BACILLUS SUBTILIS OXALATE DECARBOXYLASE: ROLES AND REGULATION

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University of East Anglia/John Innes Centre

Norwich, UK

ANDREA HALL

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Abstract

Oxalate occurs extensively in nature, being found in soil, sediment, rocks, animals, microorganisms and plants. Oxalate can be degraded biologically by an enzyme called oxalate decarboxylase. Oxalate decarboxylase was thought to be found exclusively in fungi until its recent discovery in the bacterium *Bacillus subtilis*. *B. subtilis* contains two intracellular oxalate decarboxylases, YvrK (OxdC) and YoaN (OxdD). YvrK has previously been shown to be produced at an optimum of pH 5. The oxalate decarboxylation reaction consumes a cytoplasmic proton, thus the enzyme may have a role in cytoplasmic pH maintenance or acid resistance. The aims of this study were to test whether YvrK was involved in acid resistance in *B. subtilis* and to establish the genetic organisation and regulation of *yvrK* and its neighbouring genes. In knockout mutants for genes predicted to be involved in the regulation of *yvrK* (*yvrE, yvrG, yvrH, yvrI* and *yvrL*), no discernable phenotype was noted during growth in both rich and minimal media at pH 5. Oxalate decarboxylase assays revealed that YvrI (a potential sigma factor) was essential for oxalate decarboxylase activity at both pH 7 and pH 5. YvrL (a potential membrane protein) was also shown to have an important role in oxalate decarboxylase regulation; its knockout mutant showing up-regulation (or de-repression) of enzyme activity at both pH 7 and 5. YvrE (similar in sequence to regucalcin) appeared to contribute to pH 5 levels of oxalate decarboxylase transcript, protein and activity. Wildtype *B. subtilis* oxalate decarboxylase activity under the conditions studied was shown to be entirely attributable to YvrK and activity was up-regulated at both pH 7 and 5 in cells grown in minimal medium. ^{13}C-labelled oxalate uptake experiments have shown that external oxalate was not taken up under the conditions tested. RT-PCR and primer extension analysis have shown that *yvrIHGE* and *yvrKL* form operons transcribed from single upstream promoters. Acid resistance tests showed that *B. subtilis* had negligible resistance to acid pH and that oxalate decarboxylase did not contribute to any acid resistance under the conditions tested.