The bacterial virulence factors rhamnolipids and their (*R*)-3-hydroxyalkanoate precursors activate *Arabidopsis* innate immunity through two independent mechanisms

Romain Schellenberger [a,1], Jérôme Crouzet [a,1], Arvin Nickzad [b], Lin-Jie Shu [c], Alexander Kutschera [c], Tim Gerster [c], Nicolas Borie [d], Corinna Dawid [e], Maude Cloutier [b], Sandra Villaume [a], Sandrine Dhondt-Cordelier [a], Jane Hubert [d], Sylvain Cordelier [a], Florence Mazeyrat-Gourbeyre [a], Christian Schmid [e], Marc Ongena [f], Jean-Hugues Renault [d], Arnaud Haudrechy [d], Thomas Hofmann [e], Fabienne Baillieul [a], Christophe Clément [a], Cyril Zipfel [g,h], Charles Gauthier [b], Eric Déziel [b,2], Stefanie Ranf [c,2] and Stéphan Dorey [a,2]

[a] *Université de Reims Champagne-Ardenne, RIBP EA 4707, USC INRAE 1488, SFR Condorcet FR CNRS 3417, 51100, Reims, France*

[b] *Centre Armand-Frappier Santé Biotechnologie, Institut national de la recherche scientifique (INRS), Laval, QC, H7V 1B7, Canada*

[c] *Phytopathology, TUM School of Life Sciences Weihenstephan, Technical University of Munich, Freising-Weihenstephan, 85354, Germany*

[d] *Université de Reims Champagne-Ardenne, CNRS, ICMR 7312, SFR Condorcet FR CNRS 3417, 51097, Reims France*

[e] *Food Chemistry and Molecular Sensory Science, TUM School of Life Sciences Weihenstephan, Technical University of Munich, Freising-Weihenstephan, 85354, Germany*

[f] *MiPI laboratory, SFR Condorcet FR CNRS 3417, Gembloux Agro-Bio Tech, University of Liège, Gembloux, B-5030, Belgium*

[g] *The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, Norwich, NR4 7UH, UK*

[h] *Institute of Plant and Microbial Biology, Zurich-Basel Plant Science Center, University of Zurich, Zurich, Switzerland*

[1] *These authors contributed equally to this work.*

[2] *To whom correspondence may be addressed: E.D. (eric.deziel@inrs.ca) or S.R. (ranf@wzw.tum.de) or S.D (stephan.dorey@univ-reims.fr).*

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Abstract

Plant innate immunity is activated upon perception of invasion pattern molecules by plant cell-surface immune receptors. Several bacteria of the genera *Pseudomonas* and *Burkholderia* produce rhamnolipids (RLs) from L-rhamnose and (*R*)-3-hydroxyalkanoate precursors (HAAs). RL and HAA secretion is required to modulate bacterial surface motility, biofilm development, and thus successful colonization of hosts. Here, we show that the lipidic secretome from the opportunistic pathogen *Pseudomonas aeruginosa*, mainly comprising RLs and HAAs, stimulates *Arabidopsis* immunity. We demonstrate that HAAs are sensed by the bulb-type lectin receptor kinase LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION/S-DOMAIN-1-29 (LORE/SD1-29), which also mediates medium-chain 3-hydroxy fatty acid (mc-3-OH-FA) perception, in the plant *Arabidopsis thaliana*. HAA sensing induces canonical immune signaling and local resistance to plant pathogenic *Pseudomonas* infection. By contrast, RLs trigger an atypical immune response and resistance to *Pseudomonas* infection independent of LORE. Thus, the glycosyl moieties of RLs, although abolishing sensing by LORE, do not impair their ability to trigger plant defense. Moreover, our results show that the immune response triggered by RLs is affected by the sphingolipid composition of the plasma membrane. In conclusion, RLs and their precursors released by bacteria can both be perceived by plants, but through distinct mechanisms.

Significance

Activation of plant innate immunity relies on the perception of microorganisms through self and non-self elicitors. Rhamnolipids and their precursor HAAs are exoproducts produced by beneficial and pathogenic bacteria. They are involved in bacterial surface dissemination and biofilm development. As these compounds are released in the extracellular milieu, they have the potential to be perceived by the plant immune system. Our work shows that both compounds independently activate plant immunity. We demonstrate that HAAs are perceived by the receptor protein kinase LORE. By contrast, rhamnolipids are not sensed by LORE but activate a non-canonical immune response influenced by the sphingolipid composition of the plant plasma membrane. Thus, plants are able to sense bacterial molecules as well as their direct precursors to trigger distinct immune responses.

Introduction

Plant innate immunity activation relies on detection of invasion pattern (IP) molecules that are perceived by plant cells (1, 2). Non-self-recognition IPs include essential components of whole classes of microorganisms, such as fragments of flagellin, peptidoglycans, mc-3-OH-FAs from bacteria or fragments of chitin and β-glucans from fungi and oomycetes, respectively (3, 4). Apoplastic IPs are sensed by plant plasma membrane-localized receptor kinases (RKs) or receptor-like proteins (RLPs) that function as pattern recognition receptors (PRRs) (5, 6). Activation of the immune response requires the recruitment of regulatory receptor kinases and receptor-like cytoplasmic kinases (RLCKs) by PRRs (7). Early cellular immune signaling of pattern-triggered immunity (PTI) includes ion-flux changes at the plasma membrane, rise in cytosolic Ca^{2+} levels, production of extracellular reactive oxygen species (ROS) and activation of mitogen-activated protein kinases (MAPKs) and/or Ca^{2+}-dependent protein kinases (3, 8-10). Biosynthesis and mobilization of plant hormones, including salicylic acid, jasmonic acid, ethylene, abscisic acid and brassinosteroids, ultimately modulate plant resistance to phytopathogens (11-14).

Rhamnolipids (RLs) are extracellular amphiphilic metabolites produced by several bacteria, especially *Pseudomonas* and *Burkholderia* species (15-17). Acting as wetting agents, RLs are essential for bacterial surface dissemination called swarming motility and for normal biofilm development (18-20). These glycolipids are produced from L-rhamnose and 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) precursors (15, 21). HAAs are synthesized by dimerization of (*R*)-3-hydroxyalkanoyl-CoA in *Pseudomonas*, forming congeners through the RhlA enzyme (21). The opportunistic plant pathogen *Pseudomonas aeruginosa* and the phytopathogen *Pseudomonas syringae* produce extracellular HAAs (16, 22-24). In *P. syringae*, HAA synthesis is coordinately regulated with the late-stage flagellar gene encoding flagellin (22). HAA and RL production is finely tuned and modulates the behavior of swarming migrating bacterial cells by acting as self-produced negative and positive chemotactic-like stimuli (25). RLs contribute to the alteration of the bacterial outer membrane composition, by shedding flagellin from the flagella (26) and by releasing lipopolysaccharides (LPS), resulting in an increased hydrophobicity of the bacterial cell surface (27). In mammalian cells, RLs produced by *Burkholderia plantarii* exhibit endotoxin-like properties similar to LPS, leading to the production of proinflammatory cytokines in human mononuclear cells (28, 29). They also subvert the host innate immune response through manipulation of the human beta-defensin-2 expression (30). Moreover, RLs from *Burkholderia pseudomallei* induce Interferon gamma (IFN-γ)-dependent host immune response in goat (31).

In plants, RLs induce defense responses and resistance to biotrophic and necrotrophic pathogens (32, 33). They also contribute to the biocontrol activity of the plant beneficial bacterium *P. aeruginosa* PNA1 against oomycetes (17). Recently, it was reported that the bulb-type lectin receptor kinase LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION/S-DOMAIN-1-29 (LORE/SD1-29) mediates medium-chain 3-hydroxy fatty acid (mc-3-OH-FA) sensing in *Arabidopsis thaliana* (hereafter, *Arabidopsis*) and that bacterial compounds comprising mc-3-OH-acyl building blocks including LPS and RLs do not stimulate LORE-dependent responses (34).

Here we show that the lipidic secretome produced by *P. aeruginosa* (RLsec), mostly composed of RLs and HAAs, induces *Arabidopsis* immunity. HAAs are perceived through the RK LORE. We demonstrate that, albeit not being sensed by LORE, RLs trigger an immune response characterized by an atypical defense

signature. Altogether, our results demonstrate that RLs and their precursors produced by *Pseudomonas* bacteria stimulate the plant immune response by two distinct mechanisms.

Results

RLsec from *Pseudomonas* induces *Arabidopsis* immune responses partially mediated by LORE. *Pseudomonas* species, including opportunistic plant pathogenic or plant beneficial endophytic strains, release a mixture of RL congeners and HAA precursors, here collectively termed RL secretome (RLsec) (15, 25). HPLC-MS/MS analyses of this RLsec from *P. aeruginosa* revealed the presence of mono-RLs and di-RLs at 50.9% and 44.9% of dry weight, respectively, and HAAs (3.8% of dry weight) (Supplementary Table 1, Supplementary Fig.1). RLs comprising ten-carbon long lipid tails, Rha-C_{10}-C_{10} (α-L-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoate) and Rha-Rha-C_{10}-C_{10} (α-L-rhamnopyranosyl-α-L-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoate), and C_{10}-C_{10} [(*R*)-3-(((*R*)-3-hydroxydecanoyl)oxy)decanoate] HAAs were the most abundant molecules in this lipidic secretome (37.6%, 33.1%, 2.1%, respectively). Notably, low amounts of free mc-3-OH-FAs (0.4% total), such as 3-OH-C_8, 3-OH-C_{10} and 3-OH-C_{12}, were also identified (Supplementary Table 1, Supplementary Fig.1).

First, we monitored apoplastic ROS production triggered by RLsec in *Arabidopsis* (35). Wild type (WT) plants challenged with RLsec displayed a transient extracellular ROS production, starting at six minutes and peaking at 15 minutes post elicitation (Fig. 1A). A robust ROS response was detected at concentrations of RLsec starting from 0.5 µg/mL (Fig. 1B, Supplementary Fig. 2). The ROS burst was dependent on the transmembrane NADPH oxidase RBOHD (36, 37) (Fig. 1C, Supplementary Fig. 3). RKs and RLPs mediate perception of IPs and early activation of PTI signaling (7). We monitored RLsec-triggered ROS production in *Arabidopsis* plants carrying loss-of-function mutations in genes encoding well characterized RKs and RLPs *fls2/efr1* (38, 39), *bak1*-5, *bkk1*-1, *bak1*-5/*bkk1*-1 (40), *bik1/pbl1* (41), *cerk1*-2 (42), *sobir1*-12, *sobir1*-13 (43), *dorn1*-1 (44) and *lore*-5 (45). RLsec-induced production of ROS was only reduced in *lore*-5 (Fig. 1C, Supplementary Fig. 3). Some IPs, including LPS extracts and mc-3-OH-FAs, were reported to induce a late ROS production in *Arabidopsis* (34, 46, 47). The late ROS response triggered by mc-3-OH-FAs was dependent on LORE (34). RLsec also induced a late and long-lasting ROS burst in *Arabidopsis* culminating at 6-8 hours post treatment (Fig. 2A), which was abolished in *rbohD* but not in *lore-5* mutant plants (Fig. 2A).

Next, we tested whether RLsec induces local resistance to the hemibiotrophic phytopathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst)* in *Arabidopsis* (48). RLsec pretreatment significantly enhanced resistance against *Pst* infection in WT and *lore*-5 plants (Fig. 2B). Taken together, our results show that RLsec induces immunity-related signaling events and disease resistance in *Arabidopsis* that are partially mediated by the bulb-type lectin RK LORE.

***Pseudomonas* HAAs and mc-3-OH-FAs from RLsec trigger LORE-dependent *Arabidopsis* immunity.**

By contrast to RLsec, purified RLs do not trigger LORE-dependent $[Ca^{2+}]_{cyt}$ and early ROS signaling responses (34). Because RLsec contains significant amounts of HAAs, we investigated the role of these poorly studied compounds in RLsec-triggered immunity. We compared the responses to HAA with those to mc-3-OH-FAs, known to be sensed by LORE (34) and present in low amounts in RLsec (Supplementary table 1). Side-by-side experiments with C_{10}-C_{10} HAA purified from *Pseudomonas aeruginosa* secretome and 3-OH-

C_{10} revealed that both compounds induce $[Ca^{2+}]_{cyt}$ signaling and ROS production in *Arabidopsis* plants in a dose-dependent manner (Fig. 3A and 3B, Supplementary Fig. 4 and 5). As observed upon 3-OH-C_{10} elicitation, purified C_{10}-C_{10}-induced ROS response was impaired in *rbohD* and *lore*-5 mutants (Fig. 3C). Similarly, $[Ca^{2+}]_{cyt}$ signaling triggered by C_{10}-C_{10} was impaired in *lore*-5 (Fig. 3D). In addition, C_{10}-C_{10} and 3-OH-C_{10} both triggered LORE-dependent MPK3 and MPK6 phosphorylation (Supplemental Fig. 6A). WT but not *lore*-5 mutant plants pretreated with C_{10}-C_{10} or 3-OH-C_{10} displayed enhanced resistance against *Pst* (Fig. 3E). Similar to 3-OH-FAs (34), the acyl chain length of HAA affects its immune eliciting activity, as purified C_{14}-C_{14} from *B. glumae* did neither induce ROS production nor enhanced resistance to *Pst* in *Arabidopsis* plants (Supplementary Fig. 7A and B).

Trace amount of 3-OH-C_{10} was detected in C_{10}-C_{10} purified from *P. aeruginosa* RLsec (Supplementary Table 2). To avoid any influence of potential contamination of HAAs with eliciting compounds related to purification procedure, we tested chemically synthesized C_{10}-C_{10} for the ROS and $[Ca^{2+}]_{cyt}$ responses. Synthetic C_{10}-C_{10} triggered LORE-dependent $[Ca^{2+}]_{cyt}$ signaling and ROS production in a dose-dependent manner (Fig. 4A-C). WT plants pretreated with synthetic C_{10}-C_{10} also displayed LORE-dependent enhanced resistance against *Pst* infection (Fig. 4D). LORE binds 3-OH-C_{10} through its extracellular domain (eLORE) (**34**). In a ligand depletion assay (Supplementary Fig. 8A), synthetic C_{10}-C_{10} was depleted from the low-molecular-weight fraction after size-exclusion filtration when incubated with eLORE-mCherry fusion protein transiently expressed in the apoplast of *Nicotiana benthamiana*, but not upon incubation with an apoplastic mCherry control, similarly as observed for the 3-OH-C_{10} ligand (Fig. 4E, Supplementary Fig. 8) (85). This strongly suggests binding of C_{10}-C_{10} to eLORE.

Altogether, our results show that HAAs secreted by *Pseudomonas* are sensed by *Arabidopsis* through the bulb-type lectin RK LORE, activate canonical PTI-related immune responses and provide resistance to bacterial infection.

RLs trigger LORE-independent *Arabidopsis* immune responses and resistance to *Pst*.

To investigate whether RLs activate a LORE-independent immune response, we used purified Rha-Rha-C_{10}-C_{10} and Rha-C_{10}-C_{10}, the most abundant molecules from *P. aeruginosa* RLsec. In *Arabidopsis* WT, both RL congeners induced a late and long-lasting ROS production, but as observed previously (34), no early burst (Fig. 5A). As both RL congeners gave a similar ROS signature, we only used Rha-Rha-C_{10}-C_{10} in the following experiments. The minimal concentration necessary to stimulate ROS production was 50 µM with an optimum at 100 µM (Fig. 5B). Late ROS production was compromised in *rbohD* but not in *lore*-5 mutants (Fig. 5C). Surprisingly, neither MPK3 nor MPK6 activation by Rha-Rha-C_{10}-C_{10} was detectable over a 3-hour time-course (Supplementary Fig. 6A and B). L-Rhamnose alone was inactive demonstrating that the lipid part of the RLs is necessary to trigger the immune response (Fig. 5A). *Burkholderia* species produce RL congeners with longer lipid chains than those produced by *Pseudomonas* (15). The RLsec from phytopathogenic *Burkholderia glumae* only contains congeners with fatty acid chain lengths varying from 12 to 16 carbons, in particular Rha-Rha-C_{14}-C_{14} (49, 50). Challenge of *Arabidopsis* with purified Rha-Rha-C_{14}-C_{14} from *B. glumae* did not trigger any ROS production (Fig. 5A) suggesting that the length of the fatty acid chain of RLs is critical for their eliciting activity. To determine whether RLs trigger local resistance to pathogenic *Pseudomonas* independent

of LORE, plants were pretreated with 10 µM purified Rha-Rha-C_{10}-C_{10} before *Pst* inoculation. WT plants displayed a significant enhanced resistance against *Pst* that was not compromised in *lore*-5 mutants (Fig. 5D).

To get deeper insights into the mechanisms involved in RL sensing, we used *Arabidopsis* plants carrying loss-of-function mutations in genes encoding RK and RLPs but also plasma membrane channel mutants including quintuple mechano-sensitive channels of small conductance-like (*msl4/5/6/9/10*) and double mid1-complementing activity (*mca1/2*) channel mutants (51) that could monitor changes in membrane mechanical properties. None of these mutants were affected in the long-term ROS response (Fig. 6A). Glycosylinositol phosphorylceramide (GIPC) sphingolipids were recently involved in the sensing of microbial necrosis and ethylene-inducing peptide 1-like (NLP) proteins (52). We found that the fatty acid hydroxylase *fah1/2* mutant that is disturbed in its complex sphingolipid composition (52) showed a reduced long-term ROS response (Fig. 6B). Ion leakage measurement confirmed that *fah1/2* mutant plants were less affected than WT plants by RL treatment (Fig. 6C). *fah1/2* plants are known to be more resistant to obligate biotrophic pathogens (86). We also observed that these mutants were more resistant to *Pst* (Fig. 6D). However, unlike in WT plants, challenge of *fah1/2* with RLs did not triggered enhanced resistance to *Pst* in the mutants, confirming that RL-triggered responses are compromised in these plants (Fig. 6D). Ceramide synthase *loh1* mutants are also impaired in GIPC levels but not in glucosyl ceramides (52). Interestingly, RL-triggered ROS production and ion leakage was unaltered in *loh1* plants. Altogether, our results show that RLs activate an atypical immune response in *Arabidopsis* that is LORE-independent, but which is affected by the sphingolipid composition of the plasma membrane.

Discussion

In *Pseudomonas* and *Burkholderia* species, swarming motility is intimately related to the production of extracellular surface-active RLs and HAAs (22, 25, 53-55). In addition, RL production affects bacterial biofilm architecture and increases affinity of cells for initial adherence to surfaces through increasing the cell's surface hydrophobicity (19, 56). These exoproducts are therefore at the frontline during host colonization. Our work demonstrates that both RLs and HAAs from the *Pseudomonas* lipidic secretome, referred to as RLsec here, are able to trigger *Arabidopsis* innate immunity by two distinct mechanisms.

We found that *Pseudomonas* RLs induce an atypical immune response. This response does not involve the RK LORE. Other bacterial compounds comprising mc-3-OH-acyl building blocks, but with large decorations including lipid A or LPS, lipopeptides, and *N*-acyl homoserine lactones also do not trigger LORE-dependent immune responses (34). RLs are glycolipids made of L-rhamnose linked to an HAA lipid tail (15, 21). Therefore, we propose that glycosylation of HAAs abolishes their perception by LORE. Glycosylation is known to affect the perception of IPs. Glycosylation of the flagellin from *Acidovorax avenae* on Ser^{178} and Ser^{183} prevents its perception by rice cells (57). Similarly, unglycosylated flagellin from *Pseudomonas syringae* pv. *tabaci* 6605 induces stronger defense responses in tobacco plants than glycosylated flagellin (58). In humans, glycosylation of *Burkholderia cenocepacia* flagellin significantly reduces its perception by epithelial cells (59).

We found that RL perception does not involve previously characterized RKs, RLPs or mechanosensitive channels. However, the RL response is affected by alterations in sphingolipid synthesis suggesting a role of these key membrane lipids in RL-triggered immunity. Recently GIPCs, major structural

components of the plant plasma membrane together with glucosylceramides (GlcCers), have been involved as receptors of cytotoxic NLPs (52). NLPs bind terminal monomeric hexose moieties of GIPCs. Only eudicot plants are sensing these NLPs through sphingolipid receptors. Insensitivity of monocots to NLPs is due to the length of the GIPC headgroup, consisting of three terminal hexoses compared to two in eudicots (52). *fah1/2* mutants display reduced glycosylsphingolipid (GIPCs and GlcCers) content but also lower level of ordered plasma membranes (52), suggesting that, similar to the NLP response, complex sphingolipids and/or ordered plasma membranes are necessary for the RL response. Unlike NLPs, RL responses were not significantly affected in *loh1* mutant plants also suggesting that GlcCers more than GIPC could influence RL sensing (52). Surfactin and more recently synthetic RL bolaforms and synthetic glycolipids, also active in the micromolar range, have been proposed to directly interact with plasma membrane lipids (46, 60-62). Mono- and di-RLs from *Pseudomonas* interact with phospholipids in several model membranes (63-66). In particular, RLs are able to fit into phospholipid bilayers of plant membrane models (67). In this model, the rhamnose polar heads from RLs are located near the phosphate groups from phospholipids and RL hydrophobic lipid tails are surrounded by the lipid chains from these phospholipids (67). The results obtained with these plant plasma membrane models suggest that the insertion of RLs into the lipid bilayer does not significantly affect lipid dynamics. The nature of the phytosterols could however influence the RL effect on plant plasma membrane destabilization. Subtle changes in lipid dynamics could then be linked to plant defense induction (67). Interestingly, RL bolaforms, like natural RLs are inducing a non-canonical defense signature with a long-lasting oxidative burst without MPK3 or MPK6 activation (46). We showed that the late ROS response triggered by RLs and RL-bolaforms is fully dependent on RBOHD which differs from the second ROS response observed after LPS treatment and produced by chloroplasts (47). The RL-related ROS response is not directly linked to *Arabidopsis* protection to *Pst* as some bolaforms can trigger this late ROS production without increasing resistance to *Pst* (46). However, the experiments from Luzuriaga et al. and our results on natural RLs suggest that the late ROS response is closely linked to ion leakage and may therefore be involved in membrane destabilization. This atypical defense signature triggered by two structurally different RLs, displaying amphiphilic properties and biological activities at the micromolar range, may suggest a direct interaction of these molecules with plant plasma membrane lipids, similar to what has been shown on membrane models (46).

We also demonstrated that HAAs, found in large amount in *Pseudomonas* lipidic secretome, are IPs perceived by *Arabidopsis*. HAA sensing is mediated by LORE. HAAs, in the micromolar range, induce typical PTI responses including transient ROS production, $[Ca^{2+}]_{cyt}$ signaling, and MPK3 and MPK6 phosphorylation in *Arabidopsis.* Interestingly, 3-OH-C_{10} activates similar responses but at concentrations 10 to 50 times lower. This is intriguing, because HAAs are present in much larger quantities (more than 3%) compared to 3-OH-FAs (0.3%) in the lipidic secretome (Supplemental table 1). This high amount of HAAs may compensate for their lower activity. RLs are activating an immune response at relatively high concentrations compared to both compounds. Interestingly, the RL concentration in the *P. aeruginosa* lipidic secretome is 10 to 100 times higher than HAAs and usually is in the millimolar range (23, 68). RLs are produced between 20 and 110 µM *in vivo* in mammals infected by *P. aeruginosa*, especially during cystic fibrosis disease (69-71). The high concentrations of RLs needed for plant elicitation are in the range of the concentrations produced by the bacteria.

Higher steric hindrance of HAA compared to 3-OH-FAs likely results in a lower affinity to the LORE receptor. Synthetic ethyl 3-hydroxydecanoate (Et-3-OH-C10:0) and *n*-butyl 3-hydroxydecanoate (*n*But-3-OH-C10:0), which possess unbranched ester-bound carbon chains in place of the carboxyl group, also triggered LORE-dependent immune signaling in *Arabidopsis*, while 3-branched *tert*-butyl 3-hydroxydecanoate (*t*But-3-OH-C10:0) was inactive (34). HAAs, possessing a 2-branched ester-bound headgroup, activate LORE signaling. The differences in efficacy could be explained by the different steric hindrance of the molecules. Alternatively, the additional carboxyl group could account for the LORE-eliciting activity of HAAs.

Pantoea, *Dickeya* and *Pseudomonas* bacteria, in particular the well-known phytopathogen *P. syringae* mainly produce HAAs containing 3-hydroxydecanoic acid (C_{10}) tails (15, 22, 72). By contrast, *Burkholderia* species including the phytopathogenic bacterium *B. glumae*, mainly produce HAAs comprising 3-hydroxytetradecanoic acid (C_{14}) tails (49). *Pseudomonas* C_{10}-containing HAAs activated *Arabidopsis* PTI whereas *Burkholderia* HAAs containing C_{14} fatty acid did not. Chain-length specificity was also observed for mc-3-OH-FA sensing by the LORE receptor with 3-OH-C_{10} representing the strongest immune elicitor (34). Thus, it could be hypothesized that *Arabidopsis*, and more generally *Brassicaceae* (73), are able to specifically recognize HAAs from specific bacterial species, among which several are plant opportunistic and phytopathogens (74-77). Interestingly, transcript profiles of the bean pathogen *P. syringae* pv. *syringae* B728a support a model in which leaf surface or epiphytic sites specifically favor swarming motility based on HAA surfactant production (55, 78). Low levels of HAAs contributing to motility are produced by these bacteria (22). HAA concentrations necessary to stimulate *Arabidopsis* innate immunity are in line with the concentration detected in RLsec and are produced by *Pseudomonas* (between 3 to 20% of the secretome) (23, 68, 79).

Low amounts of free mc-3-OH-FAs were found in RLsec from *P. aeruginosa* (Supplementary table 1). In *Pseudomonas*, the outer membrane lipase PagL releases 3-OH-C_{10} during synthesis of penta-acylated lipid A (34). The further fate of this 3-OH-C_{10} is unknown. RLs are able to extract LPS from the outer membrane of *P. aeruginosa* (27). Conceivably, surface-active RLs, and presumably also HAAs, could release free 3-OH-C_{10}, produced through PagL activity, along with LPS from the bacterial cell wall or outer membrane vesicles (27). Alternatively, degradation of HAAs/RLs *in planta* may also release free 3-OH-C_{10}. Acyl carrier protein (ACP)- and coenzyme A (CoA)-bound mc-3-OH-FAs are precursors of HAA/RL synthesis (21). Upon bacterial cell lysis, enzymatic or non-enzymatic degradation processes may also generate free 3-OH-C_{10} from these precursors. *In vivo*, insights into IP release have been recently obtained for flagellin. The plant glycosidase BGAL1 facilitates the release of immunogenic peptides from glycosylated flagellin, upstream of cleavage by proteases (80). The pathogen may evade detection by altering flagellin glycosylation and inhibiting the plant glycosidase. Flagellin glycosylation increases its physical stability that could contribute to the non-liberation/recognition of the flg22 epitope (58, 81). RLs are able to shed flagellin from *P. aeruginosa* flagella (26), suggesting that these biosurfactants participate in the release of this and presumably other eliciting compounds.

In conclusion, we hypothesize that when HAA- and RL-producing *Pseudomonas* colonize the leaf or root surface, they release RLs and HAAs which are necessary for surface motility, biofilm development, and thus successful colonization. Whereas *Arabidopsis* senses HAAs and mc-3-OH-FAs through the bulb-type lectin receptor kinase LORE, RLs are perceived through a LORE-independent mechanism (Figure 7). In

addition to direct activation of a non-canonical defense response in plants, RLs, by releasing other IPs from bacteria, could orchestrate a node leading to strong activation of plant immunity.

Methods

Molecules. The *P. aeruginosa* lipidic secretome used in this study was obtained from Jeneil Biosurfactant Co., Saukville, USA (JBR-599, lot. #050629). Rha-Rha-C_{10}-C_{10} and Rha-C_{10}-C_{10} were purified from this lipidic secretome mixture, as previously described (33, 34). Rha-Rha-C_{14}-C_{14} were purified from the *B. glumae* lipidic secretome (49). To obtain pure HAAs from *P. aeruginosa* or *B. glumae*, RLs were hydrolyzed using 1 M HCl in 1:1 dioxane-water boiling at reflux for 60 min. The mixture was extracted with ethyl acetate and the extracts were dried over anhydrous Na_2SO_4. After filtration, the resulting extracts were then evaporated to dryness and resuspended in 2 mL of methanol. HAAs were then isolated from digested mixture using flash chromatography on a Biotage (Stockholm, Sweden) Isorela One instrument with a SNAP Ultra C18 12g column (Biotage) using an acetonitrile/water gradient at 12 mL/min flow rate. The elution was started with 0% acetonitrile for 4.5 min and the acetonitrile concentration was raised to 100% over 28.2 min, followed by an isocratic elution of 100% acetonitrile for 13.3 min. The flash chromatography fraction containing the C_{10}-C_{10} was further separated and purified using 0.25 mm thin-layer chromatographic (TLC) plates (SiliCycle SilicaPlate F-254) and developed with *n*-hexane-ethyl acetate-acetic acid (24:74:2). The bands were scraped from the plates and the HAAs, including C_{10}-C_{10}, were extracted from the silica with chloroform-methanol (5:1). 3-OH-C_{10} was purchased from Sigma-Aldrich Saint-Quentin Fallavier, France. All compounds were dissolved in ethanol or methanol as indicated to prepare stock solutions. Final aqueous compound dilutions were prepared freshly on the days of the experiment. Control solutions contained equal amounts of ethanol or methanol (0.05% for most experiments and not exceeding 0.5% for the highest concentrations tested). Chemical synthesis of C_{10}-C_{10} is described in Datasets S1 and S2.

LC-MS analysis of HAAs. Samples were prepared by diluting stock solutions using MeOH to final concentration of 50 ppm. 16-Hydroxyhexadecanoic acid at 20 ppm was added to samples as internal standard[71]. The analyses were performed with a Quattro II triple quadrupole mass spectrometer (Micromass, Pointe-Claire, Canada) equipped with a Z-spray interface using electrospray ionization in negative mode. The capillary voltage was set at 3.5 kV and the cone voltage at 25 V. The source temperature was kept at 120°C and the desolvation gas at 150°C. The scanning mass range was from 130 to 930 Da. The instrument was interfaced to a high-performance liquid chromatograph (HPLC; Waters 2795, Mississauga, Ontario, Canada) equipped with a 100 x 4 mm i.d. Luna Omega PS C18 reversed-phase column (particle size 5 µm) using a water-acetonitrile gradient with a constant 2 mmol L^{-1} concentration of ammonium acetate (0.6 $mL.min^{-1}$). Quantification of free 3-OH-C_{10} in purified C_{10}-C_{10}, Rha-Rha-C_{10}-C_{10}, Rha-C_{10}-C_{10}, Rha-Rha-C_{14}-C_{14} or synthetic C_{10}-C_{10} were performed as reported previously (34) and are presented in Supplementary table 2.

Plant material and growth conditions. *Arabidopsis thaliana* ecotype Col-0 was used as WT parent for all experiments. Seeds from *fls2/efr1* (38, 39), *bak1*-5, *bkk1*-1, *bak1-5/bkk1-1* (40), *cerk1-2* (42), *bik1/pbl1* (41), *rbohD, msl4/5/6/9/10* and *mca1/2* (51) were provided by C. Zipfel. Seeds from *sobir1*-12 and *sobir1*-13 (43)

were provided by F. Brunner (*Center for Plant Molecular Biology, University of Tübingen, Tübingen,* PlantResponse^{TM}). Seeds from *sd1-29* (*lore-5*), Col-0^{AEQ} and *lore*-5^{AEQ} were provided by S. Ranf (45). *loh1* and *fah1/2* seed (52) were provided by I. Feussner *(University of Göttingen, Germany)*. *dorn1*-1 seeds (44) were obtained from NASC stock (SALK_042209). All mutants are in the Col-0 background. Plants were grown on soil in growth chambers at 20°C, under 12 h light / 12 h dark regime with fluorescent light of 150 µmol m^{-2} s^{-1} and 60% relative humidity.

Extracellular ROS production and calcium signaling. ROS assays were performed on 4- to 6-week-old *Arabidopsis* plants cultured on soil. Briefly, 5 mm long petiole sections were cut and placed in 150 µL of distilled water overnight in 96 wells plate (PerkinElmer) (46). Then, the protocol was conducted as previously described (82). Luminescence (relative light units, RLU) was measured every 2 min during 46 or 720 min with a Tecan Infinite F200 PRO (or a TECAN CM SPARK for Supplementary figure 6), Tecan France. Total ROS production was calculated by summing RLU measured between 4 to 46 or 4 to 720 minutes after treatment. Control was realized on petioles of WT or mutant plants. $[Ca^{2+}]_{cyt}$ measurements were done as previously described (34).

MAPK phosphorylation assays. For MAPK phosphorylation assays, 3 leaf disks (9 mm diameter) were collected from 4 to 6-week-old *Arabidopsis* plants grown on soil and incubated 8 h in distilled water. Leaf disks were mock-treated or treated with different molecules. 15 min, 1 hour, and 3 hours after treatment, plant tissues were frozen in liquid nitrogen. To extract proteins, 60 mg of leaf tissues were ground in a homogenizer Potter-Elvehjem with 60 µL of extraction buffer (0.35 M Tris-HCl (pH 6.8), 30% (v/v) glycerol, 10% (v/v) SDS, 0.6 M DTT, 0.012% (w/v) bromophenol blue). Total protein extracts were denatured for 7 min at 95°C, centrifuged at 11 000g for 5 min and 30 µL of supernatant were separated by 12% SDS-PAGE. Phosphorylation state of MAPKs by immunobloting was performed as previously described (46).

Depletion binding assay. Protein expression and ligand depletion were performed as described (85) with slight modifications. Briefly, LORE extracellular domain mCherry fusion protein (eLORE-mCherry) or mCherry control fused with N-terminal LORE signal peptide were transiently expressed in *N. benthamiana* leaf apoplast using a 1:1 mixture of *Agrobacterium tumefaciens* GV3101 carrying the respective expression plasmid or the p19 silencing suppressor (total OD_{600} 0.5). Five days post inoculation, *N. benthamiana* leaves were vacuum-infiltrated with water and apoplastic washing fluid (AWF) was collected by centrifugation (20 min, 800 *g*, 4°C). AWF was cleared by centrifugation (20 min, 8000 *g*, 4°C), filter-sterilized (pore size 0.22 µm), desalted (PD-10 Desalting Columns, Cytiva), and concentrated to a total protein concentration of 2 mg/mL (Vivaspin 20, 30 000 Da MW cut-off, Sartorius). For ligand depletion assay, AWF concentrate was mixed in 9:1 ratio (v:v) with synthetic C_{10}-C_{10} (50 µM) or 3-OH-C_{10} (5 µM) and incubated for one hour at 4°C on a rotator. Unbound ligands were separated from the mixture (Vivaspin 500, 30 000 MW cut-off, Sartorius). Content of unbound C_{10}-C_{10} and 3-OH-C_{10} in filtrates was assessed by $[Ca^{2+}]_{cyt}$ measurements using LORE overexpressing (*p35S:LORE*/*lore*-1) and *lore*-1 seedlings with a filtrate:water ratio of 1:4 (v:v).

Conductivity assay. The assay was performed as described previously (83), with few modifications. Eight leaf discs of 6-mm-diameter were incubated in distilled water overnight. One disc was transferred into 1.5 mL tube containing fresh distilled water and the corresponding elicitor concentration or ethanol for control. Conductivity measurements (three to four replicates for each treatment) were then conducted using a B-771 LaquaTwin (Horiba) conductivity meter.

***Pseudomonas syringae* culture and disease resistance assays.** *Pseudomonas syringae* pv. *tomato* strain DC3000 was grown at 28°C under stirring in King's B (KB) liquid medium supplemented with antibiotics: 50 µg mL^{-1} of rifampicin and 50 µg mL^{-1} of kanamycin. For local protection assays, 15 seeds were sown per pot and grown for 3 to 5 weeks in soil. Plants were sprayed with molecules or ethanol as control and were placed two days in high humidity atmosphere before infections. Plants were inoculated by spraying the leaves with 3 mL of a bacterial suspension at an optical density (OD_{600}) of 0.01 (0.025 % Silwet L-77, 10 mM $MgCl_2$). For each experiment, 5 pots per conditions were used (n=5). Quantification (colony forming units) of *in planta* bacterial growth was performed 3 dpi. To this end, all plant leaves from the same pot were harvested, weighed, and crushed in a mortar with 10 mL of 10 mM $MgCl_2$ and serial dilutions were performed. For each dilution, 10 µL were dropped on KB plate supplemented with appropriate antibiotics. Colony forming units (CFU) were counted after 2 days of incubation at 28°C. The number of bacteria per mg of plants fresh mass was obtained with the formula:

$$CFU.mg^{-1} = \frac{\left(\frac{N \times Vd}{Vi} \times 10^{(n-1)} \times 100\right)}{M}$$

with N equal to CFU number, Vi the volume depot on plate, Vd the total volume, n the dilution number and M the plants fresh mass.

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Author contributions

J.C. and S.D. designed the research; R.S., J.C., L.J.S, A.K., T.G., M.T., S.V., S.D.C. performed the experiments; A.N. and E.D. purified and characterized HAAs and *B. glumae* RLs; M.C. and C.G. chemically synthetized HAAs; N.B., J.H., A.H. and J.H.R., purified *P. aeruginosa* RLs; C.D. and C.S. quantified mc-3-OH-FAs in all samples; R.S., J.C., L.J.S, S.C., F.M.G., F.B., S.R., E.D. and S.D. analyzed the data; R.S., J.C. and

S.D. wrote the manuscript. M.O., J.H.R., A.H., T.H., C.Z., F.B., C.C., S.R and E.D contributed ideas, and critically revised the manuscript. All authors discussed the results and approved the manuscript.

Additional Information

Data availability

The authors declare that all data supporting the findings of this study can be found within the manuscript and its Supplementary Files.

Competing financial interests

Technical University of Munich has filed a patent application to inventors A.K., C.D., T.H., and S.R. The authors declare no financial conflicts of interest in relation to this work.
All other author(s) declare no competing financial and/or non-financial interests.

Figure legends

Fig. 1. RLsec activates LORE-dependent early immune-related responses in *Arabidopsis*. (A) Extracellular ROS production after treatment of WT leaf petioles with 50 µg/mL RLsec or EtOH as control. (B) Dose effect of RLsec on ROS production. ROS production measured after treatment of WT leaf petioles with the indicated concentrations of RLsec or EtOH as control. (C) ROS production measured after treatment of WT, *fls2/efr1*, *dorn1*-1, *bak1*-5, *bkk1*-1, *bak1*-5/*bkk1*-1, *cerk1*-2, *sobir1*-12, *sobir1*-13, *bik1/pbl1*, *lore*-5, or *rbohD* leaf petioles with 50 µg/mL RLsec. (A-C) Data are mean ± SEM (n = 6). (A-C) Experiments have been realized three times with similar results.

Fig. 2. RLsec activates LORE-independent responses in *Arabidopsis*. (A) Extracellular ROS production after treatment of WT, *lore*-5, and *rbohD* leaf petioles with 50 µg/mL RLsec or EtOH (Control). ROS production was monitored over 720 minutes. Data are mean ± SEM (n = 6). Experiments have been realized three times with similar results. (B) WT (red) and *lore*-5 (blue) *Arabidopsis* leaves were treated with 50 µg/mL Rlsec (triangle) or EtOH (square) (control) 48 h before infection. *Pst* titers were measured at 3 d.p.i. Data are individual data and mean (black line) (n = 5). Experiments have been realized three times with similar results. Letters represent results of pairwise Welch statistic test with Bonferroni correction with $P > 0.05$ (same letters) or $P \leq 0.05$ (different letters).

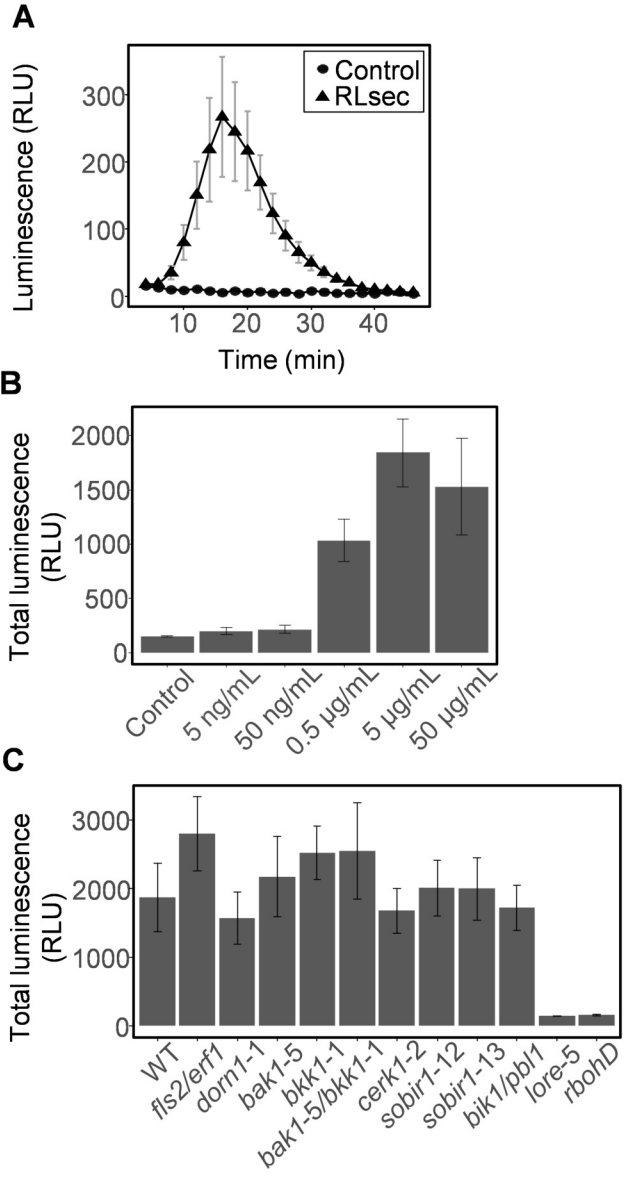
Fig. 3. Purified HAAs from *P. aeruginosa* trigger a LORE-dependent immune response in *Arabidopsis*. (A) Dose effect of 3-OH-C_{10} and C_{10}-C_{10} purified from *P. aeruginosa* on ROS production by WT leaf petioles. EtOH was used as negative control. Data are mean ± SEM (n = 6). Experiments have been realized twice with similar results. (B) Maximum (Max.) increases in $[Ca^{2+}]_{cyt}$ in *Arabidopsis* Col-0^{AEQ} seedlings treated with different concentrations of 3-OH-C_{10}, C_{10}-C_{10} purified from *P. aeruginosa* or MeOH as control. Data are mean ± SD (n = 3). Experiments have been realized twice with similar results. (C) ROS production measured after

643 treatment of WT, *lore*-5, or *rbohD* leaf petioles with 10 µM 3-OH-C_{10}, 10 µM purified C_{10}-C_{10} or EtOH as
644 control. Data are mean ± SEM (n = 6). Experiments have been realized three times with similar results. (D)
645 Maximum (Max.) increases in $[Ca^{2+}]_{cyt}$ in *Arabidopsis* Col-0^{AEQ} and *lore*-5^{AEQ} seedlings treated with 5 µM 3-
646 OH-C_{10} or purified C_{10}-C_{10}. Data are mean ± SD (n = 3). Experiments have been realized twice with similar
647 results. For B and D, the same Col-0^{AEQ} 5 µM data are presented (same experiments). (E) WT (red) and *lore*-
648 5 (blue) *Arabidopsis* leaves were treated with 10 µM 3-OH-C_{10} (triangle), 10 µM purified C_{10}-C_{10} (diamond) or
649 EtOH (square) (control) 48 h before infection. *Pst* titers were measured at 3 d.p.i. Data are individual data and
650 mean (black line) (n = 5). Experiments have been realized twice with similar results. Letters represent results
651 of pairwise Welch statistic test with Bonferroni correction with $P > 0.05$ (same letters) or $P \leq 0.05$ (different
652 letters).
653
654 **Fig. 4. Synthetic HAAs trigger a LORE-dependent immune response in *Arabidopsis*.** (A) Maximum
655 (Max.) increases in $[Ca^{2+}]_{cyt}$ in *Arabidopsis* Col-0^{AEQ} seedlings treated with different concentrations of 3-OH-
656 C_{10}, synthetic C_{10}-C_{10} or MeOH. Data are mean ± SD (n = 3). Experiments have been realized twice with
657 similar results. (B) Dose effect of synthetic C_{10}-C_{10} on ROS production by WT leaf petioles. EtOH was used as
658 negative control. Data are mean ± SEM (n = 6). Experiments have been realized twice with similar results. (C)
659 Maximum (Max.) increases in $[Ca^{2+}]_{cyt}$ in *Arabidopsis* Col-0^{AEQ} and *lore*-5^{AEQ} seedlings treated with 5 µM 3-
660 OH-C_{10} or synthetic C_{10}-C_{10}. Data are mean ± SD (n = 3). Experiments have been realized twice with similar
661 results. (D) WT (red) and *lore*-5 (blue) *Arabidopsis* leaves were treated with 10 µM 3-OH-C_{10} (triangle), 10 µM
662 synthetic C_{10}-C_{10} (diamond), or MeOH (square) (control) 48 h before infection. *Pst* titers were measured at 3
663 d.p.i. Data are individual data and mean (black line) (n = 5). Experiments have been realized twice with similar
664 results. Letters represent data of pairwise Welch statistic test with Bonferroni correction with $P > 0.05$ (same
665 letters) or $P \leq 0.05$ (different letters). (E) Depletion of 1 µM synthetic C_{10}-C_{10} or 100 nM 3-OH-C_{10} by
666 concentrated apoplastic wash fluid from *Nicotiana benthamiana* containing LORE extracellular domain
667 (eLORE)-mCherry fusion protein or apoplastic (apo-)mCherry as control as illustrated in supplemental figure
668 8A. Unbound ligand content in low-molecular-weight filtrate was analyzed by $[Ca^{2+}]_{cyt}$ measurements in LORE-
669 overexpressing (OE; red bars, grey triangles, n = 12) and *lore*-1 (blue bars, grey diamonds, n = 6) *Arabidopsis*
670 seedlings. Data are mean ± SD and individual data of three pooled experiments. Letters represent data of
671 pairwise Wilcoxon statistic test with Bonferroni correction with $P > 0.05$ (same letters) or $P \leq 0.05$ (different
672 letters).
673
674 **Fig. 5. Purified RLs trigger a LORE-independent *Arabidopsis* immune response.** (A) Extracellular late
675 ROS production after treatment of WT leaf petioles with 100 µM RLs, 100 µM L-rhamnose, or EtOH (control).
676 Data are mean ± SEM (n = 6). (B) Dose effect of Rha-Rha-C_{10}-C_{10} on late ROS production. ROS production
677 measured after treatment of WT leaf petioles with the indicated concentrations of Rha-Rha-C_{10}-C_{10} or EtOH
678 (control). Data are mean ± SEM (n = 6). (C) Late ROS production measured after treatment of WT, *lore*-5, or
679 *rbohD* leaf petioles with 100 µM Rha-Rha-C_{10}-C_{10}. Data are mean ± SEM (n = 6). (D) WT (red) and *lore-5*
680 (blue) *Arabidopsis* leaves were treated with 10 µM Rha-Rha-C_{10}-C_{10} (triangle) or EtOH (square) (control) 48 h
681 before infection. *Pst* titers were measured at 3 d.p.i. Data are individual data and mean (black line) (n = 5).

Letters represent results of pairwise t statistic test with Bonferroni correction with $P > 0.05$ (same letters) or $P \leq 0.05$ (different letters). (A-D) Experiments have been realized three times with similar results.

Fig. 6. RL perception is impacted by plasma membrane sphingolipid composition. Extracellular late ROS production after treatment of (A) WT, *cerk1*-2, *dorn1*-1, *sobir1*-12, *bak1*-5/*bkk1*-1, *bik1/pbl1*, *mca1/2*, *msl4/5/6/9/10* (*msl*), or *rbohD,* and (B) WT, *loh1*, or *fah1/2 Arabidopsis* leaf petioles with 100 µM Rha-Rha-C_{10}-C_{10} or EtOH (control). Data are mean ± SEM (n = 6). Experiments have been realized three times with similar results. (C) Electrolyte leakage induced by 100 µM Rha-C_{10}-C_{10} or EtOH (Control) on WT, *loh1*, or *fah1/2 Arabidopsis* leaf discs 24h post treatment. Data are mean ± SEM (n = 6). Letters represent results of pairwise Wilcoxon-Mann-Whitney statistic test with $P > 0.05$ (same letters) or $P \leq 0.05$ (different letters). Experiments have been realized twice with similar results. (D) WT (red) and *fah1/2* (blue) *Arabidopsis* leaves were treated with 10 µM Rha-C_{10}-C_{10} (triangle) or EtOH (square) (control) 48 h before infection. *Pst* titers were measured at 3 d.p.i. Data are individual data and mean (black line) (n = 5). Letters represent results of pairwise Welch statistic test with Bonferroni correction with $P > 0.05$ (same letters) or $P \leq 0.05$ (different letters).

Fig. 7: Proposed model for HAA and rhamnolipid perception mechanisms in *Arabidopsis* and integrated immune responses. RL-producing bacteria excrete rhamnolipids and HAAs to form biofilm and for successful colonization. *Arabidopsis* perceives HAAs *via* LORE and rhamnolipids by a LORE-independent pathway affected by sphingolipids composition of the plant plasma membrane, resulting in the activation of efficient immune responses. Parts of this scheme were created with BioRender.com.


A
Luminescence (RLU)
300
200
100
0
10
20
30
40
Time (min)
Control
RLsec
B
Total luminescence (RLU)
2000
1500
1000
500
0
Control
5 ng/mL
50 ng/mL
0.5 µg/mL
5 µg/mL
50 µg/mL
C
Total luminescence (RLU)
3000
2000
1000
0
WT
fls2/erf1
dorn1-1
bak1-5
bkk1-1
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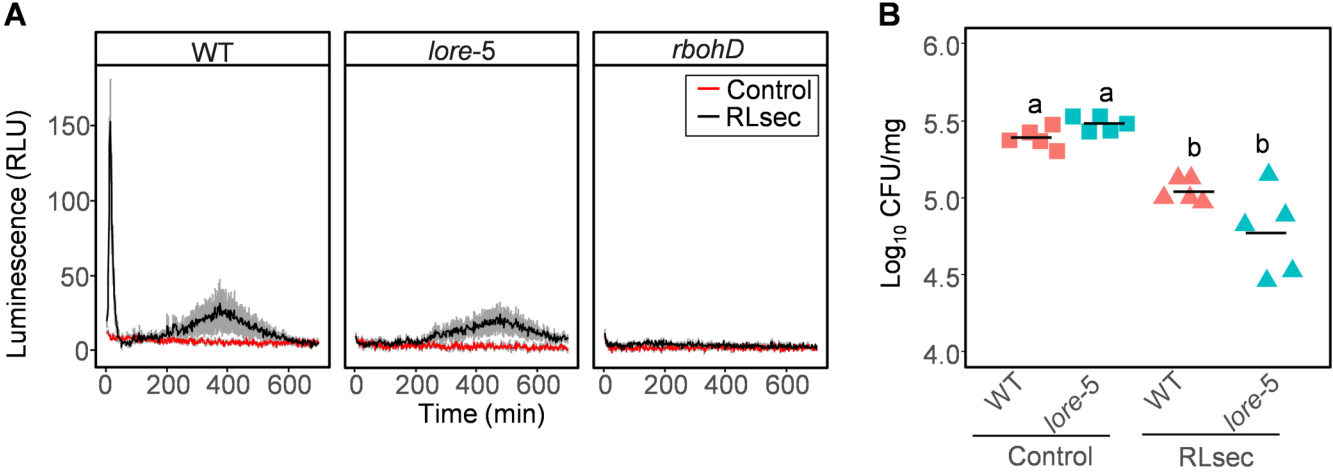
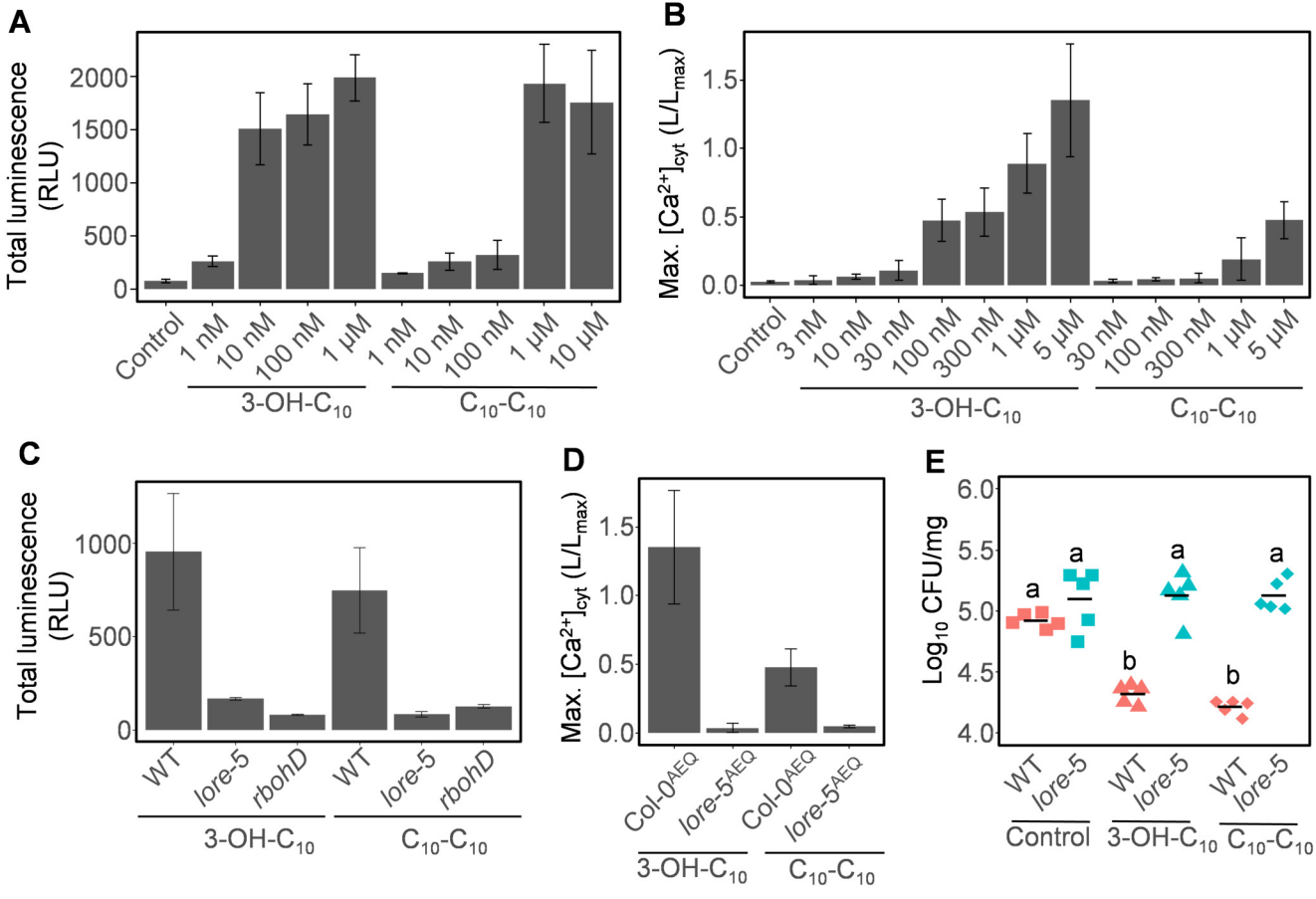
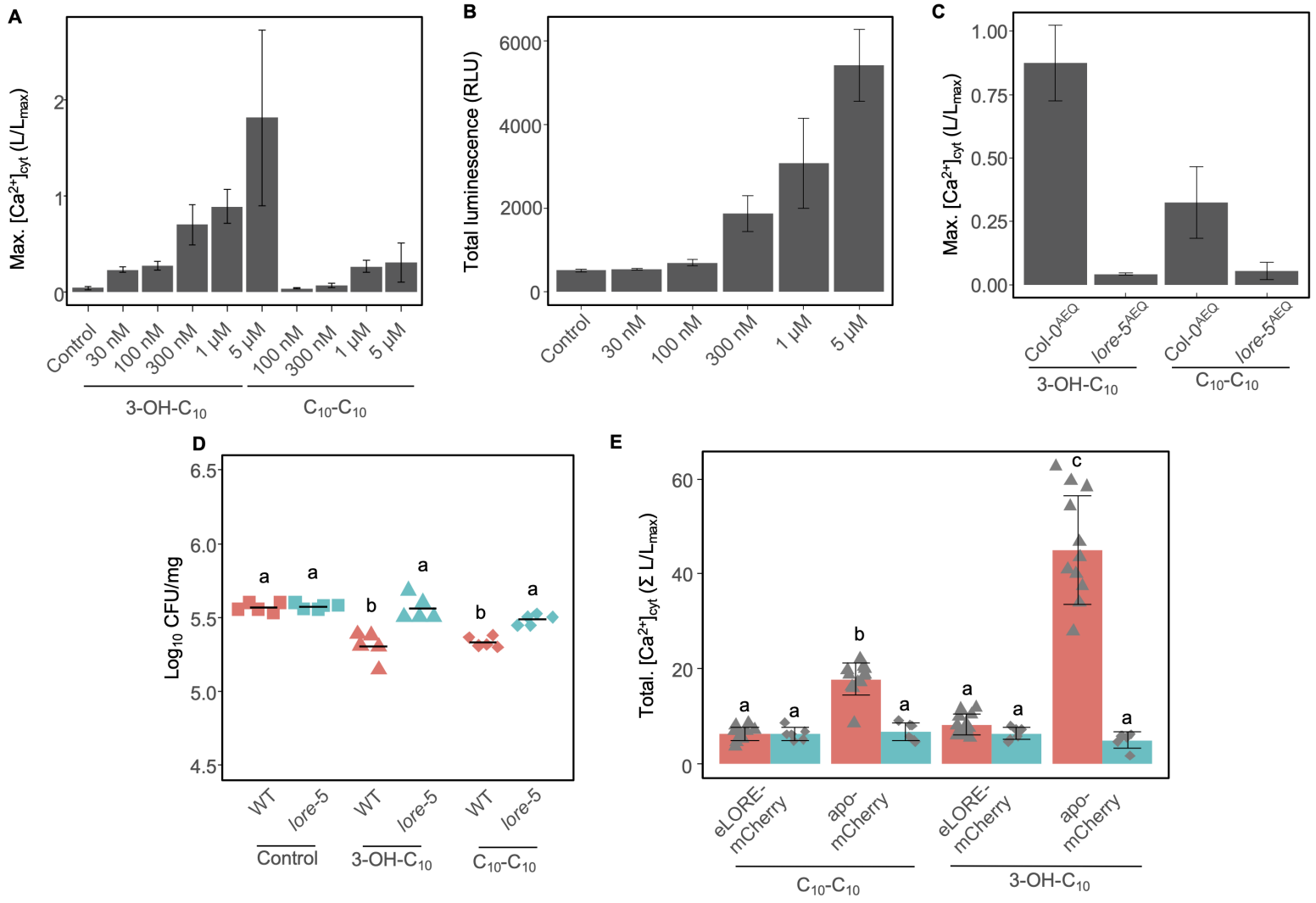
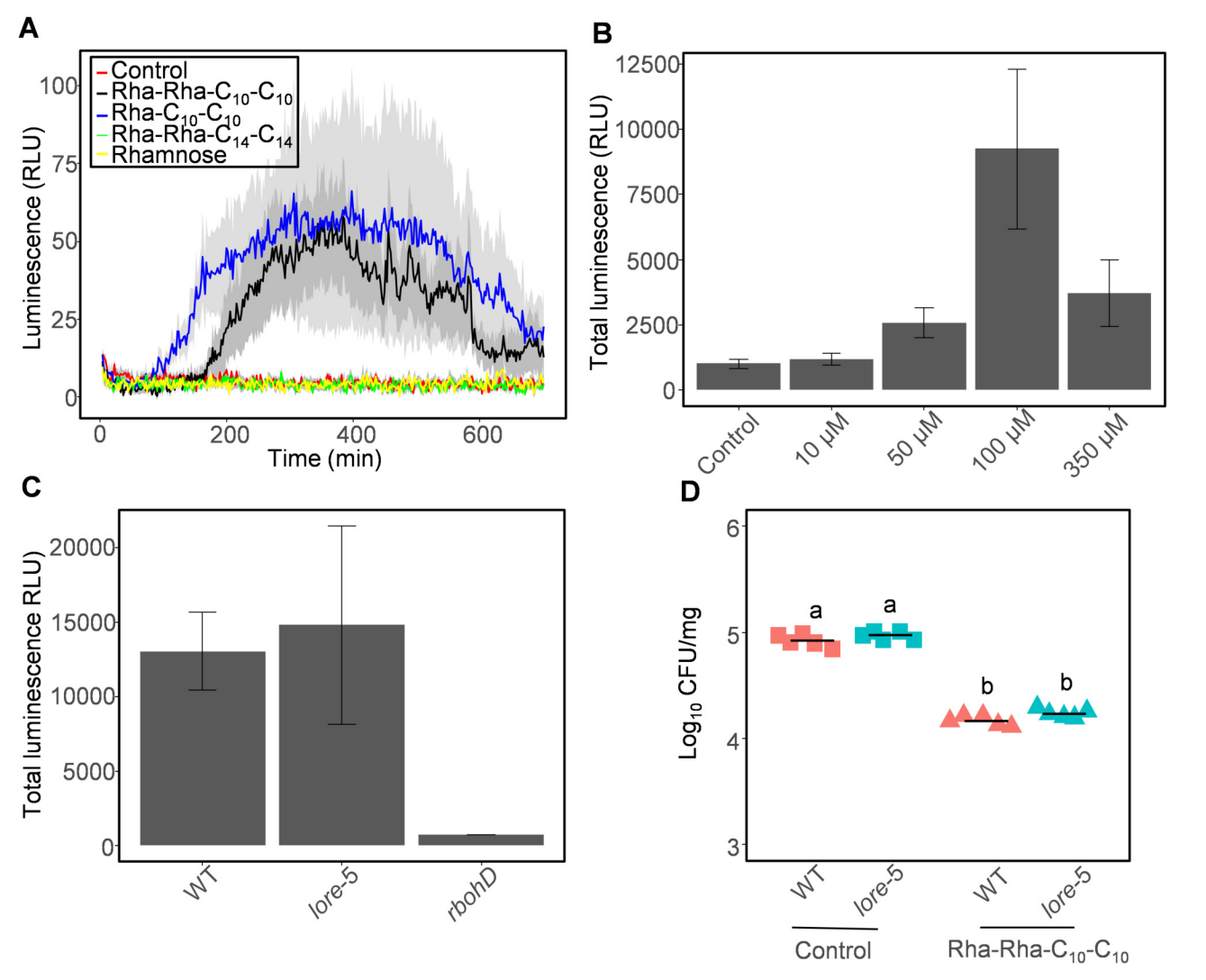
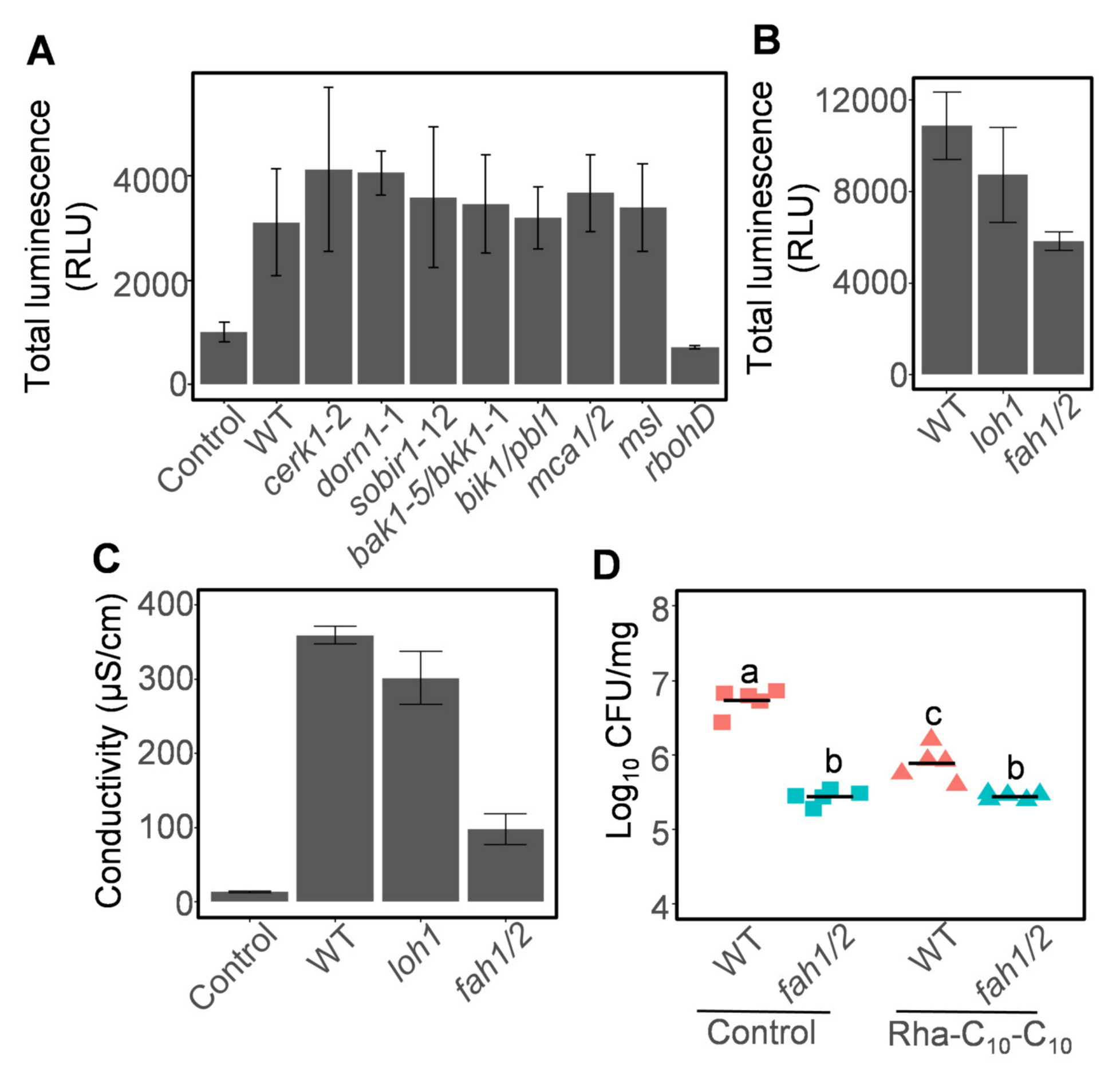
sobir1-13
bik1/pbl1
lore-5
rbohD

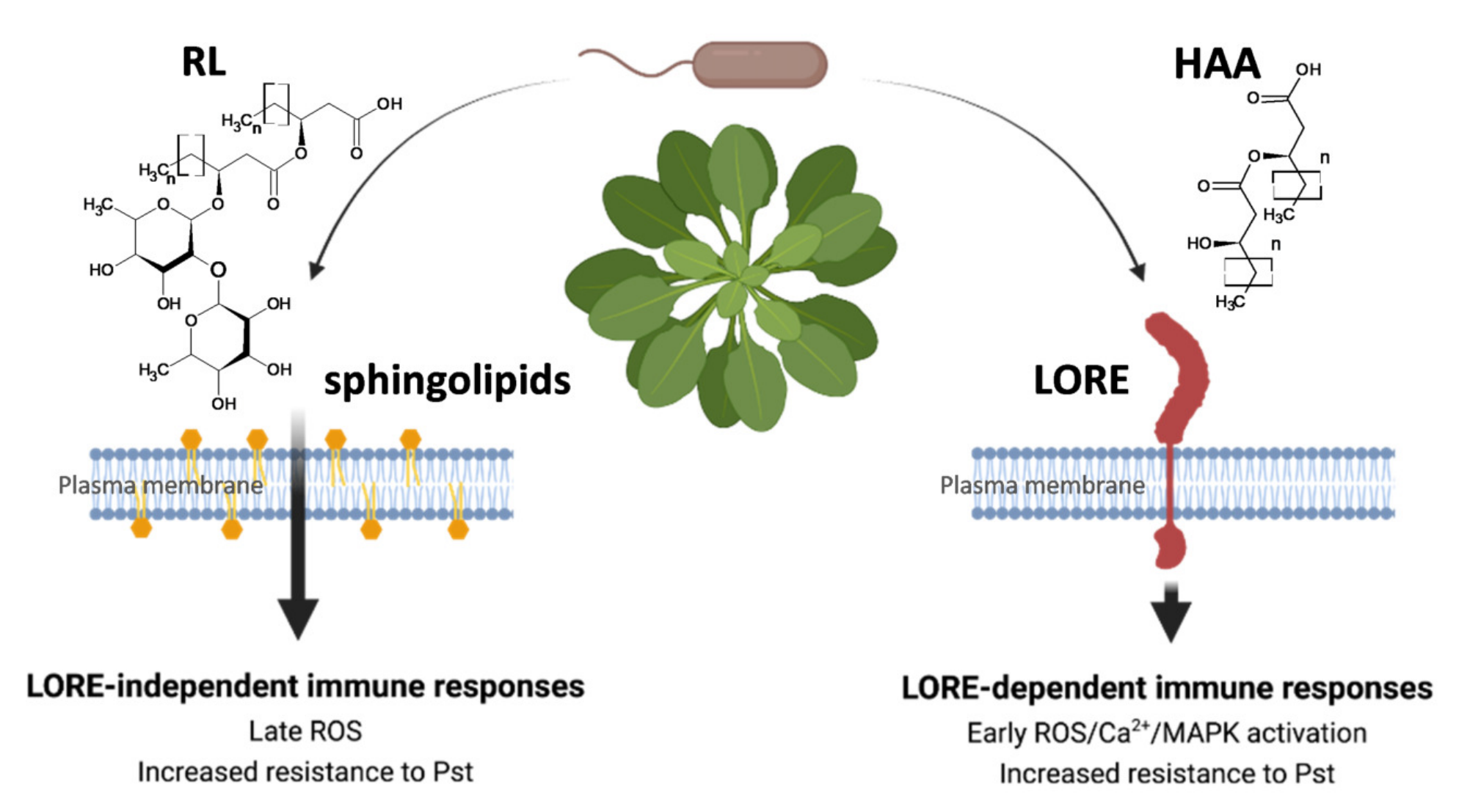

A
WT
lore-5
rbohD
Control
RLsec
Luminescence (RLU)
150
100
50
0
0
200
400
600
Time (min)
B
Log10 CFU/mg
6.0
5.5
5.0
4.5
4.0
a
a
b
b
WT
lore-5
WT
lore-5
Control
RLsec


A
Total luminescence (RLU)
2000
1500
1000
500
0
Control
1 nM
10 nM
100 nM
1 µM
1 nM
10 nM
100 nM
1 µM
10 µM
3-OH-C10
C10-C10
B
Max. [Ca2+]cyt (L/Lmax)
1.5
1.0
0.5
0.0
Control
3 nM
10 nM
30 nM
100 nM
300 nM
1 µM
5 µM
30 nM
100 nM
300 nM
1 µM
5 µM
3-OH-C10
C10-C10
C
Total luminescence (RLU)
1000
500
0
WT
lore-5
rbohD
WT
lore-5
rbohD
3-OH-C10
C10-C10
D
Max. [Ca2+]cyt (L/Lmax)
1.5
1.0
0.5
0.0
Col-0AEQ
lore-5AEQ
Col-0AEQ
lore-5AEQ
3-OH-C10
C10-C10
E
Log10 CFU/mg
6.0
5.5
5.0
4.5
4.0
a
a
a
b
b
WT
lore-5
WT
lore-5
WT
lore-5
Control
3-OH-C10
C10-C10


A
Max. [Ca2+]cyt (L/Lmax)
Control
30 nM
100 nM
300 nM
1 µM
5 µM
100 nM
300 nM
1 µM
5 µM
3-OH-C10
C10-C10
B
Total luminescence (RLU)
Control
30 nM
100 nM
300 nM
1 µM
5 µM
C
Max. [Ca2+]cyt (L/Lmax)
Col-0AEQ
lore-5AEQ
Col-0AEQ
lore-5AEQ
3-OH-C10
C10-C10
D
Log10 CFU/mg
WT
lore-5
WT
lore-5
WT
lore-5
Control
3-OH-C10
C10-C10
E
Total. [Ca2+]cyt (Σ L/Lmax)
eLORE-mCherry
apo-mCherry
eLORE-mCherry
apo-mCherry
C10-C10
3-OH-C10


A
Control
Rha-Rha-C10-C10
Rha-C10-C10
Rha-Rha-C14-C14
Rhamnose
Luminescence (RLU)
Time (min)
B
Total luminescence (RLU)
Control
10 µM
50 µM
100 µM
350 µM
C
Total luminescence RLU)
WT
lore-5
rbohD
D
Log10 CFU/mg
a
a
b
b
WT
lore-5
WT
lore-5
Control
Rha-Rha-C10-C10


A
Total luminescence (RLU)
0
2000
4000
Control
WT
cerk1-2
dorn1-1
sobir1-12
bak1-5/bkk1-1
bik1/pbl1
mca1/2
msl
rbohD
B
Total luminescence (RLU)
0
4000
8000
12000
WT
loh1
fah1/2
C
Conductivity (µS/cm)
0
100
200
300
400
Control
WT
loh1
fah1/2
D
Log10 CFU/mg
4
5
6
7
8
a
b
c
b
WT
fah1/2
WT
fah1/2
Control
Rha-C10-C10


RL
HAA
sphingolipids
LORE
Plasma membrane
Plasma membrane
LORE-independent immune responses
Late ROS
Increased resistance to Pst
LORE-dependent immune responses
Early ROS/Ca2+/MAPK activation
Increased resistance to Pst