PLANT SCIENCE

Bacterial medium-chain 3-hydroxy fatty acid metabolites trigger immunity in *Arabidopsis* plants

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In plants, cell-surface immune receptors sense molecular non–self-signatures. Lipid A of Gram-negative bacterial lipopolysaccharide is considered such a non–self-signature. The receptor kinase LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION (LORE) mediates plant immune responses to *Pseudomonas* and *Xanthomonas* but not enterobacterial lipid A or lipopolysaccharide preparations. Here, we demonstrate that synthetic and bacterial lipopolysaccharide-copurified medium-chain 3-hydroxy fatty acid (mc-3-OH-FA) metabolites elicit LORE-dependent immunity. The mc-3-OH-FAs are sensed in a chain length– and hydroxylation-specific manner, with free (*R*)-3-hydroxydecanoic acid [(*R*)-3-OH-C10:0] representing the strongest immune elicitor. By contrast, bacterial compounds comprising mc-3-OH-acyl building blocks but devoid of free mc-3-OH-FAs—including lipid A or lipopolysaccharide, rhamnolipids, lipopeptides, and acyl-homoserine-lactones—do not trigger LORE-dependent responses. Hence, plants sense low-complexity bacterial metabolites to trigger immune responses.

Plant cell–surface pattern recognition receptors sense characteristic microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs) and host-derived damage-associated patterns (DAMPs) and activate pattern-triggered immunity (*1*). Lipopolysaccharide, a glycolipid consisting of lipid A, core oligosaccharide, and O-polysaccharide, is the major outer membrane component of most Gram-negative bacteria (*2*). In humans and mammals, lipid A triggers inflammation and pyroptosis, potentially culminating in life-threatening sepsis (*2*, *3*). Lipopolysaccharide also elicits defense responses in various plant species (*4*). In *Arabidopsis thaliana* (hereafter *Arabidopsis*) and other crucifers, lipopolysaccharide preparations from *Pseudomonas* spp. and *Xanthomonas campestris*, but not from *Escherichia coli*, *Salmonella enterica*, or *Burkholderia* spp., activate characteristic immune responses through the plant-specific bulb-type lectin receptor kinase LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION/S-DOMAIN-1-29 (LORE/SD1-29) (*5*). *Pseudomonas aeruginosa* lipid A is sufficient to trigger immunity, whereas removal of the ester-linked acyl chains from lipid A abolishes its activity (*5*). Thus, lipid A with a distinctive acylation pattern appeared to be critical for immune sensing in *Arabidopsis*.

To decipher the structural features of LORE-dependent lipid A sensing, we screened lipopolysaccharide with different acylation patterns from various bacterial species and *P. aeruginosa* lipid A biosynthesis mutants for activation of cytosolic calcium ($[Ca^{2+}]_{cyt}$) signaling as an indicator of immune response in *Arabidopsis* (table S1 and figs. S1 to S4). Structurally, all tested, elicitor-active lipopolysaccharide preparations comprise a medium-length acyl chain, mostly 3-OH-decanoyl, at positions C-3 and/or C-3′ of lipid A, whereas lipopolysaccharide with 3-OH-tetradecanoyl chains at these positions was inactive. This suggests that a 3-OH-decanoyl chain is a key structural feature for LORE-dependent lipopolysaccharide sensing.

Therefore, synthetic 3-OH-FAs of varying chain length (table S2) were tested for activation of *Arabidopsis* immune responses. Free synthetic 3-OH-C10:0 (**1**; see table S2 for assignment of compound numbers; Fig. 1A) elicits increases in $[Ca^{2+}]_{cyt}$ comparable to those of *Pseudomonas* lipopolysaccharide preparations (Fig. 1B and fig. S5A). Medium-chain (mc)-3-OH-FAs (bearing 8 to 12 carbon atoms, **1** to **5**), particularly 3-OH-C10:0 (**1**), but not long-chain (lc)-3-OH-FAs (13 to 16 carbon atoms, **6** to **8**) activated LORE-dependent $[Ca^{2+}]_{cyt}$ signaling, biphasic production of reactive oxygen species (ROS), transcript accumulation of defense-related genes, and phosphorylation of mitogen-activated protein kinases (Fig. 1, C and D, and fig. S5, B to F). In plants, local MAMP application induces systemic resistance to pathogen infection (*6*). Wild-type *Arabidopsis* plants pretreated with 3-OH-C10:0 (**1**) were more resistant to *P. syringae* infection, but this systemic resistance was not activated in *Arabidopsis lore*-1 mutants or when 3-OH-C14:0 (**7**) was used (Fig. 1E and fig. S5G). Solanaceous *Nicotiana benthamiana* plants are insensitive to *Pseudomonas* lipopolysaccharide preparations but gain responsiveness upon transient expression of a LORE–green fluorescent protein (GFP) fusion (*5*). mc-3-OH-FAs (but not lc-3-OH-FAs) also induced ROS production in *N. benthamiana* leaves expressing LORE-GFP but not leaves expressing a kinase-inactive control (Fig. 1F and fig. S5H). LORE ectodomain expressed and purified from insect cells directly binds 3-OH-C10:0 (**1**) in vitro. Under these conditions, the observed binding affinity was relatively low, however (Fig. 1G and fig. S6). Taken together, this supports that LORE is a receptor for mc-3-OH-FAs.

Next, we tested whether a 3-hydroxyl and/or a carboxyl group are molecular determinants for activating LORE-mediated immunity (Fig. 2). Compared with mc-3-OH-FAs (**1** to **5**), mc-2-OH-FAs (**11** to **13**) and nonhydroxylated mc-FAs (**15** to **17**) induced only limited immune responses in *Arabidopsis* and LORE-GFP-expressing *N. benthamiana* (Fig. 2, A and B, and fig. S7, A to E). Nonhydroxylated or Δ^2-unsaturated (**19**,**20**) mc-FAs induced weak LORE-independent responses (Fig. 2, A and B, and fig. S7, A to F). The 4-OH-C10:0 (**21**) and 5-OH-C10:0 (**22**) did not elicit LORE-dependent ROS production (Fig. 2C and fig. S7G). The 3-oxodecanoic acid (3-oxo-C10:0, **23**), or its keto-enol tautomer 3-OH-decenoic acid, induced a moderate LORE-dependent $[Ca^{2+}]_{cyt}$ response (Fig. 2D and fig. S7H). Modification of the 3-hydroxyl group of 3-OH-C10:0 (**1**) with a methyl ether (**24**) or acetyl ester (**25**) or substitution with chlorine (**27**) abolished elicitor activity (Fig. 2, D and E, and fig. S7, H and J). The synthetic 3-OH-FAs used here are racemates, but *Pseudomonas* lipopolysaccharide comprises typically (*R*)-3-OH-decanoyl as primary ester-linked acyl chains (*7*) (table S1 and figs. S1 and S2). (*R*)-3-OH-C10:0 (**28**) induced stronger immune signaling than (*S*)-3-OH-C10:0 did (**29**) (Fig. 2F and fig. S8). Collectively, a free 3-hydroxyl group, preferably in the (*R*)-configuration, is critical for LORE-mediated immune sensing. 3-Decanol (**31**) or 1,3-decandiol (**32**) was inactive (Fig. 2G and fig. S9, A and B), indicating that the carboxyl function is also required for activating LORE-mediated immunity. Ester- or amide-linked moieties at the carboxyl group of 3-OH-C10:0 (**26**, **33** to **43**) gradually impaired elicitor activity with increasing hydrophobicity and size, with bulky moieties, such as *tert*-butyl (**35**) or glucosamine (**43**), rendering the 3-OH-C10:0 derivatives inactive (Fig. 2, H

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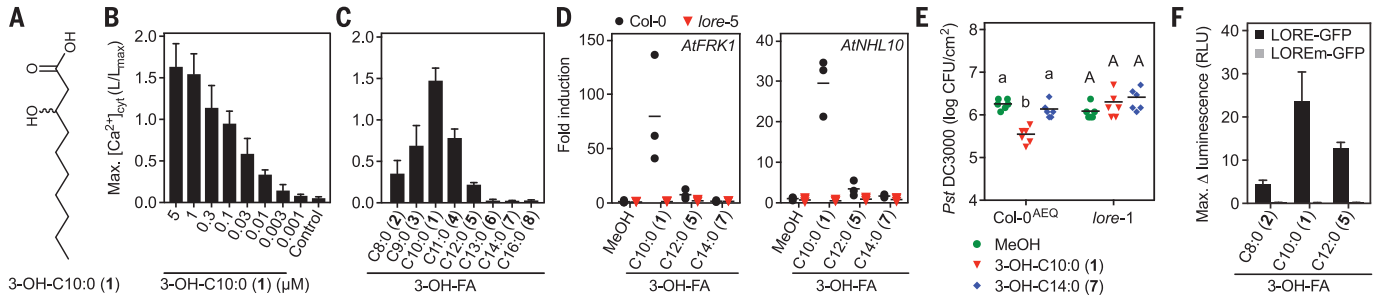

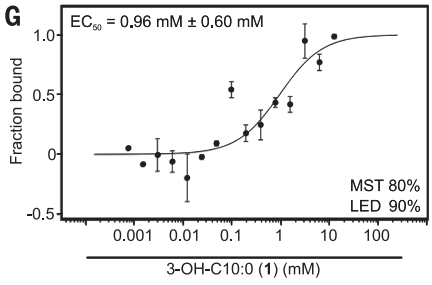

Fig. 1. The 3-OH-FAs trigger LORE-dependent immune responses in a chain length–specific manner. (**A**) Chemical structure of 3-OH-C10:0 (**1**). (**B** and **C**) Maximum (Max.) increases in $[Ca^{2+}]_{cyt}$ in *Arabidopsis* Col-0^{AEQ} seedlings treated with (B) different concentrations of 3-OH-C10:0 (**1**, stock concentration determined by quantitative nuclear magnetic resonance spectroscopy) or (C) 1 μM 3-OH-FAs of various chain length [mean ± SD, *n* = 12 (B), *n* = 3 (C)]. Kinetics are shown in fig. S5, A and B. (**D**) Defense gene expression in *Arabidopsis* seedlings 4 hours after elicitation with 3-OH-FAs (1 μM) relative to Col-0 MeOH control. Individual data (symbols) and means (bars) of three experiments are shown. (**E**) *Pst* DC3000 titer 4 days after infection in *Arabidopsis* plants pretreated with 10 μM 3-OH-FAs or MeOH for 3 days before infection. Individual data (symbols) and means (bars) of two experiments are shown (*n* = 6). Different letters indicate significant differences [one-way analysis of variance (ANOVA) with Tukey's post hoc test applied separately to Col-0^{AEQ} (small letters) and *lore*-1 (capitals), $P < 0.01$]. Similar results occur with 2 days of pretreatment (fig. S5G). CFU, colony-forming units. (**F**) Maximum ROS accumulation in leaf disks from *N. benthamiana* transiently expressing LORE-GFP or kinase-inactive LOREm-GFP elicited with 1 μM 3-OH-FAs (mean ± SE, *n* = 8). LOREm, kinase-inactive LORE variant (Lys^{516} mutated to Ala); RLU, relative light units. (**G**) Quantification of binding between 3-OH-C10:0 (**1**) and fluorescently His-tag-labeled LORE ectodomain by microscale thermophoresis (MST). Measurements were performed at a light-emitting diode (LED) power of 90% and MST power of 80%. Data points indicate the fraction of labeled LORE ectodomain bound to 3-OH-C10:0 (**1**) (mean ± SE, *n* = 4). EC_{50}, half-maximal effective concentration. Full figures including controls and additional data with alternatively labeled LORE ectodomain are provided in fig. S6. Experiments were repeated two (F and G), three (B), or four times (C) with similar results.

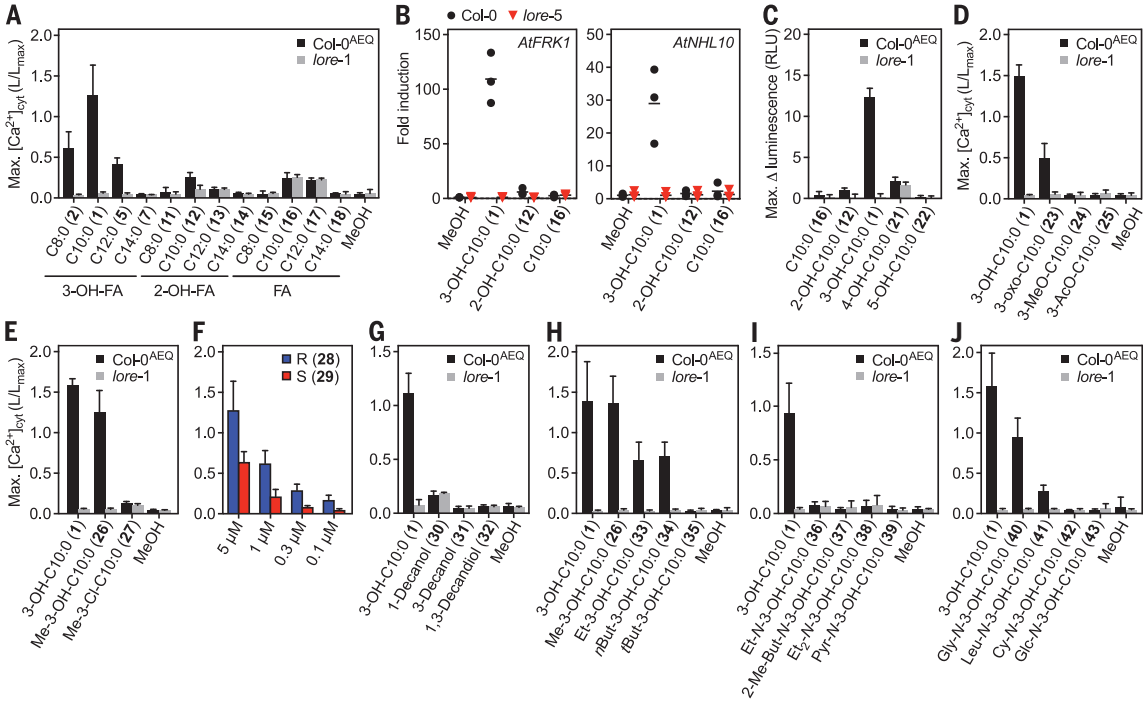

Fig. 2. The 3-OH-FAs in their free form show the strongest elicitor activity in *Arabidopsis*. (**A**) Maximum increases in $[Ca^{2+}]_{cyt}$ in *Arabidopsis* seedlings treated with FAs (5 μM) or MeOH (mean ± SD, *n* = 6). (**B**) Defense gene expression in *Arabidopsis* seedlings 4 hours after elicitation with FAs (1 μM) relative to Col-0 MeOH control. Individual data (symbols) and means (bars) of three experiments are shown. (**C**) Maximum ROS accumulation in *Arabidopsis* leaf disks treated with the indicated compounds (5 μM; mean ± SE, *n* = 8). (**D**, **E**, and **G** to **J**) Maximum increases in $[Ca^{2+}]_{cyt}$ in *Arabidopsis* seedlings treated with the indicated compounds (5 μM) or MeOH [mean ± SD, *n* = 6 (D and G to J), *n* = 3 (E)]. (**F**) Maximum increases in $[Ca^{2+}]_{cyt}$ in *Arabidopsis* seedlings treated with different concentrations of (*R*)-3-OH-C10:0 (**28**) or (*S*)-3-OH-C10:0 (**29**) (mean ± SD, *n* = 6). (A, C to J) Kinetics are shown in figs. S7 to S9. Experiments were repeated two (A, C, and G to J), three (D and E), or four times (F) with similar results.

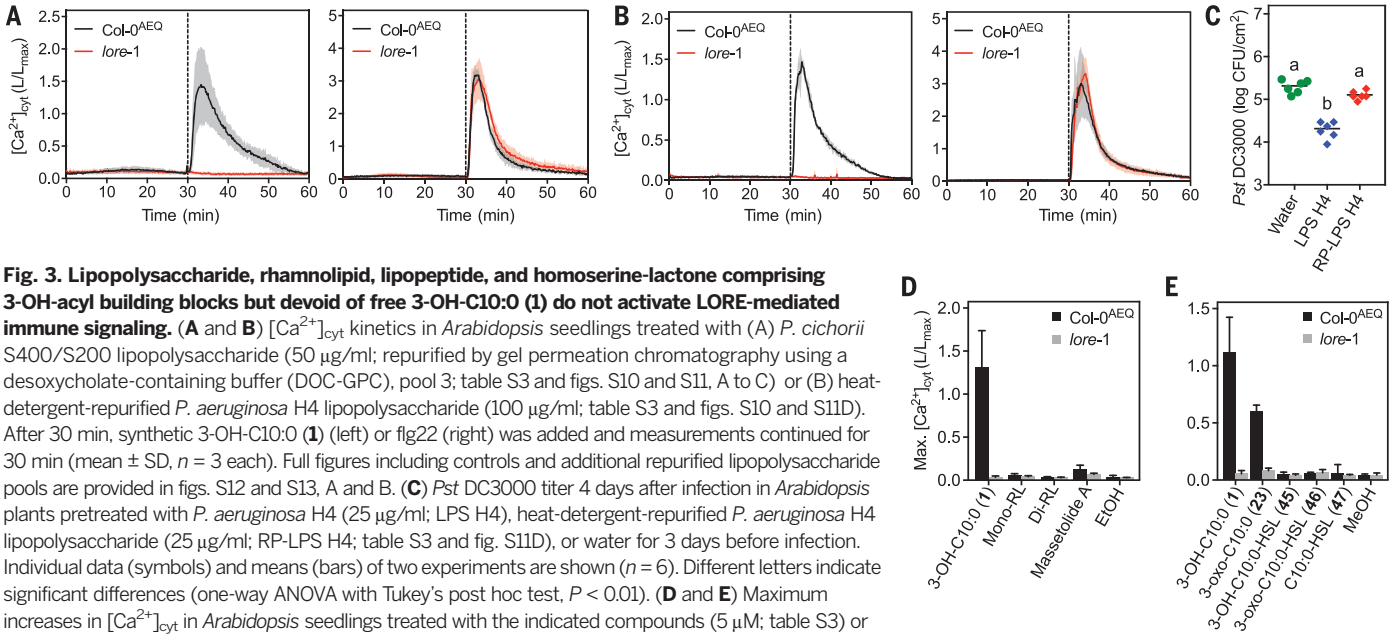

Fig. 3. Lipopolysaccharide, rhamnolipid, lipopeptide, and homoserine-lactone comprising 3-OH-acyl building blocks but devoid of free 3-OH-C10:0 (1) do not activate LORE-mediated immune signaling. (**A** and **B**) $[Ca^{2+}]_{cyt}$ kinetics in *Arabidopsis* seedlings treated with (A) *P. cichorii* S400/S200 lipopolysaccharide (50 μg/ml; repurified by gel permeation chromatography using a desoxycholate-containing buffer (DOC-GPC), pool 3; table S3 and figs. S10 and S11, A to C) or (B) heat-detergent-repurified *P. aeruginosa* H4 lipopolysaccharide (100 μg/ml; table S3 and figs. S10 and S11D). After 30 min, synthetic 3-OH-C10:0 (**1**) (left) or flg22 (right) was added and measurements continued for 30 min (mean ± SD, $n = 3$ each). Full figures including controls and additional repurified lipopolysaccharide pools are provided in figs. S12 and S13, A and B. (**C**) *Pst* DC3000 titer 4 days after infection in *Arabidopsis* plants pretreated with *P. aeruginosa* H4 (25 μg/ml; LPS H4), heat-detergent-repurified *P. aeruginosa* H4 lipopolysaccharide (25 μg/ml; RP-LPS H4; table S3 and fig. S11D), or water for 3 days before infection. Individual data (symbols) and means (bars) of two experiments are shown ($n = 6$). Different letters indicate significant differences (one-way ANOVA with Tukey's post hoc test, $P < 0.01$). (**D** and **E**) Maximum increases in $[Ca^{2+}]_{cyt}$ in *Arabidopsis* seedlings treated with the indicated compounds (5 μM; table S3) or solvent control (mean ± SD, $n = 6$). Kinetics are shown in fig. S14. EtOH, ethanol; HSL, homoserine-lactone; RL, rhamnolipid. Experiments were repeated two (D and E) or three times (A and B) with similar results.

to J, and fig. S9, B to E). Thus, mc-3-OH-FAs are the minimal motif for LORE-mediated immune activation, with free (*R*)-3-OH-C10:0 (**28**) showing the strongest activity in *Arabidopsis*.

The latter indicates that free mc-3-OH-FAs are sensed by *Arabidopsis* rather than lipid A–bound mc-3-OH-acyl chains. Indeed, unbound 3-OH-C10:0 (**1**) was detected and quantified by means of stable isotope dilution analysis combined with ultrahigh-performance liquid chromatography–mass spectrometry (SIDA-UHPLC-MS/MS) in all lipopolysaccharide and lipid A preparations that activated LORE-dependent immunity (table S3 and fig. S10). As this includes samples from different sources, including commercial suppliers, or isolated by different multistep procedures (table S3), mc-3-OH-FAs appear to copurify with lipopolysaccharide and lipid A. Detergent-based repurification depleted unbound mc-3-OH-FAs from lipopolysaccharide samples (table S3 and figs. S10 and S11). mc-3-OH-FA–depleted lipopolysaccharide samples did not activate immune signaling, whereas typical responses were induced by subsequent application of synthetic 3-OH-C10:0 (**1**) or the peptide MAMP flg22 (*8*) (Fig. 3, A and B, and figs. S12 and S13). mc-3-OH-FA–depleted lipopolysaccharide samples also did not induce systemic resistance to *P. syringae* infection in *Arabidopsis* (Fig. 3C). This hints at a separate source of free, elicitor-active mc-3-OH-FAs in bacteria rather than rapid release of mc-3-OH-FAs from lipopolysaccharide in the plant apoplast.

Several bacterial compounds, such as polyhydroxyalkanoates (*9*), rhamnolipids (*10*), lipopeptides (*11*), and *N*-acyl-homoserine-lactones (*12*), comprise 3-OH-acyl moieties, in *Pseudomonas* commonly 3-OH-decanoyl moieties (*11*, *12*). The 3-OH-decanoyl-comprising rhamnolipids (*13*) or synthetic 3-OH-C10:0- (**45**) and 3-oxo-C10:0-homoserine-lactones (**46**) did not activate LORE-mediated immune signaling in *Arabidopsis* (Fig. 3, D and E, and fig. S14). Massetolide A, a 3-OH-decanoyl-containing lipopeptide (*14*), triggered LORE-independent ROS production and a weak LORE-dependent increase in $[Ca^{2+}]_{cyt}$ (Fig. 3D and fig. S14, A and B), most likely caused by the 3-OH-C10:0 (**1**) contamination detected in this sample (table S3 and fig. S10). Taken together, this suggests that the receptor kinase LORE senses mc-3-OH-FAs in their free form but not 3-OH-decanoyl-comprising bacterial compounds.

Pseudomonas releases free (*R*)-3-OH-C10:0 (**28**) during synthesis of penta-acylated lipid A through PagL-catalyzed (*R*)-3-OH-C10:0 removal from hexa-acylated lipid A in the outer membrane (*15*, *16*). However, lipopolysaccharide preparations from *Pseudomonas* Δ*pagL* mutants still contain free 3-OH-C10:0 (**1**) and activate LORE-mediated immunity (tables S1 and S3 and figs. S3 and S4, F and G), indicating additional sources of free mc-3-OH-FAs in bacteria. Acyl carrier protein (ACP)– and coenzyme A (CoA)–bound mc-3-OH-FAs are intermediates of fatty acid metabolism. Free mc-3-OH-FAs may be released from ACP and CoA precursors through thioesterase activities (*17*) or nonenzymatically upon cell lysis in bacteria as well as in plants. In crucifers, mc-3-OH-FAs released upon tissue damage could function as DAMPs. Outer membrane vesicles, constantly released by bacteria (*18*), may deliver membrane-located or intracellular mc-3-OH-FAs. Firmicutes (*19*), honey bees (*20*), and leafcutter ants (*21*) also produce free mc-3-OH-FAs. Thus, mc-3-OH-FAs could be delivered through multiple metabolic sources. We propose that plants evolved to sense a ubiquitous low-complexity metabolite instead of different, complex microbial end products.

Humans, animals, and fungi sense metabolites, including various hydroxy fatty acids, through G protein–coupled receptors (*22*–*24*). The proinflammatory receptor GPR84 expressed in leukocytes senses mc-FAs and their 2- or 3-hydroxylated forms (*22*, *25*). GPR84 may sense mc-FAs and mc-2/3-OH-FAs of pathogenic or commensal bacteria in addition to endogenous metabolites. Therefore, our finding that lipopolysaccharide, rhamnolipid, and lipopeptide preparations from certain bacterial species may contain free mc-3-OH-FAs (table S3) is relevant to immune studies in humans and mammals, as complete removal of highly elicitor-active compounds from such biological samples is difficult (*26*, *27*). In conclusion, both plants and animals may monitor their microbial environments by sensing low-complexity microbial metabolites through cell-surface receptors (*22*, *23*, *28*).

REFERENCES AND NOTES

1. S. Ranf, *Curr. Opin. Plant Biol.* **38**, 68–77 (2017).
2. C. Alexander, E. T. Rietschel, *J. Endotoxin Res.* **7**, 167–202 (2001).
3. K. J. Kieser, J. C. Kagan, *Nat. Rev. Immunol.* **17**, 376–390 (2017).
4. S. Ranf, *PLOS Pathog.* **12**, e1005596 (2016).
5. S. Ranf *et al.*, *Nat. Immunol.* **16**, 426–433 (2015).
6. T. E. Mishina, J. Zeier, *Plant J.* **50**, 500–513 (2007).
7. U. Zähringer, B. Lindner, E. T. Rietschel, *Adv. Carbohydr. Chem. Biochem.* **50**, 211–276 (1994).
8. G. Felix, J. D. Duran, S. Volko, T. Boller, *Plant J.* **18**, 265–276 (1999).
9. R. A. Verlinden, D. J. Hill, M. A. Kenward, C. D. Williams, I. Radecka, *J. Appl. Microbiol.* **102**, 1437–1449 (2007).
10. A. M. Abdel-Mawgoud, F. Lépine, E. Déziel, *Appl. Microbiol. Biotechnol.* **86**, 1323–1336 (2010).

11. J. M. Raaijmakers, I. de Bruijn, M. J. D. de Kock, *Mol. Plant Microbe Interact.* **19**, 699–710 (2006).
12. J. M. Raaijmakers, I. de Bruijn, O. Nybroe, M. Ongena, *FEMS Microbiol. Rev.* **34**, 1037–1062 (2010).
13. A.-L. Varnier *et al.*, *Plant Cell Environ.* **32**, 178–193 (2009).
14. J. T. de Souza, M. de Boer, P. de Waard, T. A. van Beek, J. M. Raaijmakers, *Appl. Environ. Microbiol.* **69**, 7161–7172 (2003).
15. R. K. Ernst *et al.*, *J. Bacteriol.* **188**, 191–201 (2006).
16. J. Geurtsen, L. Steeghs, J. T. Hove, P. van der Ley, J. Tommassen, *J. Biol. Chem.* **280**, 8248–8259 (2005).
17. Z. Zheng *et al.*, *Appl. Environ. Microbiol.* **70**, 3807–3813 (2004).
18. L. Katsir, O. Bahar, *PLOS Pathog.* **13**, e1006306 (2017).
19. J. Sjögren, J. Magnusson, A. Broberg, J. Schnürer, L. Kenne, *Appl. Environ. Microbiol.* **69**, 7554–7557 (2003).
20. N. Weaver, N. C. Johnston, R. Benjamin, J. H. Law, *Lipids* **3**, 535–538 (1968).
21. H. Schildknecht, K. Koob, *Angew. Chem. Int. Ed. Engl.* **10**, 124–125 (1971).
22. C. C. Blad, C. Tang, S. Offermanns, *Nat. Rev. Drug Discov.* **11**, 603–619 (2012).
23. C. D. Hanlon, D. J. Andrew, *J. Cell Sci.* **128**, 3533–3542 (2015).
24. C. Braunsdorf, D. Mailänder-Sánchez, M. Schaller, *Cell. Microbiol.* **18**, 1188–1200 (2016).
25. M. Suzuki *et al.*, *J. Biol. Chem.* **288**, 10684–10691 (2013).
26. M. Hirschfeld, Y. Ma, J. H. Weis, S. N. Vogel, J. J. Weis, *J. Immunol.* **165**, 618–622 (2000).
27. S. Ranf, D. Scheel, J. Lee, *Mol. Plant Pathol.* **17**, 1165–1169 (2016).
28. F. Boutrot, C. Zipfel, *Annu. Rev. Phytopathol.* **55**, 257–286 (2017).

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/364/6436/178/suppl/DC1
Materials and Methods
Figs. S1 to S14
Tables S1 to S3
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Bacterial medium-chain 3-hydroxy fatty acid metabolites trigger immunity in *Arabidopsis* plants

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A fatty acid triggers immune responses

Plants and animals respond to the microbial communities around them, whether in antagonistic or mutualistic ways. Some of these interactions are mediated by lipopolysaccharide—a large, complex, and irregular molecule on the surface of most Gram-negative bacteria. Studying the small mustard plant *Arabidopsis*, Kutschera *et al.* identified a 3-hydroxydecanoyl chain as the structural element sensed by the plant's lectin receptor kinase. Indeed, synthetic 3-hydroxydecanoic acid alone was sufficient to produce a response. A small microbial metabolite may thus suffice to trigger immune responses.

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