQ82905.1| putative methyltransferase [Lactobacillus reuteri DSM 20016];>	5	3	0	2	2	4	28.3	20.85	187
>gi|183227331|dbj|BAG27847.1| 30S ribosomal protein S19 [Lactobacillus fermentum IFO 3956];>	3	3	2	3	2	2	34.4	10.489	93
>gi|148530907|gb|ABQ82906.1| Phosphopantetheine adenylyltransferase [Lactobacillus reuteri DSM 20016];>	5	5	5	5	4	3	27.2	19.361	173
>gi|148530479|gb|ABQ82478.1| Silent information regulator protein Sir2 [Lactobacillus reuteri DSM 20016];>	8	5	3	4	3	5	47	26.454	232
>gi|148531951|gb|ABQ83950.1| anaerobic cobaltochelatase [Lactobacillus reuteri DSM 20016];>	4	1	3	0	2	3	17	29.176	259
>gi|148531918|gb|ABQ83917.1| UspA domain protein [Lactobacillus reuteri DSM 20016];>	3	1	3	2	2	1	18.7	17.168	155
>gi|148531910|gb|ABQ83909.1| Alcohol dehydrogenase GroES domain protein [Lactobacillus reuteri DSM 20016	4	3	2	3	3	3	32.9	34.921	328
>gi|148530411|gb|ABQ82410.1| phosphoribosylformylglycinamidine synthase, purS [Lactobacillus reuteri DSM 20016];>	2	1	0	2	0	0	32.9	9.3323	82
>gi|148531126|gb|ABQ83125.1| GCN5-related N-acetyltransferase [Lactobacillus reuteri DSM 20016];>	2	1	2	2	2	2	19.2	20.109	177
>gi|148531274|gb|ABQ83273.1| Hydroxyethylthiazole kinase [Lactobacillus reuteri DSM 20016];>	3	2	2	3	3	2	18.2	29.028	269

>gi|227185635|gb|EEI65706.1| protein of hypothetical function DUF322 [Lactobacillus reuteri CF48-3A];>	3	3	2	3	3	3	10.7	13.014	122
>gi|183225127|dbj|BAG25644.1| hypothetical protein [Lactobacillus reuteri JCM 1112];>	6	5	1	4	3	5	24.7	40.161	352
>gi|112944062|gb|ABI26332.1| phosphoglycerate mutase [Lactobacillus reuteri];>	4	2	3	4	4	4	20.6	24.692	218
>gi|227070057|gb|EEI08435.1| tetratricopeptide repeat family protein [Lactobacillus reuteri MM2-3];>	7	3	1	4	4	5	26	50.211	434
>gi|148531988|gb|ABQ83987.1| microcompartments protein [Lactobacillus reuteri DSM 20016];>	4	0	3	1	0	1	32.4	24.945	238
>gi|148530346|gb|ABQ82345.1| hypothetical protein Lreu_0070 [Lactobacillus reuteri DSM 20016];>	2	2	0	1	1	0	12.3	29.491	261
>gi|148531170|gb|ABQ83169.1| LrgB family protein [Lactobacillus reuteri DSM 20016];>	3	1	2	2	3	2	20.3	26.306	246
>gi|148530942|gb|ABQ82941.1| hydroxymethylglutaryl-CoA synthase [Lactobacillus reuteri DSM 20016	6	4	3	4	3	5	19.2	42.757	385
>gi|148530993|gb|ABQ82992.1| SSU ribosomal protein S21P [Lactobacillus reuteri DSM 20016];>	2	2	2	2	2	2	30.2	7.7178	63
>gi|148530848|gb|ABQ82847.1| RNA methyltransferase, TrmH family, group 2 [Lactobacillus reuteri DSM 20016];>	2	2	2	2	2	2	17.2	19.557	169
>gi|227070559|gb|EEI08890.1| possible gamma-glutamyl-gamma-aminobutyrate hydrolase [Lactobacillus reuteri MM2-3];>	1	1	0	1	1	1	7.3	27.281	247
>gi|148530473|gb|ABQ82472.1| nucleoside diphosphate kinase [Lactobacillus reuteri DSM 20016];>	5	1	3	3	3	2	40.1	16.772	147
>gi|148531727|gb|ABQ83726.1| LSU ribosomal protein L2P [Lactobacillus reuteri DSM 20016	6	2	2	2	4	2	27.8	30.47	281
>gi|148530818|gb|ABQ82817.1| Xaa-Pro aminopeptidase, Metallo peptidase, MEROPS family M24B [Lactobacillus reuteri DSM 20016	7	4	3	5	5	2	32	40.615	369
>gi|148530567|gb|ABQ82566.1| amino acid ABC transporter substrate-binding protein, PAAT family [Lactobacillus reuteri DSM 20016];>	3	3	0	0	1	0	12.9	28.516	263
>gi|148531875|gb|ABQ83874.1| phage shock protein C, PspC [Lactobacillus reuteri DSM 20016];>	2	2	0	1	2	2	18.1	9.3228	83
>gi|227185529|gb|EEI65600.1| translation factor SUA5 [Lactobacillus reuteri CF48-3A];>	2	0	1	1	2	1	7.9	38.904	354
>gi|227070164|gb|EEI08539.1| protein of hypothetical function DUF896 [Lactobacillus reuteri MM2-3];>	5	2	1	2	4	2	61.6	10.422	86
>gi|148531439|gb|ABQ83438.1| farnesyl-diphosphate synthase [Lactobacillus reuteri DSM 20016];>	4	2	2	3	2	3	26.2	31.64	290
>gi|194453403|gb|EDX42300.1| molybdenum cofactor synthesis domain protein [Lactobacillus reuteri 100-23];>	4	3	2	1	2	3	14.3	44.908	405
>gi|148530805|gb|ABQ82804.1| protein of unknown function DUF1292 [Lactobacillus reuteri DSM 20016];>	2	1	1	0	1	1	33.7	11.401	98
>gi|194453194|gb|EDX42092.1| glutamate racemase [Lactobacillus reuteri 100-23];>	3	2	1	0	3	1	24.7	29	267
>gi|148531492|gb|ABQ83491.1| metal dependent phosphohydrolase [Lactobacillus reuteri DSM 20016];>	2	0	2	0	0	0	13.2	23.131	204

>gi|148531008|gb|ABQ83007.1| RNA polymerase, sigma 70 subunit, RpoD [Lactobacillus reuteri DSM 20016];>	6	4	1	2	2	4	18.9	43.385	380
>gi|148530597|gb|ABQ82596.1| DNA polymerase III, subunits gamma and tau [Lactobacillus reuteri DSM 20016];>	3	2	0	0	1	0	7.9	68.85	611
>gi|148530416|gb|ABQ82415.1| formyltetrahydrofolate-dependent phosphoribosylglycinamide formyltransferase [Lactobacillus reuteri DSM 20016];>	4	1	3	0	2	0	34.7	21.163	190
>gi|148530327|gb|ABQ82326.1| Recombination helicase AddA [Lactobacillus reuteri DSM 20016];>	4	0	0	0	2	4	5.2	159.71	1392
>gi|148530604|gb|ABQ82603.1| protein of unknown function DUF972 [Lactobacillus reuteri DSM 20016];>	2	2	2	2	2	2	12.9	13.587	116
>gi|148531497|gb|ABQ83496.1| LSU ribosomal protein L20P [Lactobacillus reuteri DSM 20016];> r	4	4	4	4	4	4	27.4	13.515	117
>gi|148530839|gb|ABQ82838.1| dipeptidase A, Cysteine peptidase, MEROPS family C69 [Lactobacillus reuteri DSM 20016];>	2	1	2	2	2	2	6.1	51.591	458
>gi|148530511|gb|ABQ82510.1| protein tyrosine phosphatase [Lactobacillus reuteri DSM 20016];>	2	2	2	1	0	1	23.4	17.398	154
>gi|148532023|gb|ABQ84022.1| hypothetical protein Lreu_1783 [Lactobacillus reuteri DSM 20016];>	3	0	1	3	3	3	11.5	43.26	373
>gi|227185295|gb|EEI65366.1| hypothetical extracellular protein [Lactobacillus reuteri CF48-3A];>	1	1	0	1	0	1	4.5	26.125	247
>gi|148531416|gb|ABQ83415.1| condensin subunit Smc [Lactobacillus reuteri DSM 20016];>	2	0	0	1	1	2	2.7	135.2	1187
>gi|148531410|gb|ABQ83409.1| 16S rRNA processing protein RimM [Lactobacillus reuteri DSM 20016	5	3	3	3	3	3	32.1	19.219	168
>gi|148531623|gb|ABQ83622.1| hypothetical protein Lreu_1373 [Lactobacillus reuteri DSM 20016];>	2	0	0	0	2	0	7.8	46.111	398
>gi|148531785|gb|ABQ83784.1| hypothetical protein Lreu_1539 [Lactobacillus reuteri DSM 20016	3	2	2	1	2	0	21.9	16.827	146
>gi|148531533|gb|ABQ83532.1| Dihydropteroate synthase [Lactobacillus reuteri DSM 20016	5	0	1	1	4	2	19.4	43.584	387
>gi|227071502|gb|EEI09802.1| cyanide hydratase [Lactobacillus reuteri MM2-3];>	1	1	0	0	0	1	6	30.185	266
>gi|130893190|gb|ABO32597.1| ATP-dependent Clp protease ATP-binding subunit ClpC [Lactobacillus reuteri];>	5	0	2	3	1	2	6.1	92.89	830
>gi|183224980|dbj|BAG25497.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	1	1	1	1	0	1	5.2	22.333	194
>gi|148531472|gb|ABQ83471.1| transcription elongation factor GreA [Lactobacillus reuteri DSM 20016];>	4	1	3	2	2	4	35.4	18.019	158
>gi|148531003|gb|ABQ83002.1| GTP-binding protein Era [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	7	33.79	301
>gi|148530525|gb|ABQ82524.1| holo-acyl-carrier-protein synthase [Lactobacillus reuteri DSM 20016	3	1	1	2	1	2	56.7	13.167	120
>gi|148531035|gb|ABQ83034.1| dihydrofolate reductase [Lactobacillus reuteri DSM 20016];>	2	1	2	2	1	2	13.6	18.66	162
>gi|194453686|gb|EDX42583.1| ATP-cone domain protein [Lactobacillus reuteri 100-23];>	2	1	0	0	0	1	13.5	18.122	155

>gi|183225937|dbj|BAG26453.1| formate-tetrahydrofolate ligase [Lactobacillus fermentum IFO 3956];>	2	2	2	1	2	1	2.5	59.287	553
>gi|148531450|gb|ABQ83449.1| LSU ribosomal protein L21P [Lactobacillus reuteri DSM 20016];>	2	1	1	1	0	0	26.5	11.256	102
>gi|148531622|gb|ABQ83621.1| lipopolysaccharide biosynthesis protein [Lactobacillus reuteri DSM 20016];>	3	1	2	2	1	2	18.2	22.766	209
>gi|148530899|gb|ABQ82898.1| Dihydrolipoyllysine-residue succinyltransferase [Lactobacillus reuteri DSM 20016];>	2	1	0	1	0	1	5.2	48.372	444
>gi|148531272|gb|ABQ83271.1| hypothetical protein Lreu_1011 [Lactobacillus reuteri DSM 20016];>	2	1	1	0	2	0	4.5	72.576	629
>gi|148531138|gb|ABQ83137.1| type I site-specific deoxyribonuclease, HsdR family [Lactobacillus reuteri DSM 20016	2	1	1	1	1	1	2.6	118.77	1041
>gi|148531407|gb|ABQ83406.1| LSU ribosomal protein L19P [Lactobacillus reuteri DSM 20016];>	6	3	5	4	5	4	33.6	14.824	128
>gi|148531298|gb|ABQ83297.1| hypothetical protein Lreu_1037 [Lactobacillus reuteri DSM 20016	2	0	2	0	1	1	5.2	69.932	630
>gi|227186326|gb|EEI66397.1| conserved hypothetical protein [Lactobacillus reuteri CF48-3A];>	2	1	1	1	1	1	78.9	4.2448	38
>gi|148530286|gb|ABQ82285.1| SSU ribosomal protein S18P [Lactobacillus reuteri DSM 20016];>	5	3	4	3	3	4	39.7	9.1536	78
>gi|148530720|gb|ABQ82719.1| thymidine kinase [Lactobacillus reuteri DSM 20016	1	0	1	0	0	1	7.9	22.171	191
>gi|148530635|gb|ABQ82634.1| protein translocase subunit secA [Lactobacillus reuteri DSM 20016];>	5	3	4	3	2	2	5.1	90.351	787
>gi|148531876|gb|ABQ83875.1| hypothetical protein Lreu_1635 [Lactobacillus reuteri DSM 20016	2	1	2	0	2	0	14.7	31.711	279
>gi|148530347|gb|ABQ82346.1| Uracil phosphoribosyltransferase [Lactobacillus reuteri DSM 20016];>	4	2	2	2	1	3	25.1	20.252	179
>gi|183224052|dbj|BAG24569.1| beta-phosphoglucomutase [Lactobacillus reuteri JCM 1112];>	5	3	2	0	0	1	35.6	24.587	225
>gi|148531763|gb|ABQ83762.1| hypothetical protein Lreu_1517 [Lactobacillus reuteri DSM 20016];>	2	1	1	1	1	2	19.1	13.119	115
>gi|148531954|gb|ABQ83953.1| precorrin-3 methyltransferase [Lactobacillus reuteri DSM 20016];>	5	2	3	2	3	4	19.9	26.233	241
>gi|148532142|gb|ABQ84141.1| Hydratase/decarboxylase [Lactobacillus reuteri DSM 20016];>	2	1	0	0	2	1	11.1	29.863	270
>gi|148530374|gb|ABQ82373.1| Na+ dependent nucleoside transporter domain protein [Lactobacillus reuteri DSM 20016];>	3	0	2	2	1	2	8.2	44.04	403
>gi|183224607|dbj|BAG25124.1| pyruvate dehydrogenase complex E1 component alpha subunit [Lactobacillus reuteri JCM 1112];>	3	3	3	2	1	1	15.4	41.361	371
>gi|148530966|gb|ABQ82965.1| ribosome-binding factor A [Lactobacillus reuteri DSM 20016];>	2	2	2	2	1	2	19.3	13.412	119
>gi|148531512|gb|ABQ83511.1| L-glutaminase [Lactobacillus reuteri DSM 20016];>	3	2	1	2	1	2	12.4	33.392	306
>gi|227070371|gb|EEI08733.1| diaminohydroxyphosphoribosylaminopyrimidine deaminase / 5-amino-6-(5-phosphoribosylamino)uracil reductase [Lactobacillus reuteri MM2-3];>	3	1	3	1	0	0	11	39.658	355
>gi|148531144|gb|ABQ83143.1| GTP cyclohydrolase II [Lactobacillus reuteri DSM 20016];>	4	2	1	1	1	3	16	43.927	393
>gi|112943293|gb|ABI26303.1| pyruvate/2-oxoglutarate dehydrogenase complex	4	4	2	1	2	1	11.6	50.725	475

dihydrolipoamide dehydrogenase (E3) component [Lactobacillus reuteri									
>gi|148531413|gb|ABQ83412.1| signal recognition particle subunit FFH/SRP54 (srp54) [Lactobacillus reuteri DSM 20016];>	5	4	2	2	1	0	10.2	54.114	481
>gi|148531292|gb|ABQ83291.1| SMC domain protein [Lactobacillus reuteri DSM 20016];>	2	2	0	1	0	1	3.2	118.56	1033
>gi|227071080|gb|EEI09398.1| metallo-beta-lactamase [Lactobacillus reuteri MM2-3];>	3	1	3	2	2	2	3.8	70.828	628
>gi|148531719|gb|ABQ83718.1| LSU ribosomal protein L24P [Lactobacillus reuteri DSM 20016];>	2	2	1	1	1	2	10.8	10.975	102
>gi|227070756|gb|EEI09083.1| ABC superfamily ATP binding cassette transporter, ABC/membrane protein [Lactobacillus reuteri MM2-3	1	1	0	1	0	0	1.7	72.445	666
>gi|148530633|gb|ABQ82632.1| amidophosphoribosyltransferase-like protein [Lactobacillus reuteri DSM 20016];>	2	2	2	1	2	2	3.5	26.055	226
>gi|183225571|dbj|BAG26088.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	6	4	1	1	3	3	28.1	27.624	249
>gi|183224651|dbj|BAG25168.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	4	4	3	3	3	3	56	8.6646	75
>gi|148530351|gb|ABQ82350.1| peptidase C26 [Lactobacillus reuteri DSM 20016];>	2	1	1	1	2	1	20.9	26.977	244
>gi|148531602|gb|ABQ83601.1| bacterial peptide chain release factor 3 (bRF-3) [Lactobacillus reuteri DSM 20016	3	2	2	1	2	2	5.9	59.477	525
>gi|148532084|gb|ABQ84083.1| ErfK/YbiS/YcfS/YnhG family protein [Lactobacillus reuteri DSM 20016];>	1	1	1	0	1	1	6.4	24.726	218
>gi|189485784|gb|AAU08014.2| inactive glucansucrase [Lactobacillus reuteri];>	1	0	0	1	1	0	0.6	179.41	1619
>gi|148531187|gb|ABQ83186.1| NrdI family protein [Lactobacillus reuteri DSM 20016	2	0	0	0	2	2	17.1	18.082	158
>gi|148530779|gb|ABQ82778.1| FolC bifunctional protein [Lactobacillus reuteri DSM 20016	2	0	1	0	2	0	8.9	47.999	437
>gi|119390607|pdb|2NT8|A Chain A, Atp Bound At The Active Site Of A Pduo Type Atp:co(I) Rrinoid Adenosyltransferase From Lactobacillus Reuteri;>	3	2	3	1	2	2	13.5	25.598	223
>gi|227070045|gb|EEI08423.1| possible reductase [Lactobacillus reuteri MM2-3];>	1	1	1	1	1	1	8	14.924	125
>gi|227185113|gb|EEI65184.1| conserved hypothetical protein [Lactobacillus reuteri CF48-3A];>	3	2	3	1	1	2	38.8	8.7612	80
>gi|148530703|gb|ABQ82702.1| hypothetical protein Lreu_0433 [Lactobacillus reuteri DSM 20016];>	2	1	1	0	0	1	22.9	12.718	109
>gi|148531183|gb|ABQ83182.1| penicillin-binding protein, 1A family [Lactobacillus reuteri DSM 20016	4	2	0	1	0	1	6.2	82.075	754
>gi|6707064|gb|AAF25576.1|AF120104_1 mucus binding protein precursor [Lactobacillus reuteri ATCC 53608	2	2	0	0	0	0	7.8	357.95	3269
>gi|148530566|gb|ABQ82565.1| amino acid ABC transporter ATP-binding protein, PAAT family [Lactobacillus reuteri DSM 20016];>	1	1	0	0	0	1	6	27.538	249
>gi|227186386|gb|EEI66457.1| ArsR family transcriptional regulator [Lactobacillus reuteri CF48-3A];>	4	4	3	1	3	2	22.6	12.411	106
>gi|148530664|gb|ABQ82663.1| RNAse R [Lactobacillus reuteri DSM 20016];>	3	1	0	0	0	3	4.6	91.867	801

>gi|148531240|gb|ABQ83239.1| Alpha/beta hydrolase fold-3 domain protein [Lactobacillus reuteri DSM 20016];>	4	4	3	2	4	2	16	34.992	312
>gi|148531644|gb|ABQ83643.1| malate dehydrogenase (NAD) [Lactobacillus reuteri DSM 20016];>	3	1	1	2	1	3	20.8	33.353	307
>gi|148530504|gb|ABQ82503.1| LSU ribosomal protein L31P [Lactobacillus reuteri DSM 20016];>	1	1	1	1	0	1	17.3	9.123	81
>gi|148531537|gb|ABQ83536.1| 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase [Lactobacillus reuteri DSM 20016	4	0	2	1	3	3	28.2	19.815	170
>gi|183225679|dbj|BAG26196.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112	2	0	0	0	1	1	12.9	18.055	170
>gi|183224626|dbj|BAG25143.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112];>	4	1	0	0	1	3	22.4	35.43	304
>gi|148530719|gb|ABQ82718.1| domain of unknown function DUF1727 [Lactobacillus reuteri DSM 20016];>	2	2	1	2	2	2	5.6	50.191	445
>gi|148530512|gb|ABQ82511.1| LemA family protein [Lactobacillus reuteri DSM 20016	4	1	1	1	3	2	22.2	21.33	189
>gi|194454712|gb|EDX43609.1| Septum formation initiator [Lactobacillus reuteri 100-23];>	2	1	1	0	0	2	19.5	14.007	118
>gi|183225630|dbj|BAG26147.1| propanediol dehydratase reactivation protein PduH [Lactobacillus reuteri JCM 1112];>	4	2	2	2	2	3	34.6	14.213	127
>gi|183224761|dbj|BAG25278.1| hypothetical protein [Lactobacillus reuteri JCM 1112];>	1	0	0	1	0	0	4.9	21.003	184
>gi|183225866|dbj|BAG26382.1| pyrimidine operon regulator [Lactobacillus fermentum IFO 3956];>	3	1	1	2	0	2	19	20.167	179
>gi|148531324|gb|ABQ83323.1| 4-oxalocrotonate tautomerase [Lactobacillus reuteri DSM 20016];>	3	3	2	2	2	2	47.5	6.8159	61
>gi|148531498|gb|ABQ83497.1| LSU ribosomal protein L35P [Lactobacillus reuteri DSM 20016];>	2	2	2	2	2	2	32.3	7.615	65
>gi|194453567|gb|EDX42464.1| hypothetical protein Lreu23DRAFT_3980 [Lactobacillus reuteri 100-23];>	1	1	0	1	1	1	1.1	96.477	880
>gi|148530282|gb|ABQ82281.1| DNA gyrase subunit B [Lactobacillus reuteri DSM 20016];>	3	2	0	0	2	2	4.6	72.528	649
>gi|148530732|gb|ABQ82731.1| ATP synthase F0 subcomplex B subunit [Lactobacillus reuteri DSM 20016	4	0	1	2	3	1	18	19.254	172
>gi|148530621|gb|ABQ82620.1| ABC transporter related [Lactobacillus reuteri DSM 20016];>	2	0	1	1	0	0	5.3	73.56	645
>gi|148532010|gb|ABQ84009.1| transcriptional regulator, AsnC family [Lactobacillus reuteri DSM 20016];>	3	1	2	2	1	1	22.4	17.758	156
>gi|148530673|gb|ABQ82672.1| UDP-N-acetylmuramate dehydrogenase [Lactobacillus reuteri DSM 20016];>	2	0	0	1	0	2	12.8	32.389	298
>gi|194454703|gb|EDX43600.1| alanine racemase [Lactobacillus reuteri 100-23];>	2	1	1	0	0	1	8.3	41.257	375
>gi|148530894|gb|ABQ82893.1| protein of unknown function DUF1447 [Lactobacillus reuteri DSM 20016];>	3	2	1	2	3	2	21.4	8.3122	70
>gi|227184517|gb|EEI64588.1| possible acetolactate decarboxylase [Lactobacillus reuteri CF48-3A];>	3	3	2	2	2	2	9.3	27.057	246

>gi|148530982|gb|ABQ82981.1| hypothetical protein Lreu_0717 [Lactobacillus reuteri DSM 20016];>	2	1	1	2	2	2	22.3	13.889	112
>gi|227071855|gb|EEI10143.1| penicillin-binding protein [Lactobacillus reuteri MM2-3];>	4	2	2	1	1	2	5.4	76.108	699
>gi|148531436|gb|ABQ83435.1| DNA replication and repair protein RecN [Lactobacillus reuteri DSM 20016];>	2	1	2	2	0	0	4.5	62.895	559
>gi|148530684|gb|ABQ82683.1| Cof-like hydrolase [Lactobacillus reuteri DSM 20016];>	2	2	1	0	1	1	7.8	30.206	268
>gi|148532149|gb|ABQ84148.1| aminotransferase, class I and II [Lactobacillus reuteri DSM 20016];>	3	1	1	1	2	1	13.2	43.732	386
>gi|148531784|gb|ABQ83783.1| aldose 1-epimerase [Lactobacillus reuteri DSM 20016	2	1	2	0	1	0	14.2	37.927	345
>gi|148530636|gb|ABQ82635.1| bacterial peptide chain release factor 2 (bRF-2) [Lactobacillus reuteri DSM 20016	3	2	1	2	1	1	15.4	37.463	332
>gi|148531007|gb|ABQ83006.1| DNA primase [Lactobacillus reuteri DSM 20016	3	0	0	0	3	0	4.5	71.586	621
>gi|227070716|gb|EEI09044.1| NAD-dependent epimerase/dehydratase [Lactobacillus reuteri MM2-3];>	2	2	0	0	1	0	11.2	27.234	242
>gi|148531462|gb|ABQ83461.1| hypothetical protein Lreu_1204 [Lactobacillus reuteri DSM 20016	2	1	0	2	0	1	31	6.3153	58
>gi|183227545|dbj|BAG28061.1| ribose 5-phosphate isomerase [Lactobacillus fermentum IFO 3956];>	2	1	0	0	0	2	12.4	24.932	226
>gi|133930415|gb|ABO43779.1| L-lactate dehydrogenase [Lactobacillus reuteri];>	5	1	1	2	3	3	16.3	33.427	312
>gi|227070037|gb|EEI08415.1| S-adenosyl-L-methionine-dependent methyltransferase [Lactobacillus reuteri MM2-3];>	1	1	1	1	1	0	5.5	26.69	238
>gi|148531530|gb|ABQ83529.1| malate dehydrogenase (NAD) [Lactobacillus reuteri DSM 20016	5	1	1	1	4	2	14.7	33.353	312
>gi|148531744|gb|ABQ83743.1| Deoxyadenosine kinase [Lactobacillus reuteri DSM 20016	1	0	1	1	1	1	4.7	24.647	213
>gi|148530796|gb|ABQ82795.1| Holliday junction DNA helicase subunit RuvA [Lactobacillus reuteri DSM 20016];>	2	0	0	0	2	0	18.1	22.298	199
>gi|148531529|gb|ABQ83528.1| phosphomethylpyrimidine kinase [Lactobacillus reuteri DSM 20016];>	3	3	1	1	0	2	15.9	29.317	271
>gi|183226376|dbj|BAG26892.1| conserved hypothetical protein [Lactobacillus fermentum IFO 3956];>	2	0	1	0	0	1	18.7	15.706	134
>gi|148531904|gb|ABQ83903.1| hypothetical protein Lreu_1664 [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	8	12.648	113
>gi|148532031|gb|ABQ84030.1| Homoserine O-succinyltransferase [Lactobacillus reuteri DSM 20016];>	2	1	1	0	1	1	11.6	32.139	275
>gi|183227422|dbj|BAG27938.1| aspartate-ammonia ligase [Lactobacillus fermentum IFO 3956];>	3	0	0	2	2	2	8.6	38.795	336
>gi|183226154|dbj|BAG26670.1| peptide chain release factor 2 [Lactobacillus fermentum IFO 3956];>	2	2	2	2	2	1	7	39.961	355
>gi|183225888|dbj|BAG26404.1| tyrosyl-tRNA synthase [Lactobacillus fermentum IFO 3956];>	2	0	1	1	0	2	5.5	46.933	417
>gi|183224304|dbj|BAG24821.1| hypothetical protein [Lactobacillus reuteri JCM 1112];>	3	2	1	1	1	0	27.1	11.379	96

>gi|227070646|gb|EEI08976.1| SPOUT methyltransferase superfamily protein [Lactobacillus reuteri MM2-3];>	2	1	2	2	2	2	10.4	19.733	173
>gi|337729425|emb|CCC04555.1| conserved hypothetical protein [Lactobacillus reuteri ATCC 53608]	2	1	1	1	1	2	9.4	26.142	223
>gi|227070121|gb|EEI08497.1| possible malate dehydrogenase [Lactobacillus reuteri MM2-3];>	3	3	1	1	2	3	8.1	39.19	360
>gi|148530588|gb|ABQ82587.1| large conductance mechanosensitive channel protein [Lactobacillus reuteri DSM 20016	3	2	2	1	1	1	21.1	13.773	123
>gi|183225058|dbj|BAG25575.1| phage major head protein [Lactobacillus reuteri JCM 1112];>	1	1	0	0	1	0	3.8	43.887	394
>gi|183225285|dbj|BAG25802.1| muramidase [Lactobacillus reuteri JCM 1112];>	4	2	2	3	3	3	14	55.89	492
>gi|148530863|gb|ABQ82862.1| protein of unknown function YGGT [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	8.1	9.7797	86
>gi|148531635|gb|ABQ83634.1| methionine aminopeptidase, type I [Lactobacillus reuteri DSM 20016];>	2	0	1	0	1	0	7	31.665	285
>gi|148531335|gb|ABQ83334.1| SSU ribosomal protein S14P [Lactobacillus reuteri DSM 20016];>	3	3	1	2	2	2	32.6	10.399	89
>gi|183224586|dbj|BAG25103.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112	3	1	1	0	0	1	12.6	26.479	230
>gi|112944038|gb|ABI26331.1| folylpolyglutamate synthase [Lactobacillus reuteri];>	2	0	1	0	2	0	8.5	48.081	437
>gi|337728982|emb|CCC04102.1| glucose-inhibited division protein B [Lactobacillus reuteri ATCC 53608];>	2	1	0	1	2	1	16.6	26.683	241
>gi|227185796|gb|EEI65867.1| rod shape-determining protein MreC [Lactobacillus reuteri CF48-3A];>	1	1	1	0	0	1	4.1	31.961	291
>gi|148531188|gb|ABQ83187.1| putative RNA methylase [Lactobacillus reuteri DSM 20016	1	1	1	1	0	0	5	44.845	398
>gi|183224889|dbj|BAG25406.1| flavodoxin [Lactobacillus reuteri JCM 1112];>	2	1	0	0	1	2	10.5	19.402	172
>gi|148532090|gb|ABQ84089.1| hypothetical protein Lreu_1851 [Lactobacillus reuteri DSM 20016];>	1	1	1	1	0	1	26.2	7.0627	61
>gi|148530594|gb|ABQ82593.1| ribonucleoside-diphosphate reductase class Ib alpha subunit [Lactobacillus reuteri DSM 20016];>	3	1	1	0	1	1	7.7	82.738	723
>gi|183227462|dbj|BAG27978.1| cation-transporting ATPase [Lactobacillus fermentum IFO 3956	3	1	0	1	1	1	3	100.37	921
>gi|148531079|gb|ABQ83078.1| hypothetical protein Lreu_0815 [Lactobacillus reuteri DSM 20016	1	1	0	1	0	0	9.8	14.305	123
>gi|148530393|gb|ABQ82392.1| branched-chain amino acid transport [Lactobacillus reuteri DSM 20016];>	1	1	0	1	0	1	8.8	12.954	114
>gi|148531487|gb|ABQ83486.1| LSU ribosomal protein L32P [Lactobacillus reuteri DSM 20016	1	1	1	0	0	0	23.7	6.5727	59
>gi|148531751|gb|ABQ83750.1| cold-shock DNA-binding protein family [Lactobacillus reuteri DSM 20016];>	1	1	0	1	0	1	19.7	7.3269	66
>gi|148531427|gb|ABQ83426.1| protein kinase [Lactobacillus reuteri DSM 20016];>	3	2	3	2	2	2	7.1	70.819	634
>gi|183225432|dbj|BAG25949.1| conserved hypothetical protein [Lactobacillus reuteri JCM 1112	1	1	0	1	1	1	2.9	35.145	313
>gi|148531205|gb|ABQ83204.1| 2,5-didehydrogluconate reductase [Lactobacillus reuteri DSM 20016	3	0	2	0	1	0	12.7	34.433	300

>gi|227071659|gb|EEI09952.1| conserved hypothetical protein [Lactobacillus reuteri MM2-3	2	0	1	0	0	2	10.4	19.844	173
>gi|148530914|gb|ABQ82913.1| SSU ribosomal protein S15P [Lactobacillus reuteri DSM 20016	1	1	1	1	1	1	7.9	10.416	89
>gi|227070978|gb|EEI09301.1| cobalt-precorrin-2 C(20)-methyltransferase [Lactobacillus reuteri MM2-3	1	0	1	0	1	1	6.7	26.806	240
>gi|148531465|gb|ABQ83464.1| protein of unknown function DUF910 [Lactobacillus reuteri DSM 20016	1	1	0	1	0	0	12.7	9.4478	79
>gi|336448118|gb|AEI56733.1| P4 family prophage LambdaSa04 protein [Lactobacillus reuteri SD2112	2	1	1	1	1	2	1.7	85.238	751
>gi|183226040|dbj|BAG26556.1| hypoxanthine-guanine phosphoribosyltransferase [Lactobacillus fermentum IFO 3956];>	2	0	1	2	1	1	9.3	20.442	182
>gi|183226928|dbj|BAG27444.1| chorismate synthase [Lactobacillus fermentum IFO 3956];>	3	2	1	1	2	1	7.5	41.636	388
>gi|148532169|gb|ABQ84168.1| ATP-grasp protein-like protein [Lactobacillus reuteri DSM 20016];>	2	1	2	1	1	0	6.8	48.604	414
>gi|148531424|gb|ABQ83423.1| thiamine diphosphokinase [Lactobacillus reuteri DSM 20016];>	1	0	0	0	1	1	8.3	24.536	216
>gi|148531267|gb|ABQ83266.1| hypothetical protein Lreu_1006 [Lactobacillus reuteri DSM 20016];>	2	2	0	0	1	0	14.7	16.149	143
>gi|148531174|gb|ABQ83173.1| protein of unknown function DUF1440 [Lactobacillus reuteri DSM 20016	1	1	0	1	1	1	7.2	18.375	166
>gi|148530686|gb|ABQ82685.1| two component transcriptional regulator, winged helix family [Lactobacillus reuteri DSM 20016];>	2	1	1	1	1	2	11.7	27.76	239
>gi|148531107|gb|ABQ83106.1| hypothetical protein Lreu_0843 [Lactobacillus reuteri DSM 20016];>	1	1	1	0	0	0	4.2	23.788	215
>gi|148531647|gb|ABQ83646.1| GCN5-related N-acetyltransferase [Lactobacillus reuteri DSM 20016];>	3	2	3	2	2	1	21.9	17.195	151
>gi|148531071|gb|ABQ83070.1| transcriptional regulator, XRE family [Lactobacillus reuteri DSM 20016];>	3	0	2	2	1	2	28	9.7862	82
>gi|227070084|gb|EEI08461.1| metal-dependent hydrolase [Lactobacillus reuteri MM2-3];>	2	2	1	1	1	1	10	18.134	160
>gi|148531021|gb|ABQ83020.1| condensin subunit ScpB [Lactobacillus reuteri DSM 20016];>	2	0	0	0	0	2	11.4	22.54	201
>gi|148531173|gb|ABQ83172.1| glycoside hydrolase, clan GH-D [Lactobacillus reuteri DSM 20016];>	2	1	1	0	0	0	2.7	84.003	729
>gi|148530329|gb|ABQ82328.1| acetyl-CoA acetyltransferase [Lactobacillus reuteri DSM 20016];>	4	2	3	0	0	2	14.3	41.055	392
>gi|183224876|dbj|BAG25393.1| carbamoyl-phosphate synthase large subunit [Lactobacillus reuteri JCM 1112];>	2	0	0	0	2	1	3.3	92.789	825
>gi|148532172|gb|ABQ84171.1| glucose inhibited division protein A [Lactobacillus reuteri DSM 20016];>	2	1	0	0	0	2	4	71.918	647
>gi|148531678|gb|ABQ83677.1| isochorismatase hydrolase [Lactobacillus reuteri DSM 20016];>	4	0	1	1	0	2	18.9	19.239	164
>gi|227070750|gb|EEI09077.1| conserved hypothetical protein [Lactobacillus reuteri MM2-3];>	3	0	0	1	1	1	14.9	25.173	221

>gi|148530499|gb|ABQ82498.1| Domain of unknown function DUF1934 [Lactobacillus reuteri DSM 20016];>	2	0	2	0	0	1	18.2	15.33	132
>gi|148530487|gb|ABQ82486.1| periplasmic solute binding protein [Lactobacillus reuteri DSM 20016];>	2	2	1	0	0	0	7.3	34.078	303
>gi|148532114|gb|ABQ84113.1| transcriptional regulator, HxlR family [Lactobacillus reuteri DSM 20016];>	3	3	2	1	0	2	17.2	14.22	122
>gi|183227079|dbj|BAG27595.1| hemolysin [Lactobacillus fermentum IFO 3956];>	2	1	1	1	1	2	4.1	29.737	271
>gi|227185320|gb|EEI65391.1| 2-dehydropantoate 2-reductase [Lactobacillus reuteri CF48-3A];>	1	1	0	1	1	1	3.2	35.797	315
>gi|148530753|gb|ABQ82752.1| aldo/keto reductase [Lactobacillus reuteri DSM 20016];>	3	2	0	0	2	1	11.1	32.082	280
>gi|133930471|gb|ABO43807.1| Smf [Lactobacillus reuteri];>gi|148531043|gb|ABQ83042.1| DNA protecting protein DprA [Lactobacillus reuteri DSM 20016];>	2	2	1	1	0	0	5.5	31.8	291
>gi|148531957|gb|ABQ83956.1| precorrin-6Y C5,15-methyltransferase (decarboxylating), CbiT subunit [Lactobacillus reuteri DSM 20016];>	2	0	1	0	0	1	9.2	20.387	184
>gi|337729525|emb|CCC04656.1| putative replication initiator protein [Lactobacillus reuteri ATCC 53608]	2	0	1	1	0	1	1.7	102.72	909
>gi|148530648|gb|ABQ82647.1| amino acid ABC transporter substrate-binding protein, PAAT family [Lactobacillus reuteri DSM 20016];>	1	1	1	1	1	1	5.8	33.273	294
>gi|183227290|dbj|BAG27806.1| conserved hypothetical protein [Lactobacillus fermentum IFO 3956	2	1	0	2	1	2	6.3	36.249	335

Appendix 3

List of publications

Oral presentations

22nd March 2012. *Using SILAC to identify potential mucus binding proteins in Lactobacillus reuteri*. Mass Spectacular JIC conference centre.

24th March 2012. *What are calories? Measuring calories in food* Hands on demonstration. Science in Norwich.

1st April 2010. *How do gut microbes bind to mucus?* SGM – Edinburgh.

Poster presentations

Jeffers F., Mulholland F., Etzold S., Roos S., MacKenzie D.A., and Juge N. *Exploring molecular interactions of Lactobacillus reuteri with mucus by quantitative proteomics*. Exploring Human Host-Microbiome Interactions in Health and Disease, Wellcome Trust, Genome Campus, Hinxton, Cambridge. 8th-10th May 2012.

Jeffers F., MacKenzie D., and Juge N. *How do gut microbes bind to mucus?* SET for Britain, Houses of Parliament, London. 14th March 2011.

Jeffers F,. Fuell C., MacKenzie D.A., Tailford L.E., Bongaerts R.J., and Juge N. *Mucin-lectin binding assessed by flow cytometry and mass spectrometry.* RSC Carbohydrates in Dundee Conference 30th-31st August 2010.

Jeffers F., MacKenzie D.A., Fuell C., Tailford L.E., Bongaerts R. and Juge N. *Mucin – lectin interactions assessed by flow cytometry*. RSC Carbohydrate Group Meeting: Sugars in Norwich, IFR, 15th-16th September 2009.

Jeffers F., MacKenzie D.A., Fuell C., Tailford L.E. and Juge N. *How do gut microbes bind to mucus.* RSC Carbohydrate Group Meeting: Sugars in Norwich, IFR, 15th-16th September 2009.

Jeffers F., MacKenzie D.A., Fuell C., Tailford L.E., Bongaerts R. and Juge N. *How do commensal gut microbes bind to mucus?* IFR Student Showcase May 2009.

Posters and conference talks (given by others)

MacKenzie D.A., Crost E.H., Etzold S., Kober O., **Jeffers F**., Le Gall G., Fuell C., Tailford L.E. and Juge N. *Investigating the role of mucins in the interaction between gut bacteria and the host.* 8th INRA-Rowett Symposium on Gut Microbiology, Gut Microbiota: Friend or Foe? Polydome Congress Centre, Clermont-Ferrand France 17th-20th June 2012.

Kober O., Schreiber O., **Jeffers F**., Wakenshaw L., Roos S., Holm L. and Juge N. *Functional modulation of the rodent mucus layer by gut bacteria.* Molecular mechanisms of inflammatory bowel disease, Biochemical Society, Durham 20th-22nd March 2011.

Kober O.K., **Jeffers F**., Hemmings A., Wakenshaw L., MacKenzie D.A. and Juge N. *Functional modulation of the murine mucus layer by gut bacteria*. British Society of Immunology Congress, Liverpool, 6th-10th December 2010.

MacKenzie D.A., **Jeffers F**., Tailford L.E., Parris A., Williams M.R., Hemmings A.M. and Juge N. *Structural and functional analysis of a mucus-binding protein repeat: role in bacterial adhesion to mucus.* BBSRC-INRA-WUR Workshop on Food and Gut Health, IFR, 6th-8th October 2010.
MacKenzie D.A., **Jeffers F**., Etzold S,. Kober O., Hemmings A. and Juge N. *Structure and function of Lactobacillus reuteri mucus binding proteins in the maintenance of gut homeostasis.* 3rd ASM Conference on Beneficial Microbes, Miami, Florida, USA. 25th-29th October 2010.

MacKenzie D.A., **Jeffers F**., Etzold S., Parris A., Fais M., Tailford L.E., Fairhurst S., Williams M.R., Field R., Hemmings A.M. and Juge N. *Gut bacteria – host interaction: the role of mucus binding proteins.* INRA-Rowett Symposium on Gut Microbiology, new insights into gut microbial ecosystems. Aberdeen Exhibition and Conference Centre 22nd-25th June 2010.

MacKenzie D.A., **Jeffers F**., Tailford L.E., Ruiz-Moyano S., Parris A., Williams M.R., Parker M.L., Hemmings A.M. and Juge N. *Structural and functional analysis of a mucus-binding protein repeat: role in bacterial adhesion to mucus.*4th International Probiotic Conference, Kosice, Slovakia 15th-17th June 2010.

MacKenzie D.A., **Jeffers F**., Tailford L.E., Parris A., Williams M.R., Hemmings A.M. and Juge N. *Structural and functional analysis of a mucus binding protein repeat:role in bacterial adhesion to mucus.* Microbes in Norwich, JIC Conference Centre 27th November 2009.

Fuell C., MacKenzie D.A., Tailford L.E., **Jeffers F**. and Juge N. *Mucin analysis by mass spectrometry*. RSC Carbohydrate Group Meeting: Sugars in Norwich, IFR, 15th-16th September 2009.

MacKenzie D.A., **Jeffers F**., Tailford L.E., Parris A., Williams M.R., Hemmings A.M. and Juge N. *Structural and functional analysis of a mucus binding protein repeat:role in bacterial adhesion to mucus.*RSC Carbohydrate Group Meeting: Sugars in Norwich, IFR, 15th-16th September 2009.

Fuell C., **Jeffers F**., MacKenzie D.A., Tailford L.E., Bongaerts R.J. and Juge N. *Mucin-lectin binding assessed by flow cytometry and mass spectrometry.* 15th European Carbohydrate Symposium. 19th-24th July 2009.

Publication (with other projects)

Jeffers F., Fuell C., Tailford L.E., MacKenzie D.A., Bongaerts R.J. and Juge N*. Mucin-lectin interactions assessed by flow cytometry.* Carbohydrate Research (2010) 345, 1486-1491.

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