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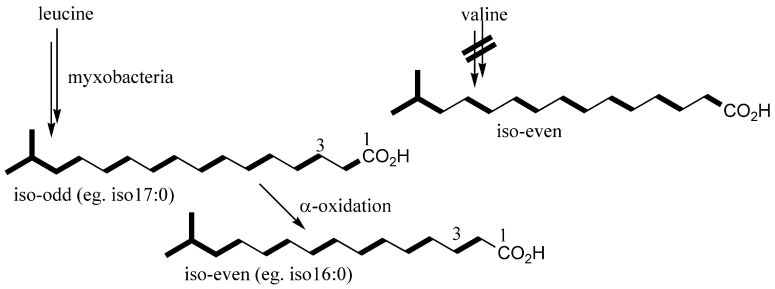

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Biosynthesis of Iso-Fatty Acids in Myxobacteria: Iso-Even Fatty Acids Are Derived by α-Oxidation from Iso-Odd Fatty Acids

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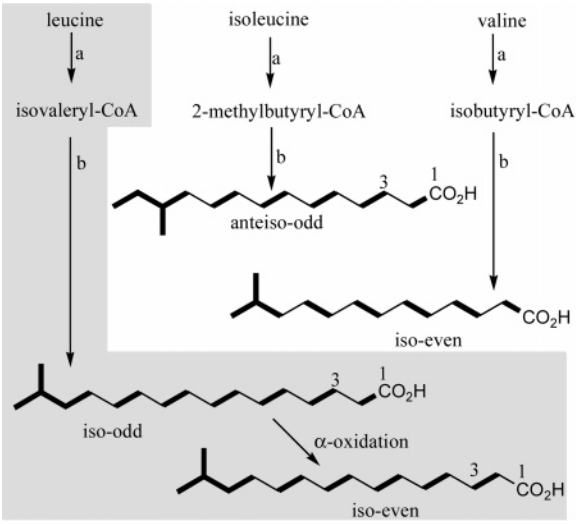
Iso- and anteiso-fatty acids (FA) play an important role in controlling membrane fluidity in various bacteria.[1] Furthermore, they and their precursors are important building blocks for various secondary metabolites.[2] The biosynthesis of iso- and anteiso-FA was elucidated 30 years ago,[3] establishing the starting units isovaleryl-CoA (IV-CoA), isobutyryl-CoA (IB-CoA), and 2-methylbutyryl-CoA (2MB-CoA) derived from the branched-chain amino acids leucine, valine, and isoleucine for the formation of iso-odd, iso-even, and anteiso-odd fatty acids, respectively (Scheme 1).[1]

The first step in the amino acid degradation is the transamination to the corresponding α-keto carboxylic acids, which are then further processed by the branched-chain keto acid dehydrogenase complex (Bkd) into the thioesters IV-CoA, IB-CoA, and 2MB-CoA. Standard textbook biochemistry involves the degradation of all three branched-chain amino acids by a single Bkd complex, which explains the loss of all three branched-chain carboxylic thioesters in case of an inactivated Bkd complex. This knowledge was applied to produce new antibiotics by feeding the nonnatural starting unit cyclohexane carboxylic acid to Bkd mutants (*bkd*) of the avermectin producing strain *Streptomyces avermitilis.* This led to a priming of the avermectin megasynthase with this unnatural compound and resulted in the production of the superior anthelminthic doramectin.[4,5] Contrary to streptomycetes, disruption of the *bkd* genes in myxobacteria does not result in a total depletion of the three thioesters and all products derived thereof.[6−8] Instead, a reduction in the production of the antibiotic myxothiazol (derived from IV-CoA) and iso-FA could be observed because of an alternative pathway to IV-CoA that branches from hydroxymethylglutaryl-CoA, a central intermediate in the mevalonate-dependent isoprenoid biosynthesis. This alternative pathway is strongly induced in a *bkd* mutant and almost not detectable in wild-type cells.[7,9]

During further analysis of this new pathway, we observed incorporation of labeled leucine into both iso-odd and iso-even fatty acids of the myxothiazol producer *Stigmatella aurantiaca* (in the wild type and in the *bkd* mutant), indicating a cross-connection between these two fatty acid families, which are usually specifically primed by leucine- and valine-derived starters, respectively.

To elucidate the underlying mechanism, we conducted additional feeding experiments with wild-type strains and *bkd* mutants of myxobacteria and *Streptomyces violaceoruber* as a control and analyzed the relative amount of fatty acids and incorporation rates by GC and GC−MS. Whereas in *S. violaceoruber* iso-odd FA were labeled exclusively by leucine and iso-even FA exclusively by valine, labeling of iso-FA in *S. aurantiaca* was very weak (Table 1). This is most likely due to the well-known biosynthesis of leucine using the transamination product of valine as intermediate.[1] Feeding of IVA and IBA (the free carboxylic acids of the final leucine and valine degradation products) to *bkd* mutants of *S. aurantiaca* and the myxobacterium *M. xanthus* also showed some important

Scheme 1. Biosynthesis of Iso-Fatty Acids[a]



[a] Only the pathway in gray boxes takes place in myxobacteria. Transaminase and Bkd complex (a) and fatty acid biosynthesis (b). Starting units (IV-CoA, IB-CoA, and 2MB-CoA) and extender units (derived from malonyl-CoA) of the different fatty acids are shown in bold.

Table 1. Relative Amount and Incorporation of [D_{10}]leucine, [D_8]valine, and FA [D_7]iso17:0 into FA Iso15:0 and FA Iso16:0 in *S. aurantiaca* DW4/3-1, *M. xanthus*, and *S. violaceoruber*[a]

			iso15:0	iso16:0
S. aurantiaca	wt		22	5
		valine	22 (2)	8 (3)
		leucine	22 (17)	8 (16)
		iso17:0	10 (58)	4 (58)
	bkd		12	3
		IVA	24	5
		IBA	17	11
M. xanthus	wt		37	0
		valine	38 (0)	0
		leucine	38 (16)	0
	bkd		15	5
		IVA	34	1
		IBA	11	12
S. violaceoruber			9	16
		valine	6 (0)	22 (6)
		leucine	9 (16)	14 (0)

[a] Enrichment (in %) of selected fatty acids after feeding of [D_{10}]leucine, [D_8]valine, and FA [D_7]iso17:0 in brackets. wt = wild type, *bkd* = Bkd mutant strain.

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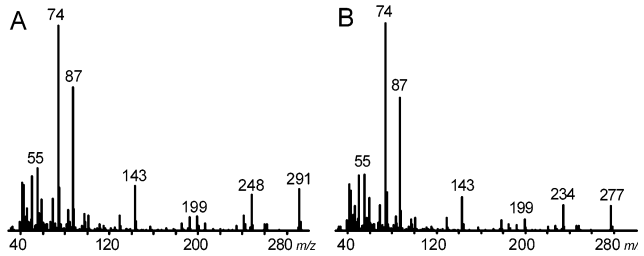

Figure 1. MS spectra of [D_7]iso17:0 methyl ester (A; [$C_{17}H_{29}D_7O_2^+$] = 291) and [D_7]iso16:0 methyl ester (B; [$C_{16}H_{27}D_7O_2^+$] = 277) after feeding of [D_7]iso17:0 to *S. aurantiaca*.

differences: Addition of either IVA or IBA increased the amount of iso-odd and iso-even FA in *S. aurantiaca*, whereas the increase in *M. xanthus* was only specific for the expected FA family (Table 1). The *bkd* mutants were used in these experiments instead of the wild-type strains because the wild type showed no increase in the different fatty acid families after the corresponding amino or carboxylic acids were added.

The obtained results indicate that myxobacterial FA biosynthesis has the usual broad range specificity and is able to use IVA, IBA, and 2MBA (in *M. xanthus*, data not shown) as starting units. The fact that no valine is incorporated into iso-FA and that almost no anteiso-FA are found in myxobacteria indicates an unusually specific Bkd complex. In addition, the data confirmed the biosynthetic connection between iso-odd and iso-even FA in *S. aurantiaca*. To determine if the required biochemistry takes place at the level of the starting unit or on the fatty acid itself, we fed labeled FA [15,16,16′-D_7]iso17:0 and analyzed its incorporation into iso-FA (Table 1, Figure 1). Both β-oxidation ([D_7]iso15:0) and α-oxidation ([D_7]iso16:0) were observed in high yields (58% of both FAs were derived from degradation of [D_7]iso17:0 FA).[10]

To determine the amount of α-oxidation, we fed [$^{13}C_2$]acetate to *S. aurantiaca* and determined the different isotopes in the fatty acid mixture by ^{13}C NMR. Although the fatty acid mixture could not be analyzed for specific fatty acids because of overlapping signals of the methylene groups in the NMR spectra, the incorporation pattern of acetate into the carboxylic acid terminus and into the ω-ends of nonbranched and iso-FA could be determined after hydrogenation of the mixture and comparison with reference data. It was possible to determine the incorporation for at least a three-carbon chain (Figure 2 and Supporting Information) from both ends. In the experiment with *S. aurantiaca*, a second doublet with $J_{2,3}$ = 34.5 Hz was obtained for C2 resulting from the incorporation of an intact C2/C3 acetate unit besides the expected C1/C2 unit ($J_{1,2}$ = 57.4 Hz) in the FA mixture. From the integrals of the C2−^{13}C satellites it can be concluded that 26% of all fatty acids in *S. aurantiaca* are derived from α-oxidation. However, no similar correlation can be obtained from the fatty acid composition itself (e.g., iso16:0 and iso17:0 are present in almost equal amounts), indicating the α-oxidation to be chain-length specific.

The production of 2-hydroxy FAs is essential for the biosynthesis of shingolipids. In cucumbers, humans, and the bacterium *Shingomonas paucimobilis*, previous reports correlate the production of these FA to α-oxidation.[11−14] At least in *S. aurantiaca*, which

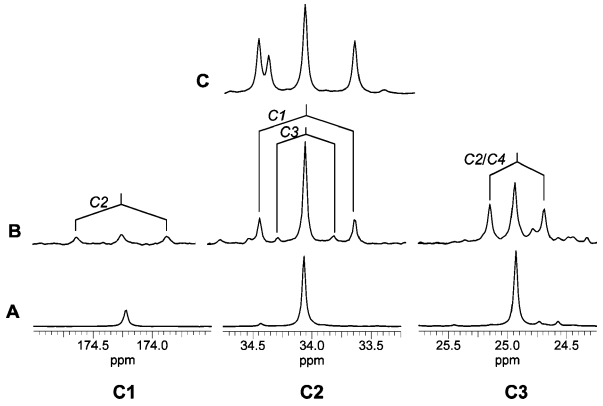

Figure 2. ^{13}C NMR signals of the carboxyl end of fatty acids isolated from *S. aurantiaca* (A and B) and *S. violaceoruber* (C). Natural abundance signals (A); after feeding of [1,2-$^{13}C_2$]acetate to strain DW4/3−1 (B); after feeding of [1,2-$^{13}C_2$]acetate to strain 3844-33C (C, the signal at 34.5 ppm corresponds to the ω-3 carbon of anteiso-FA). For assignments of carbon atoms C1−C3, see Scheme 1. ^{13}C-coupling to the different carbon atoms is indicated by brackets.

does not contain spingolipids, FA α-oxidation seems to be a general way to generate fatty acid diversity, which is normally obtained by using different starting units. The narrow fatty acid profile that would result from the highly specific Bkd complex that degrades only leucine is broadened by α-oxidation to form fatty acids not accessible from the fatty acid biosynthesis.

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Supporting Information Available: Materials and methods and detailed fatty acid analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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