Novel *Xanthomonas campestris* Long-Chain-Specific 3-Oxoacyl-Acyl Carrier Protein Reductase Involved in Diffusible Signal Factor Synthesis

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**ABSTRACT** The precursors of the diffusible signal factor (DSF) family signals of *Xanthomonas campestris* pv. *campestris* are 3-hydroxyacyl-acyl carrier protein (3-hydroxyacyl-ACP) thioesters having acyl chains of 12 to 13 carbon atoms produced by the fatty acid biosynthetic pathway. We report a novel 3-oxoacyl-ACP reductase encoded by the *X. campestris* pv. *campestris* XCC0416 gene (*fabG2*), which is unable to participate in the initial steps of fatty acyl synthesis. This was shown by the failure of FabG2 expression to allow growth at the nonpermissive temperature of an *Escherichia coli* temperature-sensitive strain. However, when transformed into the *E. coli* strain together with a plasmid bearing the *Vibrio harveyi* acyl-ACP synthetase gene (*aasS*), growth proceeded, but only when the medium contained octanoic acid. In vitro assays showed that FabG2 catalyzes the reduction of long-chain (≥C11) 3-oxoacyl-ACPs to 3-hydroxyacyl-ACPs but is only weakly active with shorter-chain (C4, C6) substrates. FabG1, the housekeeping 3-oxoacyl-ACP reductase encoded within the fatty acid synthesis gene cluster, could be deleted in a strain that overexpressed *fabG2* but only in octanoic acid-supplemented media. Growth of the *X. campestris* pv. *campestris* Δ*fabG1* strain overexpressing *fabG2* required *fabH* for growth with octanoic acid, indicating that octanoyl coenzyme A is elongated by *X. campestris* pv. *campestris* fabH. Deletion of *fabG2* reduced DSF family signal production, whereas overproduction of either FabG1 or FabG2 in the Δ*fabG2* strain restored DSF family signal levels.

**IMPORTANCE** Quorum sensing mediated by DSF signaling molecules regulates pathogenesis in several different phytopathogenic bacteria, including *Xanthomonas campestris* pv. *campestris*. DSF signaling also plays a key role in infection by the human pathogen *Burkholderia cepacia*. The acyl chains of the DSF molecules are diverted and remodeled from a key intermediate of the fatty acid synthesis pathway. We report a *Xanthomonas campestris* pv. *campestris* fatty acid synthesis enzyme, FabG2, of novel specificity that seems tailored to provide DSF signaling molecule precursors.

**KEYWORDS** *Xanthomonas*, fatty acids, quorum sensing

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The phytopathogenic bacterium *Xanthomonas campestris* pv. *campestris* is the causal agent of black rot, which is probably the most important disease of cruciferous plants worldwide (1–3). Upon infection of the host plant, *X. campestris* pv. *campestris* produces a range of extracellular enzymes which collectively play essential roles in pathogenesis (3). The production of these factors is regulated by quorum-sensing (QS)
mechanisms (Fig. 1A) mediated by the diffusible signal factor (DSF) family of fatty acids (1, 2). The first *X. campestris* pv. *campestris* DSF signal characterized was cis-11-methyl-2-dodecenoic acid (11-Me-C12:Δ2) (4, 5). Other DSF family signals have since been identified in *X. campestris* pv. *campestris*, including cis-2-dodecenoic acid (C12:Δ2; BDSF), cis-11-methyldeca-2,5-dienoic acid (11-Me-C12:Δ2,5; CDSF), and cis-10-methyl-2-dodecenoic acid (10-Me-C12:Δ2; IDSF) (6, 7) (Fig. 1B).

In *X. campestris* pv. *campestris*, a cluster of genes designated *rpfABCDEFG* (*rpf* stands for regulation of pathogenicity factors) is involved in the biosynthesis, perception, transduction, and turnover of DSF family signaling molecules (2, 8, 9). The synthesis of DSF family signaling molecules is dependent on RpfF, an enoyl-acyl carrier protein (enoyl-ACP) hydratase/thioesterase. RpfF is a bifunctional enzyme that not only catalyzes the dehydration of 3-hydroxyacyl-ACPs to cis-2-enoyl-ACPs but also cleaves the acyl-ACP thioester bonds to produce free fatty acids (6, 10) (Fig. 1A). The complex pathway that regulates pathogenicity is beyond the scope of this report, and thus readers are referred to recent reviews (1, 2).

Bacteria utilize primarily a disassociated fatty acid synthase system for de novo production of fatty acids (11, 12). The flexible nature of this system allows the diversion of intermediates to other end products, including lipid A (13, 14), quorum-sensing signal molecules (15, 16), and vitamin cofactors (17, 18). The *X. campestris* pv. *campestris* genome contains all of the genes known to be required for fatty acid synthesis, although the synthesis mechanism has received little study. The precursors of the DSF family signals are 12- or 13-carbon 3-hydroxyacyl-ACP molecules (6, 10) derived by 3-oxoacyl-ACP reductase (OAR)-catalyzed reduction of 3-oxoacyl-ACPs (Fig. 1B). Overexpression of FabG1 led to a significant increase in the production of DSF family signals (6).

The *X. campestris* pv. *campestris* genome carries four putative OAR genes: *fabG1* (XCC1018), *fabG2* (XCC0416), *fabG3* (XCC4003), and *fabG4* (XCC0384) (Fig. 1C). XCC1018 (fabG7) is located within a cluster of fatty acid synthesis genes, and 69.1% of the residues of the FabG1 protein are identical to those of *Escherichia coli* FabG. The active-site triad (Ser, Tyr, and Lys) and the N-terminal cofactor-binding sequence

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**FIG 1** DSF family signaling cascade and 3-oxoacyl-ACP reductase candidates. (A) Schematic model of the DSF family signaling cascade. DSF quorum sensing involves a complex series of protein-protein and protein-ligand interactions that are described in the introduction. (B) Production of precursors of DSF family signals and synthesis of DSF family signals. FabF/B, long-chain 3-oxoacyl-ACP synthases; OAR, 3-oxoacyl-ACP reductase; RpfF, DSF family synthase. (C) The cofactor-binding sequence (Gly motif [GlyXXXGlyXGly]) is overlined. Catalytic triad residues (Ser-Tyr-Lys) are marked by asterisks. Alignment was constructed with Clustal W based on identical residues. OM, outer membrane; IM, inner membrane; EC, E. coli.
defined by the *E. coli* FabG X-ray crystal structures are conserved in *X. campestris* pv. *campestris* FabG1 (19, 20) (Fig. 1C). Therefore, given these motifs, together with its genome location, FabG1 was considered to play the major role in the reduction of 3-oxoacyl-ACPs for the synthesis of the phospholipid fatty acyl chains. In contrast, FabG3 seems involved in the biosynthesis of xanthomonadin polyketides (21). XCC0384 (fabG4) is located in a putative biotin synthesis operon but contains neither the conserved catalytically active triad nor the N-terminal cofactor-binding sequence and thus seems unlikely to have OAR activity (Fig. 1C).

The remaining OAR candidate, FabG2, is encoded by a lone gene located far from the above-mentioned genes. Although alignments showed that FabG2 is only 32.4% identical to *E. coli* FabG, it contains the typical active-site triad and the N-terminal cofactor-binding sequence (Fig. 1C). Based on these data, it was reasonable to hypothesize that fabG2 encodes a functional 3-oxoacyl-ACP reductase. However, given that of FabG1, the role of FabG2 seemed unlikely to be involved in bulk fatty acid synthesis. A possible FabG2 role is DSF synthesis. We report that FabG2 is a novel OAR that specifically reduces long-chain substrates.

**RESULTS**

**fabG2 encodes an OAR of novel specificity.** To determine whether FabG2 has OAR activity, we transformed the *E. coli* fabG(Ts) strain CL104 with a plasmid that expressed FabG2 under arabinose control and assayed growth at 42°C. Strain CL104 lacks 3-oxoacyl-ACP reductase activity at 42°C and is unable to grow at that temperature (22). As a control, we similarly expressed the housekeeping OAR, FabG1, with the expectation that it would allow growth of strain CL104 at 42°C, and that was the case (Fig. 2A).

In contrast, strain CL104 expressing FabG2 failed to grow at 42°C either in the presence or the absence of arabinose induction (Fig. 2A), and thus FabG2 seemed to lack OAR activity. However, because overexpression of FabG2 in the wild-type *X. campestris* pv. *campestris* strain Xc1 increased DSF production (see below), it seemed that FabG2 might specifically reduce 3-oxoacyl-ACPs to provide substrates for DSF synthesis and be unable to reduce short-chain 3-oxoacyl-ACPs. If so, when provided with a sufficiently long 3-oxoacyl-ACP substrate, FabG2 should functionally replace *E. coli* FabG. This hypothesis was tested by expressing both FabG2 and the AasS acyl-ACP synthetase (23) in *E. coli* CL104 and testing for growth at 42°C on plates containing octanoic acid. In this scenario, AasS converted exogenous octanoic acid to octanoyl-ACP, which was elongated to 3-oxodecanoyl-ACP. FabG2 then reduced this product to 3-hydroxydecanoyl-ACP, as described above. Fatty acid synthesis was thus primed, and growth at 42°C proceeded. Both enzymes were required (Fig. 2B).

In a second approach, we expressed both FabG2 and *X. campestris* pv. *campestris* FabH in *E. coli* CL104 and tested growth in the presence or absence of octanoic acid at 42°C. In this second scenario, *X. campestris* pv. *campestris* FabH condensed octanoyl coenzyme A (octanoyl-CoA) with malonyl-ACP to produce 3-oxodecanoyl-ACP (24), which FabG2 reduced to 3-hydroxydecanoyl-ACP, as described above. Fatty acid synthesis was thus primed, and growth at 42°C proceeded. Both enzymes were required (Fig. 2B). These results argued strongly that FabG2 is a 3-oxoacyl-ACP reductase that specifically reduces long-chain substrates.

To test whether a specific fatty acid chain length was required to support growth, plates supplemented with 100 μg/ml of straight-chain saturated fatty acids with chain lengths of C₄ to C₁₆ were tested (Fig. 2B and C). Only the C₆ and C₈ fatty acids supported the growth of derivatives of strain CL104 that expressed FabG2 plus either AasS or *X. campestris* pv. *campestris* FabH (Fig. 2B and C). The failure of butyric acid to support growth can be attributed to the inability of AasS to use this substrate (16) and/or the weak activity of FabG2 with 3-oxohexanoyl-ACP observed *in vitro* (see below). Longer fatty acids (>C₈) failed to support the growth of >C₈ acids because they feed into the pathway past the branch point for unsaturated fatty acid synthesis (see below).
FabG2 preferentially reduces long-chain 3-oxoacyl-ACPs in vitro. Recombinant hexahistidine-tagged FabG2 was expressed in *E. coli* and purified to homogeneity (see Materials and Method). Purified FabG2 had the size expected from the sequence of the tagged protein (26.4 kDa) (see Fig. S1A in the supplemental material). Size exclusion chromatography indicated that FabG2 is a multimer (trimer or tetramer) in solution (Fig. S1B).

**FIG 2** Complementation of the *E. coli fabG*(Ts) strain CL104 by expression of *X. campestris pv. campestris* enzymes. (A) Growth of *E. coli fabG*(Ts) strain CL104 containing plasmids that express various FabG proteins at 42°C. The FabG1, FabG2, and *E. coli* FabG proteins were expressed from plasmids pHZ003, pHZ004, and pTWH21, respectively, which were derived from using the compatible vectors pBAD24M and pBAD33. To allow entry of exogenous fatty acids into the *E. coli* fatty acid synthesis pathway, a plasmid encoding either AasS (pYF186) or *X. campestris pv. campestris* FabH (pYHY56) was used to obtain the results shown in panel B or C, respectively. "Vectors" denotes the empty vectors. No Ara, without arabinose induction; Plus Ara, arabinose induction. The medium was RB agar. (B) Growth at 42°C of *E. coli* strain CL104 carrying the plasmids expressing FabG2 or *E. coli fabG* in the presence or absence of AasS expression on RB plates supplemented with various fatty acids (see Materials and Methods). No FA, no fatty acid supplementation; C4, butyric acid supplementation; C6, hexanoic acid supplementation; C8, octanoic acid supplementation; C10, decanoic acid supplementation; C12, dodecanoic acid supplementation; C14, tetradecanoic acid supplementation; C16, hexadecanoic acid supplementation. The lack of growth on >C8 fatty acids is because their chain lengths are past the C8 to C10 branch points for synthesis of the unsaturated fatty acids required for membrane function. (C) Growth of *E. coli* strain CL104 containing a plasmid expressing FabG2 or *E. coli FabG* as in panel B, except that the expressed octanoate entry enzyme was *X. campestris pv. campestris* FabH in place of AasS. Fatty acid supplementation was as described for panel B.
To study FabG2, the initiation reactions of fatty acid synthesis were reconstructed from the purified *E. coli* proteins FabD, FabA, FabI, and FabH plus an *X. campestris* pv. *campestris* OAR (either FabG1 or FabG2). The reaction products were analyzed by conformationally sensitive gel electrophoresis (Fig. 3A). Use of the complete fatty acid synthesis cycle avoided unstable intermediates and reversible reactions. FabD converted malonyl-CoA to malonyl-ACP, and FabH reacted with acetyl-CoA to produce 3-oxobutyryl-ACP. The OAR reduced 3-oxobutyryl-ACP to 3-hydrobutyryl-ACP. Dehydration by FabA gave trans-2-crotonyl-ACP, which FabI reduced to butyryl-ACP, a stable product. As expected, FabG1 addition resulted in robust butyryl-ACP synthesis (Fig. 3A, lane 2), whereas FabG2 gave only trace amounts of butyryl-ACP (Fig. 3A, lane 3). Thus, FabG2 only very weakly reduces 3-oxobutyryl-ACP, consistent with its inability to support the growth of *E. coli* CL104 at 42°C (Fig. 2).

The successful octanoic acid supplementation argued that FabG2 was an OAR active with medium-chain-length substrates (Fig. 3B). To test this *in vitro*, we incubated *E. coli* FabB with malonyl-ACP and octanoyl-ACP or dodecanoyl-ACP to give 3-oxodecanoyl-ACP or 3-oxotetradecanoyl-ACP, respectively. Addition of either FabG1or FabG2 and FabA to these reaction mixtures gave a mixture of 3-hydroxyacyl-ACP and enoyl-ACP species (Fig. 3B). These products were more definitively analyzed by mass spectrometry (MS). The mass peaks (m/z) formed with octanoyl-ACP and either FabG2 or FabG1 were similar. These were holo-ACP (mass, 8,980) and octanoyl-ACP (mass, 9,106). The 3-hydroxydecanoyl-ACP generated a new peak at a mass of 9,150, whereas FabA dehydration of 3-hydroxydecanoyl-ACP produced a mixture of trans-2- and cis-3-
Pseudomonas aeruginosa fatty acid synthesis pathway. In synthase III condenses malonyl-ACP with synthesis (chain lengths (C6 to C8) to produce longer-chain 3-oxoacyl-ACPs that prime fatty acid FabH cannot accept octanoyl-CoA (34.5 campestris strains expressing incorporate labeled octanoate into long-chain fatty acids. In contrast, tested (Fig. S4B) and both fatty acids were required. Hence, the lack of unsaturated fatty acid strain was plated with decanoic acid and oleic acid supplementation, the strain grew due to a lack of unsaturated fatty acid synthesis pathway. FabH is responsible for the entry of octanoic acid into the long-chain fatty acid synthesis pathway. This was tested by use of a E. coli FabH (214.3 ± 32.5 μM). The Kcat values for FabG1 and FabG2 were 29.0 ± 6.0 s⁻¹ and 40.9 ± 8.1 s⁻¹, respectively. Overexpression of FabG2 plus supplementation with octanoic acid allows deletion of the fabG1 gene. The physiological functions of FabG2 were tested by disruption of fabG1 and fabG2 using suicide plasmids carrying in-frame gene deletions (Fig. S2). A ΔfabG2 deletion strain was readily generated (Fig. S2C), but no fabG1 deletion strain could be isolated. Only the single-crossover integrant strain HZ1 was obtained (Fig. S2), which indicated that X. campestris pv. campestris fabG1 is essential. However, since FabG2 restored E. coli CL104 growth in the presence of exogenous octanoic acid, we plated the fabG1 single-crossover integrant (strain HZ1) on medium containing octanoic acid to allow the second crossover to give a fabG1 deletion, but this failed. Arguing that differential expression levels of the two genes might explain this failure, we measured their transcription and found that fabG1 transcription was 5- to 7-fold higher than that of fabG2 (data not shown). Given these data, we overexpressed FabG2 using the vector pSRK-Gm (25) in the single-crossover strain and selected for growth in the presence of octanoic acid, which produced the ΔfabG1 strain HZ6 (ΔfabG1/pfabG2) (Fig. S2F).

Deletion of fabG2 did not affect growth on NaCl-yeast extract-glycerol (NYG) plates, whereas the ΔfabG1/pfabG2 strain that overproduced FabG grew only when the plates contained octanoic acid (Fig. 4AB; Fig. S3A). Our finding that the ΔfabG1/pfabG2 strain (HZ6) grew when provided with octanoic acid or (less so) with hexanoic acid in the absence of AasS expression argued that X. campestris pv. campestris contained an enzyme that converted the C6 and C8 acids to the ACP thioesters required to enter the fatty acid synthesis pathway. In Pseudomonas aeruginosa PA3286a, novel 3-oxoacyl-ACP synthase III condenses malonyl-ACP with β-oxidation-derived acyl-CoAs of medium-chain lengths (C6 to C8) to produce longer-chain 3-oxoacyl-ACPs that prime fatty acid synthesis (26). Since in vitro X. campestris pv. campestris FabH uses octanoyl-CoA in place of octanoyl-ACP (24) and X. campestris pv. campestris encodes two acyl-CoA synthetases, RpfB and FadD (XCC1017), X. campestris pv. campestris may have an enzyme functionally analogous to PA3286. This was tested by use of a ΔfabG2 derivative of a strain in which E. coli FabH replaced X. campestris pv. campestris FabH (24). E. coli FabH cannot accept octanoyl-CoA (27), and hence this strain is unable to incorporate labeled octanoate into long-chain fatty acids. In contrast, X. campestris pv. campestris strains expressing X. campestris pv. campestris FabG2 as the sole FabG elongated [1-14C]octanoic acid, but not [1-14C]acetate, whereas the X. campestris pv. campestris ΔfabH strain expressing E. coli FabH elongated only [1-14C]acetate. The wild-type strain Xc1 elongated both precursors (Fig. 5). Hence, X. campestris pv. campestris FabH is responsible for the entry of octanoic acid into the long-chain fatty acid synthesis pathway.

Growth of the ΔfabG1/pfabG2 strain on plates supplemented with other fatty acids (Fig. 4B) was also tested. As seen when E. coli CL104 complemented with FabG2 was tested (Fig. 2), fatty acids (>C8) were unable to support growth. Presumably, this was due to a lack of unsaturated fatty acid synthesis. Indeed, when the ΔfabG1/pfabG2 strain was plated with decanoic acid and oleic acid supplementation, the strain grew (Fig. S4B) and both fatty acids were required. Hence, the lack of unsaturated fatty acid
synthesis is indeed responsible for the inability of fatty acids (>C₈) to support the growth of the Δ fabG1/p fabG2 strain.

To test whether FabG2 has long-chain 3-oxoacyl-ACP reductase activity in its native bacterium, we analyzed the fatty acid compositions of strain HZ6 (Δ fabG1/p fabG2) and

![Diagram](image-url)

**FIG 4** Growth of *X. campestris* pv. *campestris* Δ fabG strains on supplemented or unsupplemented NYG plates. (A) Growth of Δ fabG strains. WT, wild-type *X. campestris* pv. *campestris* strain Xc1; Δ fabG1 + p fabG2, pH2009 strain. (B) Complementation of the Δ fabG1 strain by plasmid-borne fabG2 in the presence of various fatty acid supplements. Designations: WT, wild-type strain Xc1; Δ fabG1 + p fabG2, Δ fabG1 mutant strain carrying fabG2 plasmid pH2009. Fatty acid designations are as described for Fig. 2.

![Diagram](image-url)

**FIG 5** Incorporation of [1-14C]octanoate or [1-14C]acetate into methyl esters derived from the phospholipid fatty acids of *X. campestris* pv. *campestris* or *E. coli* strains (see Materials and Methods). (A to C) Autoradiograms of argentation thin-layer chromatographic analyses of *X. campestris* pv. *campestris* strains (A and B) and *E. coli* strains (C). The labeled precursor is given above the autoradiograms. (A and B) Lane 1, wild-type strain Xc1; lane 2, Δ fabG1 strain expressing FabG2 from pH2009; lane 3, Δ fabG2 strain HZ3; lane 4, Δ fabG2 strain expressing FabG2 (strain HZ4); lane 5, *X. campestris* pv. *campestris* Δ fabH complemented with *E. coli* fabH (strain T-3). (C) *E. coli* fabG(Ts) strain CL104 derivatives were labeled at 42°C. Lane 1, plasmid pTWH21 encoding *E. coli* FabG plus the vector pBAD33; lane 2, vectors pBAD24M and pBAD33; lane 3, plasmid pFFJ86 expressing *V. harveyi* AasS plus the vector pBAD24M; lane 4, plasmid pH2004 expressing FabG2 plus the vector pBAD33; lane 5, plasmids pH2004 and pYFJ86 expressing FabG2 and AasS, respectively; lane 6, plasmid pYYH56 expressing *X. campestris* pv. *campestris* FabH plus the vector pBAD24M; lane 7, plasmids expressing FabG2 and *X. campestris* pv. *campestris* fabH (pH2004 and pYYH56, respectively). SFA, saturated fatty acid; UFA, unsaturated fatty acid.
wild-type strain Xc1 grown in NYG liquid medium containing octanoic acid by gas chromatography (GC)-MS (Table S3). The species of fatty acids produced by strain HZ6 (ΔfabG1/pfabG2) were essentially the same as those produced by the wild-type strain Xc1 grown in NYG liquid medium. Deletion of fabG2 resulted in reduced production of DSF family signaling molecules. 3-Hydroxyacyl-ACPs of 12 or 13 carbon atoms are the precursors of the DSF family signals (Fig. 1) (6, 10). To determine whether FabG2 preferentially produces such substrates, we assayed the production of DSF family signals in the ΔfabG2 mutant strain grown to stationary phase in NYG medium using high-performance lipid chromatography. The production of both DSF and BDSF by the ΔfabG2 mutant strain (HZ3) was <50% of the production of wild-type strain Xc1 (Fig. 6A). Upon complementation with a plasmid expressing wild-type FabG2, the ΔfabG2 strain increased its production of both DSF family signals (Fig. 6A), implying that FabG2 is involved in DSF family signal production. However, complementation with a plasmid overexpressing FabG1 also restored DSF family signal production to the ΔfabG2 strain to levels similar to those produced by the strain overexpressing FabG2 (Fig. 6A). Hence, although FabG2 has a significant role in DSF family signal synthesis, it is not the sole source of 3-hydroxydodecanoyl-ACPs. Indeed, overexpression of each FabG in wild-type strain Xc1 gave DSF family signals levels 50% higher than the Xc1 levels (Fig. 6B). Hence, the level of 3-oxoacyl-ACP reductase activity rather than of the specific OAR is the important parameter in the production of DSF family signals.

DISCUSSION

In the present study, we identified FabG2 as a novel OAR that specifically reduces long-chain 3-oxoacyl-ACPs to 3-hydroxyacyl-ACPs. Unlike FabG1, FabG2 cannot replace
E. coli FabG in the general fatty acid synthesis pathway. However, in the presence of an enzyme that allows exogenous fatty acids to enter the fatty acid synthetic pathway (either AasS or X. campestris pv. campestris FabH) and exogenous octanoic acid, growth was allowed at the nonpermissive temperature. Moreover, in the native bacterium, fabG1 could be deleted only when FabG2 was overexpressed and the medium contained octanoic acid. These observations argued that the failure of FabG2 to perform all of the 3-oxoacyl-ACP reductions required for general fatty acid synthesis was due to the strain’s absent or weak ability to reduce the first 3-oxoacyl-ACP of the pathway, 3-oxobutyryl-ACP. Indeed, in vitro FabG2 was only weakly active with short-chain 3-oxobutyryl-ACP and 3-oxohexanoyl-ACP but readily reduced long-chain 3-oxoacyl-ACPs, with the 10-carbon substrate being the most active.

Although X. campestris pv. campestris FabG2 preferentially reduces long-chain 3-oxoacyl-ACP substrates and deletion of fabG2 decreases the ability of X. campestris pv. campestris to produce DSF family signals, FabG2 is not required for the production of DSF family signals. Indeed, overexpression of either FabG2 or FabG1 in the wild-type strain Xc1 significantly increased the production of the DSF family signaling molecules. FabG1 is the housekeeping X. campestris pv. campestris OAR and is required for normal X. campestris pv. campestris growth, although fabG1 can be deleted from the X. campestris pv. campestris genome provided that FabG2 is overexpressed in the presence of exogenous octanoic acid. Therefore, it seems that the role of FabG2 is to maintain a sufficiently high level of OAR activity for DSF family signal production.

**MATERIALS AND METHODS**

**Materials.** Moravek supplied the radioactive precursors. Sigma-Aldrich provided cis-11-methyl-2-dodecenoic acid and cyclic-di-GMP. Ni-agarose columns were from Invitrogen. Agilent Technologies provided HC-C18 high-performance liquid chromatography (HPLC) columns. All other reagents were of the highest available quality. Sangon Biotechnology Co. synthesized the oligonucleotide primers.

**Bacterial strains, plasmids, and growth conditions.** The strains, plasmids, and primers used in this study are listed in Table S1 in the supplemental material. Luria-Bertani (LB) medium was used as the rich medium for E. coli growth at 37°C. Escherichia coli fabGT(Ts) mutant strain CL104 was grown in LB medium (10 g/liter tryptone, 10 g/liter NaCl, and 1 g/liter yeast extract) (LB with one-fifth yeast extract) at 30°C (22). The X. campestris pv. campestris strains were grown in NYG medium (in grams per liter, peptone, 5; yeast extract, 3; and glycerol, 20 [pH 7.0]). Where required, antibiotics were added at the following concentrations: 100 μg/ml sodium ampicillin, 30 μg/ml kanamycin sulfate, 30 μg/ml (for E. coli) or 10 μg/ml (for X. campestris pv. campestris) gentamicin sulfate, and 50 μg/ml rifampin. L-Arabinose was used at a final concentration of 0.01%. Isopropyl-β-D-thiogalactoside (IPTG) was used at a final concentration of 1 mM. Bacterial growth in liquid medium was determined by measuring the optical density at 600 nm (OD600) using a Bioscreen-C automated growth curve analysis system (OY Growth Curves).

**Assay of FabG1 and FabG2 activities in vitro.** Malonyl-ACP was synthesized from holo-ACP and malonyl-CoA with E. coli FabD. Acyl-ACPs (C6 ACP to C14 ACP) were synthesized from fatty acids, ATP, and E. coli holo-ACP with AasS, as described previously (23). The reaction products were resolved with conformationally sensitive gel electrophoresis on 20% or 17.5% polyacrylamide gel containing a urea concentration optimized for the separation. The gel was stained with Coomassie brilliant blue R250.

To verify the products of the FabG2-catalyzed reaction, the acyl-ACP derivatives were purified from 500 μl of the above-described reaction mixture by the method of Zhao et al. (31). Their molecular masses...
were determined with matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) MS (Bruker Autoflex III) as previously described (32).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/mBio.00596-18.

FIG S1, DOCX file, 0.1 MB.
FIG S2, DOCX file, 0.1 MB.
FIG S3, DOCX file, 0.1 MB.
TABLE S1, DOCX file, 0.1 MB.
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