

# Straight-Chain Fatty Acids Are Dispensable in the Myxobacterium *Myxococcus xanthus* for Vegetative Growth and Fruiting Body Formation

Helge B. Bode,<sup>1\*</sup> Michael W. Ring,<sup>1</sup> Dale Kaiser,<sup>2</sup> Anna C. David,<sup>1</sup>  
Reiner M. Kroppenstedt,<sup>3</sup> and Gertrud Schwär<sup>1</sup>

*Institut für Pharmazeutische Biotechnologie, Universität des Saarlandes, 66041 Saarbrücken, Germany*<sup>1</sup>; *Departments of Biochemistry and Developmental Biology, Stanford University, Stanford, California 94305*<sup>2</sup>; and *Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), 38124 Braunschweig, Germany*<sup>3</sup>

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**Inactivation of the MXAN\_0853 gene blocked the production in *Myxococcus xanthus* of straight-chain fatty acids which otherwise represent 30% of total fatty acids. Despite this drastic change in the fatty acid profile, no change in phenotype could be observed, which contrasts with previous interpretations of the role of straight-chain fatty acids in the organism's development.**

Myxobacteria have very complex fatty acid (FA) profiles, with up to 40 different compounds belonging to all known classes of FAs (2, 27). The most dominant fatty acid usually belongs to the iso-branched fatty acids (iso-FAs), which contribute up to 80% of total FAs in some species. All other fatty acids are straight-chain fatty acids (scFAs), whereas anteiso-FAs are found only as trace compounds in myxobacteria. Interestingly, mono- and diunsaturated species as well as 2-OH and 3-OH derivatives of these FAs are common in myxobacteria, and iso-2-OH FAs especially have been proposed as chemotaxonomic markers to identify myxobacteria and other gliding bacteria (7). The resulting abundance of different fatty acids is unexpected because several bacteria from different environments, like *Escherichia coli*, bacilli, and actinomycetes, live with much simpler fatty acid profiles. One might argue that this mixture allows myxobacteria to maintain their membrane fluidity over a broad temperature range, but except for some thermophilic species (8), almost all myxobacteria described are mesophiles, most of which grow only in a narrow temperature range. Additionally, myxobacteria possess members of different FA families thought to fulfill the same functions (e.g., iso- and unsaturated FAs) (13). Even FAs with more than one functional group can be observed in substantial amounts.

It was postulated previously that some of these FAs play a role in the complex life cycle of myxobacteria which culminates in the formation of fruiting bodies containing heat- and desiccation-resistant myxospores (11, 22). The model organism for studying the development of and sporulation in myxobacteria is *Myxococcus xanthus*, for which different extracellular signals and regulators have already been described. These are required for *M. xanthus* to proceed through the developmental program (12). However, the underlying biochemistry is barely understood. It was shown previously by Toal et al. that disruption of the branched-chain keto acid dehydrogenase (Bkd)

complex results in a developmental phenotype with almost no aggregates or spores formed under starvation conditions (25). The Bkd complex is involved in the degradation of the branched-chain amino acids leucine, valine, and isoleucine to isovaleryl-coenzyme A (isovaleryl-CoA), isobutyryl-CoA, and 2-methylbutyryl-CoA, respectively. These thioesters are the starting units for iso- and anteiso-fatty acids and for several secondary metabolites from different bacteria (e.g., avermectin from *Streptomyces avermitilis* [6] and myxothiazol [24], myxalamides [23], and aurafuron [16] from *Stigmatella aurantiaca*). In all myxobacteria analyzed so far, iso-FAs are the dominant fatty acid family. Consequently, mutations of the *bkd* genes (also called *esg* from *E*-signal [4]) result in a dramatic decrease in the amount of iso-FAs. Interestingly, not a complete loss of iso-FAs was observed, which is due to the presence of a novel alternative pathway to isovaleryl-CoA branching from the well-known mevalonate-dependent isoprenoid biosynthesis pathway (17, 18). This pathway is unique to myxobacteria and is highly induced in *bkd* mutants. Since the wild-type fatty acid profile and the normal life cycle can be restored in the *bkd* mutant by adding isovalerate, it was speculated that an iso-FA or a corresponding lipid is involved in the development of *M. xanthus* (5, 25).

Additional work by Rosenbluh and Rosenberg (20) and Varon et al. (26) has identified unsaturated FAs and the corresponding major lipid phosphatidylethanolamine (PE) as autocides involved in cell lysis, which is required for the development of *M. xanthus*. Furthermore, Kearns et al. have recently shown that the PE consisting of the unsaturated fatty acid 16:1 $\omega$ 5c acts as a chemotaxis signal in *M. xanthus* under starvation conditions by facilitating direct movement during fruiting body formation (14).

**MXAN\_0853 encodes an acetyl-CoA-specific FabH.** To clarify the function of fatty acids during the growth and development of *M. xanthus*, we have constructed several mutants impaired in the biosynthesis of specific fatty acids. During this program we have identified three  $\beta$ -ketoacyl-acyl carrier protein synthase IIIs (FabHs) by doing a BLAST-P (1) search of the M1genome sequence (10) for *Myxococcus xanthus*

\* Corresponding author. Mailing address: Institut für Pharmazeutische Biotechnologie, Universität des Saarlandes, P.O. Box 151150, 66041 Saarbrücken, Germany. Phone: 49 681 3025494. Fax: 49 681 3025473. E-mail: h.bode@mx.uni-saarland.de.

DK1622, using FabHs from *E. coli* (NCBI accession no. AAC74175) and *Bacillus subtilis* (accession no. NP\_389015) as query sequences. FabHs are required for the initiation of FA biosynthesis, catalyzing the first elongation of the CoA thioester-activated starting unit carboxylic acid (acetyl-CoA or butyryl-CoA for scFAs or isovaleryl-CoA or isobutyryl-CoA for iso-FAs) with the first acyl carrier protein-bound malonyl extender unit (9). One of the *fabH* genes present in the genome, Mx\_1291 (1,002 bp, encoding 334 amino acids; also named MXAN\_0853 [see [www.tigr.org](http://www.tigr.org)]), was not located near other genes related to FA biosynthesis, but its product showed high similarity over the full length of the protein to FabHs from several bacteria, including *B. subtilis* (28% identity; 48% similarity), *E. coli* (30% identity; 43% similarity), and other bacteria. MXAN\_0853 in the wild-type strain DK1622 was disrupted by plasmid integration, resulting in strain DK5614. To this end, an internal 586-bp fragment of MXAN\_0853 was amplified by PCR using the primer pair 1291-1 (5'-CGGCGC TCTCAGCAACG-3') and 1291-2 (5'-TGCCCGATGAAG GAGACG-3') and cloned into plasmid pCR2.1-TOPO (Invitrogen). The resulting plasmid was purified from *E. coli* TOP10 (Invitrogen) and introduced into *M. xanthus* DK1622 by electroporation as described previously (10). Because the plasmid cannot replicate in *M. xanthus*, kanamycin-resistant electroporants result from homologous recombination. These incorporate the plasmid into the chromosome and thereby disrupt MXAN\_0853, which was verified as described previously using a PCR protocol based on a plasmid-specific and a gene-specific primer pair (10). Whole-cell FA analysis of DK1622 and DK5614, done as described previously (2, 17), showed almost a complete loss of scFAs in the mutant when the mutant was vegetatively grown in Casitone-based CTT medium (15) (Table 1). The level of scFAs decreased from 34% to 1.5% of total FAs. Interestingly, the most abundant scFA, 16:1 $\omega$ 5c, was completely lost, whereas its diunsaturated derivative, 16:2 $\omega$ 5c  $\omega$ 11c, was still produced in trace amounts. This indicates that the corresponding desaturase activities are still high in DK5614, catalyzing the consecutive insertion of two double bonds into palmitate and therefore most likely leading to complete conversion of the intermediary FA, 16:1 $\omega$ 5c (M. W. Ring, G. Schwär, and H. B. Bode, unpublished data).

Two other genes, MXAN\_0215 and MXAN\_7353 (see [www.tigr.org](http://www.tigr.org)), showed high similarity and identity to MXAN\_0853 and to FabH genes from other bacteria, but disruption of the genes did not lead to a change in phenotype in the resulting mutants. This indicates that MXAN\_0215 and MXAN\_7353 might have complementary functions with respect to starting unit specificity and that both are involved in the formation of iso-FAs.

scFAs can be derived from either of the different starting units, acetyl-CoA or butyryl-CoA. In order to distinguish between these possibilities and to determine the origin of the residual scFAs in DK5614, deuterated butyric acid (1 mM [ $^2\text{H}_7$ ]-butyric acid) was fed to the wild type and the mutant, and its incorporation into the FAs as a starter unit was determined by gas chromatography-mass spectrometry analysis of the FA picolinyl esters ([www.lipidlibrary.co.uk](http://www.lipidlibrary.co.uk)) resulting from alkaline hydrolysis of whole cells and the subsequent reaction of the free FAs with 3-hydroxymethylpyridine in the presence

of *N,N'*-dicyclohexylcarbodiimide and 4-dimethylamino pyridine, similarly to a published procedure (3). Whereas 14% of the major scFA 16:1 $\omega$ 5c was labeled in the wild type, the incorporation in DK5614 was 100%. This indicates that the residual amount of scFAs in the mutant is not a result of incorporation of acetyl-CoA as a starting unit in FA biosynthesis but of butyryl-CoA, which results from the activity of other FabH enzymes encoded in the genome. Similar results were obtained for scFAs with 15 carbons which are derived from elongation of propionyl-CoA (as shown by feeding experiments with deuterated propionate [data not shown]) and which are also still produced in the mutant, although at a reduced level compared to the wild type, and therefore seem to be synthesized at least in part by an alternative FabH as well. Although it is not clear if all three *fabH* genes identified in the genome are involved in FA biosynthesis, it is evident that disruption of MXAN\_0853 is not lethal as has been described for the unique *fabH* gene in *Streptomyces coelicolor* (19).

Despite the fact that the change in the FA profile seems to be severe, no change in phenotype could be observed for DK5614. We analyzed the vegetative growth in liquid culture and on agar plates at temperatures between 20 and 37°C. Additionally, swarming on hard and soft agar, which is indicative of possible motility defects of the adventurous and social motility systems (21), was investigated for the wild type and the mutant as described previously (10). Furthermore, analyses of fruiting body formation and sporulation according to published procedures (10) showed no differences between the wild type and the mutant with respect to number and timing of aggregates and spores, even if tested at different cell densities.

**Conclusions.** On the one hand, scFAs seem to be dispensable in *M. xanthus*, since no change in phenotype could be detected for DK5614. On the other hand, a decrease in the iso-FAs leads to multiple defects, as exemplified by *bkd* mutants. *bkd* mutants show a reduction in the levels of iso-FAs to about 50% of the wild-type level (2, 17, 18, 25), and their aggregation and sporulation are strongly delayed and reduced (4).

It has previously been shown that diunsaturated FAs (16:1 $\omega$ 5c  $\omega$ 11c and iso-17:2 $\omega$ 5c  $\omega$ 11c) are potent autolytic FAs, inducing the cell lysis required for development in *M. xanthus* (26). Interestingly, the overall levels of diunsaturated FAs are the same in the mutant and the wild type (8.4% in both strains) due to a strong increase in the level of iso-17:2 $\omega$ 5c  $\omega$ 11c, which might be indicative of their biological importance. The level of monounsaturated FAs which are also potent autolytic FAs is less in the mutant (13.1% versus 19.5% in the wild type). Furthermore, no single FA is present in the mutant at a level comparable to that of 16:1 $\omega$ 5c in the wild type (9.5%). PE containing this FA has been proposed to be a chemotaxis signal in *M. xanthus* under developmental conditions. Moreover, the level of this lipid present in wild-type cells is sufficient for signaling (14). Since the level of this FA in the PE fraction increases about 30% during development (14), a role in development was suggested. The DK5614 mutant produces only trace amounts of 16:1 $\omega$ 5c, amounting to no more than 1/10 of the active concentration calculated for a monolayer of cells (14). Because we could not exclude de novo biosynthesis of this FA during development, we analyzed the FA profile of DK5614 during fruiting body formation. However, 16:1 $\omega$ 5c increased from undetectable trace amounts (Table 1) to only

TABLE 1. Fatty acid composition of cells of *M. xanthus* DK1622 (wild type) and DK5614 (MXAN\_0853)

Fatty acid <sup>a</sup>	% of total fatty acids in:	
	DK1622	DK5614
iso-11:0	0.26	0.79
iso-13:0	0.84	1.22
iso-15:0	40.81	60.98
iso-3-OH-15:0	1.47	1.96
iso-15:1 $\omega$ 9c	1.10	5.74
iso-17: $\omega$ 0	3.30	4.94
iso-2-OH-17:0	2.20	2.29
iso-3-OH-17:0	2.01	2.65
iso-17:1 $\omega$ 11c	1.36	3.9
iso-17:1 $\omega$ 5c	1.84	2.5
iso-17:2 $\omega$ 5c $\omega$ 11c	3.23	8.11
9:0	0.22	
10:0	0.68	
12:0	0.15	
13:0	0.33	
14:0	5.53	
14:1 $\omega$ 5c	2.23	
14:1 $\omega$ 9c	0.39	
15:0	1.67	
15:1 $\omega$ 10c	2.21	0.21
15:1 $\omega$ 4c	2.57	0.72
16:0	1.42	
2-OH-16:0	0.23	
3-OH-16:0	0.49	
16:1 $\omega$ 11c	0.90	
16:1 $\omega$ 5c	9.52	
16:2 $\omega$ 5c $\omega$ 11c	5.18	0.38
NI (6.931)	2.26	0.79
NI (9.288)	4.22	2.1
NI (9.899)	0.28	
NI (16.575)	1.11	0.31

<sup>a</sup> For nonidentified (NI) fatty acids, retention times are given in parentheses (minutes).

1.7% of total FAs (data not shown). Feeding experiments with D<sub>7</sub>-butyrate during fruiting body formation showed that 92% of 16:1 $\omega$ 5c is labeled, a result similar to that obtained from vegetative cells and, furthermore, confirming the activity of another FabH enzyme responsible for the incorporation of butyryl-CoA as a starting unit in FA biosynthesis. Importantly, the level of this or other  $\omega$ 5c-unsaturated FAs is still too low to act as a signal as proposed previously (14). Since the mutant completes normal development, these new results suggest either that the lipid derived from 16:1 $\omega$ 5c is not essential for development or that it can be replaced by some other lipid.

Although we have identified the first gene involved in FA biosynthesis in *M. xanthus* and characterized its function by means of comparative chemical analysis, the explanation of the unusual complexity of the FA profile in myxobacteria is still elusive. Our group is performing further work regarding additional genes involved in FA biosynthesis to shed more light on this unknown aspect of myxobacterial biology.

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