LORE-mediated immune sensing of bacterial metabolites

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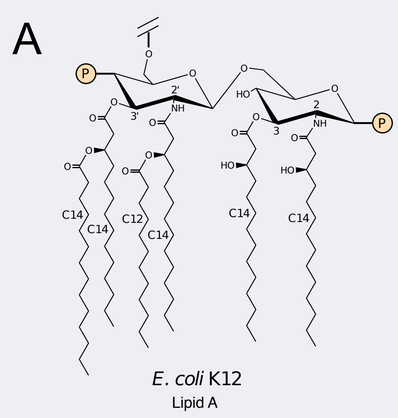
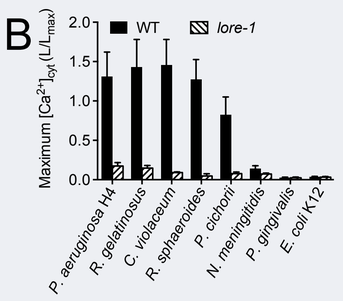
LORE senses mc-3-OH-FAs to activate PTI

Plant cell-surface Pattern Recognition Receptors (PRRs) sense Microbe-Associated Molecular Patterns (MAMPs). Lipid A of Gram-negative bacterial lipopolysaccharide (LPS) is considered such a MAMP. The receptor kinase LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION (LORE or SD1-29) mediates immune responses to *Pseudomonas* lipid A and LPS preparations [1]. We demonstrate now that synthetic and bacterial, lipopolysaccharide-co-purified medium chain 3-hydroxy fatty acid (mc-3-OH-FA) metabolites elicit LORE-dependent immunity [2]. By contrast, bacterial compounds comprising mc-3-OH-acyl building blocks, but devoid of free mc-3-OH-FAs, including lipid A and LPS, do not trigger LORE-dependent responses. Hence, plants sense low-complexity bacterial metabolites to trigger immune responses.

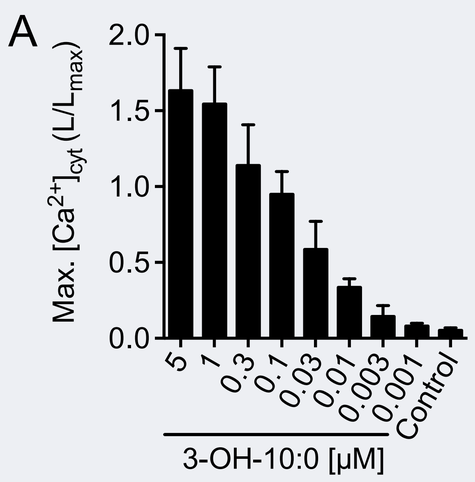


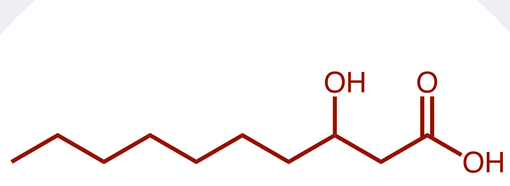


C

Bacterial species	LORE-dependent PTI	Lipid A structure 3'-Acyl chains	3-Acyl chains
Escherichia coli	-	C14	C14
Burkholderia cepacia	-	C14	C14
Pectobacterium carotovorum	-	C14/C12	C14
Neisseria meningitides	+ / -	C12	C12
Pseudomonas aeruginosa	+	C10	(C10)
Xanthomonas campestris	+	C10-C13	C10-C13
Rhodobacter sphaeroides	+	C10	C10
Rubrivivax gelatinosus	+	C10	C10
Chromobacterium violaceum	+	C10	C10

Fig. 1 LPS preparations trigger LORE-dependent PTI. (**A**) Typical lipid A structures. (**B**) $[Ca^{2+}]_{cyt}$ elevations in Arabidopsis induced by LPS from different bacteria`l species [25 µg/mL] [1]. (**C**) LORE-dependent PTI responses correlate with lipid A structures.





3-hydroxy decanoic acid
3-OH-C10:0

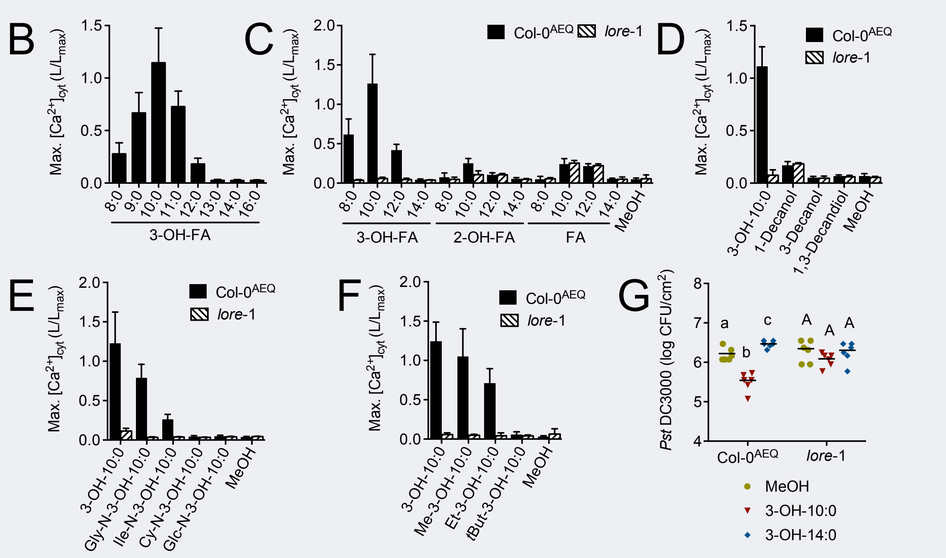


Fig. 2 LORE senses mc-3-OH-FAs to activate PTI. (**A**) Max. $[Ca^{2+}]_{cyt}$ elevations induced by different concentrations of 3-OH-C10:0 (n=12, SD). (**B-F**) Max. $[Ca^{2+}]_{cyt}$ elevations induced by (**B**) different 3-OH-FAs (1µM, n=12, SD), (**C**) 3-OH-, 2-OH- and non-hydroxylated FAs (5µM, n=6, SD), (**D**) C10:0-alcohol derivatives (5µM, n=6, SD) or (**EF**) 3-OH-C10:0 with carboxyl group substituted with amides (**E**, 5µM, n=6, SD) or esters (**F**, 5µM, n=9, SD). (**G**) *Pst* DC3000 titre 4 dpi in plants pre-treated with 10 µM 3-OH-FAs or MeOH for 3 days (1-ANOVA, Tukey's post test, $p<0.01$).

3-OH-FA-depleted LPS is not sensed in Arabidopsis

A

Sample	Sample conc.	Free 3-OH-C10:0 [µM]
Water control		<LOQ
3-OH-C10:0	20 µM	22.41
P. aeruginosa H4 LPS	100 µg/mL	5.38
Repurified *P. aeruginosa* H4 LPS	400 µg/mL	<LOQ

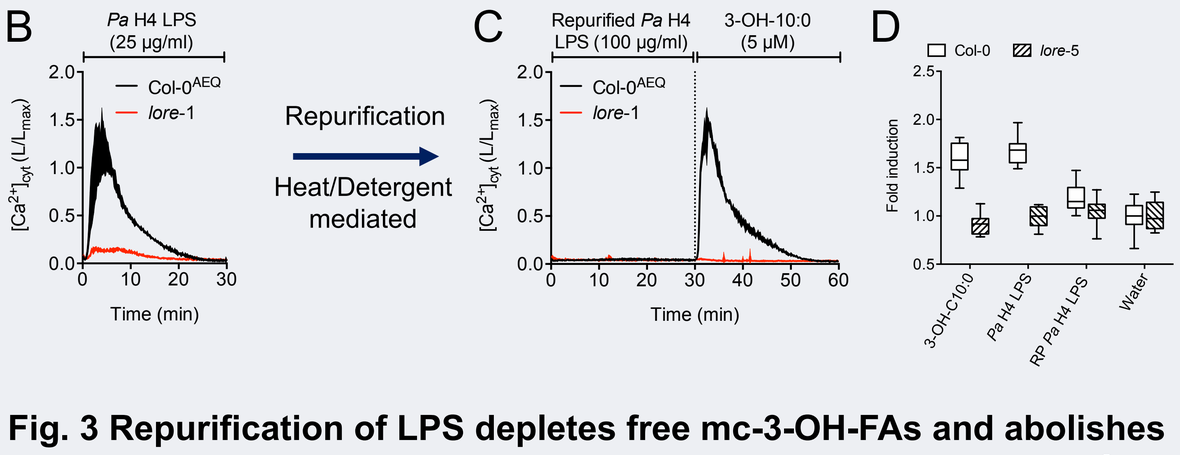
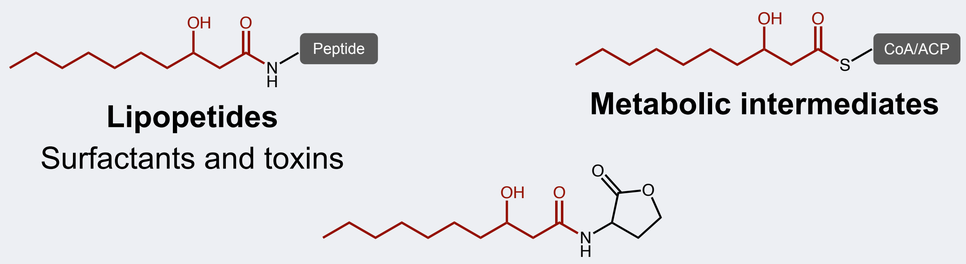


Fig. 3 Repurification of LPS depletes free mc-3-OH-FAs and abolishes immune responses. (**A**) Quantification of free 3-OH- C10:0. (**BC**) $[Ca^{2+}]_{cyt}$ elevations before (**B**) and after (**C**) repurification of *P. aeruginosa* (*Pa*) H4 LPS. (**D**) Quantification of peroxidase secretion before and after repurification of *P. aeruginosa* (*Pa*) H4 LPS.

3-OH-FA are important metabolic precursors and metabolites in bacteria



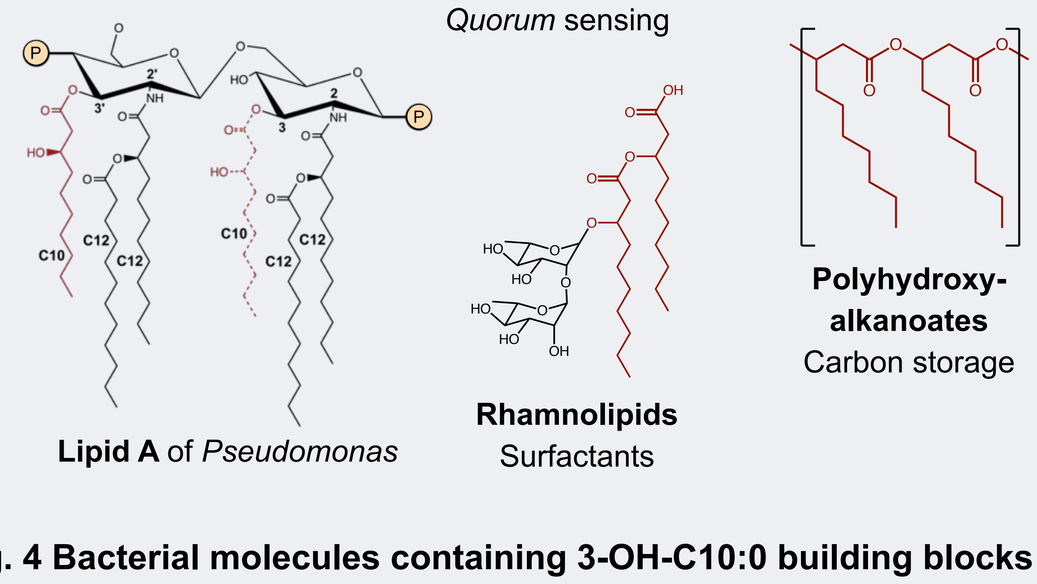


Fig. 4 Bacterial molecules containing 3-OH-C10:0 building blocks

References and acknowledgements

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[2] A. Kutschera*, C. Dawid*, N. Gisch, C. Schmid, L. Raasch, T. Gerster, M. Schäffer, E. Smakowska-Luzan, Y. Belkhadir, A. C. Vlot, C. E. Chandler, R. Schellenberger, D. Schwudke, R. K. Ernst, S. Dorey, R. Hückelhoven, T. Hofmann, S. Ranf. Bacterial medium chain 3-hydroxy fatty acid metabolites trigger immunity in Arabidopsis plants (in revision)

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