The myxobacterial lipidome

Unusual structural features and specific changes during fruiting body development

Dissertation

zur Erlangung des Grades

des Doktors der Naturwissenschaften

der Naturwissenschaftlich-Technischen Fakultät III

Chemie, Pharmazie, Bio- und Werkstoffwissenschaften

der Universität des Saarlandes

von

Michael W. Ring

Saarbrücken

2009

Tag des Kolloquiums:	26.03.2010
Dekan:	Prof. Dr.-Ing. Stefan Diebels
Berichterstatter:	Prof. Dr. Helge B. Bode
	Prof. Dr. Rita Bernhardt
	Prof. Dr. Jörn Piel

Zusammenfassung

Myxobakterien sind Gram-negative Bodenbakterien, die sich durch ihren komplexen Lebenszyklus auszeichnen. Bei Nährstoffmangel bilden sie multizelluläre Aggregate, die als Fruchtkörper bezeichnet werden, und in denen sich die Zellen zu Myxosporen differenzieren, die gegen Hitze und Austrocknung resistent sind. Die biochemischen Veränderungen während dieses Vorgangs sind größtenteils unbekannt.

Die vorliegende Arbeit befasst sich mit der Analyse des Lipidoms im Modellorganismus *Myxococcus xanthus*, vor allem während der Entwicklung von Fruchtkörpern. Neben tieferen Einblicken in die myxobakterielle Fettsäurebiosynthese zeigten die Untersuchungen eine vermehrte Bildung von ungewöhnlichen verzweigtkettigen Etherlipiden unter Entwicklungsbedingungen, ebenso wie das Auftreten von Vesikeln, die hauptsächlich aus Triacylglycerol bestehen. Eine weitere Beobachtung war die Aktivierung der α-Oxidation von Fettsäuren, bei der eine Familie von stereospezifischen Fettsäure-α-Hydroxylasen eine entscheidende Rolle spielt. Außerdem ergaben sich Hinweise auf das Vorkommen von Sphingolipiden, was für Bakterien ebenfalls ungewöhnlich ist.

Die gewonnenen Erkenntnisse sollen dazu dienen, den Lipidstoffwechsel und auch das komplexe Regulationsnetzwerk, das dem Lebenszyklus der Myxobakterien zugrunde liegt, besser zu verstehen. Enzyme des bakteriellen Lipidstoffwechsels stellen interessante Targets für die Entwicklung neuer Antibiotika dar, und die Stoffwechselwege der Myxobakterien sind möglicherweise auf pathogene Bakterien übertragbar. Außerdem greifen die Biosynthesewege mehrerer myxobakterieller Sekundärstoffe auf den Lipidstoffwechsel zurück, so dass die erhaltenen Daten auch beitragen werden zum Verständnis des Zusammenspiels von Primär- und Sekundärstoffwechsel, das vielversprechende Leitstrukturen für die pharmazeutische und landwirtschaftliche Nutzung hervorbringt.

Abstract

Myxobacteria are Gram-negative soil bacteria with a complex life cycle. In response to starvation, they form multicellular aggregates named fruiting bodies, in which the cells differentiate into heat- and desiccation-resistant myxospores. The biochemical changes during this process are mostly unknown.

In this study, the lipidome of the model organism *Myxococcus xanthus* has been analyzed, in particular during the time course of fruiting body development. Besides deeper insights into fatty acid biosynthesis in these organisms, the analyses revealed an accumulation of unusual branched-chain ether lipids and the formation of triacylglycerol vesicles under developmental conditions. Furthermore, an activation of fatty acid α-oxidation could be observed, and a family of stereospecific fatty acid α-hydroxylases involved in this process has been identified. Evidence is presented that myxobacteria contain sphingolipids as another unusual structural feature in the lipidome.

These data will help to shed more light into the lipid metabolism and the mechanisms governing the complex life cycle of myxobacteria. Enzymes of the bacterial lipid metabolism are interesting targets for the development of antibacterial agents, and the results gained from myxobacteria may give helpful hints to find homologous enzymes in pathogenic bacteria. Furthermore, the biosynthesis of several myxobacterial secondary metabolites relies on certain reactions from lipid metabolism. The data presented here will help to understand how primary and secondary metabolism work together to produce promising lead structures for pharmaceutical and agricultural use.

In dieser Arbeit enthaltene Publikationen

Bode, H. B., M. W. Ring, D. Kaiser, A. C. David, R. M. Kroppenstedt and G. Schwär (2006)
Straight-chain fatty acids are dispensable in the myxobacterium *Myxococcus xanthus* for vegetative growth and fruiting body formation
J. Bacteriol. **188** (15), 5632-5634

Bode, H. B., M. W. Ring, G. Schwär, R. M. Kroppenstedt, D. Kaiser and R. Müller (2006)
3-Hydroxy-3-methylglutaryl-coenzyme A (CoA) synthase is involved in the biosynthesis of isovaleryl-CoA in the myxobacterium *Myxococcus xanthus* during fruiting body formation
J. Bacteriol. **188** (18), 6524-6528

Ring, M. W., G. Schwär, V. Thiel, J. S. Dickschat, R. M. Kroppenstedt, S. Schulz and H. B. Bode (2006)
Novel iso-branched ether lipids as specific markers of developmental sporulation in the myxobacterium *Myxococcus xanthus*
J. Biol. Chem. **281** (48), 36691-36700

Bode, H. B., M. W. Ring, G. Schwär, M. O. Altmeyer, C. Kegler, I. R. Jose, M. Singer and R. Müller (2009)
Identification of additional players in the alternative biosynthesis pathway to isovaleryl-CoA in the myxobacterium *Myxococcus xanthus*
Chembiochem **10** (1), 128-140

Hoiczyk, E., M. W. Ring, C. A. McHugh, G. Schwär, E. Bode, D. Krug, M. O. Altmeyer, J. Z. Lu and H. B. Bode (2009)

Lipid body formation plays a central role in cell fate determination during developmental differentiation of *Myxococcus xanthus*

Mol. Microbiol. **74** (2), 497-517

Ring, M. W., E. Bode, G. Schwär and H. B. Bode (2009)

Functional analysis of desaturases from the myxobacterium *Myxococcus xanthus*

FEMS Microbiol. Lett. **296** (1), 124-130

Ring, M. W., G. Schwär and H. B. Bode (2009)

Biosynthesis of 2-hydroxy and iso-even fatty acids is connected to sphingolipid formation in myxobacteria

Chembiochem **10** (12), 2003-2010

Weitere Publikationen

Folgende Publikationen entstanden während der Zeit meiner Arbeit, sind jedoch nicht Bestandteil dieser Dissertation:

Gross, F., M. W. Ring, O. Perlova, J. Fu, S. Schneider, K. Gerth, S. Kuhlmann, A. F. Stewart, Y. Zhang and R. Müller (2006)

Metabolic engineering of *Pseudomonas putida* for methylmalonyl-CoA biosynthesis to enable complex heterologous secondary metabolite formation

Chem. Biol. **13** (12), 1253-1264

Garcia, R. O., H. Reichenbach, M. W. Ring and R. Müller (2009)

Phaselicystidaceae fam. nov., *Phaselicystis flava* gen. nov., sp. nov., an arachidonic acid-containing soil myxobacterium

Int. J. Syst. Evol. Microbiol. **59**, 1524-1530

Chatterjee, I., S. Schmitt, C. F. Batzilla, S. Engelmann, A. Keller, M. W. Ring, R. Kautenburger, W. Ziebuhr, M. Hecker, K. T. Preissner, M. Bischoff, R. A. Proctor, H. P. Beck, H.-P. Lenhof, G. A. Somerville, M. Herrmann (2009)

Staphylococcus aureus ClpC ATPase is a late growth phase effector of metabolism and persistence

Proteomics **9** (5), 1152-1176

Botella, L., N. D. Lindley, and L. Eggeling (2009)

Formation and metabolism of methylmalonyl coenzyme A in *Corynebacterium glutamicum*

J. Bacteriol. **191** (8), 2899-2901

(Mitarbeit von M. W. Ring in Danksagung genannt)

Lorenzen, W., M. W. Ring, G. Schwär, and H. B. Bode (2009)

Isoprenoids are essential for fruiting body formation in *Myxococcus xanthus*

J. Bacteriol. **191** (18), 5849-5853

Tagungsbeiträge

Posterpräsentationen

Ring, M. W., G. Schwär, R. M. Kroppenstedt, S. Schulz and H. B. Bode
Biochemistry of fruiting body formation in *Myxococcus xanthus:* Two novel ether lipids
18. Irseer Naturstofftage, Irsee (02/2006)

Ring, M. W. and H. B. Bode
Identification of lipid-biomarkers during fruiting body formation in *Myxococcus xanthus*
VAAM Jahrestagung, Osnabrück (04/2007)

Kurzvorträge

Ring, M. W. and H. B. Bode
Lipid profile changes during myxobacterial development
European Myxobacterial Meeting, Marburg (02/2006)

Ring, M. W., G. Schwär and H. B. Bode
Specific changes in the lipidome of *Myxococcus xanthus* during development
34th International Conference on the Biology of the Myxobacteria, Granada, Spanien (07/2007)
Die Teilnahme an dieser Tagung wurde gefördert durch einen Meeting Attendance Grant der Federation of European Microbiological Societies (FEMS).

Table of contents

1. Introduction

1.1. Myxobacteria and their outstanding life cycle

Myxobacteria are Gram-negative, obligate aerobic bacteria constituting the order *Myxococcales* in the Delta group of *Proteobacteria*. This order is again subdivided into the three suborders *Cystobacterineae*, *Sorangiineae* and *Nannocystineae* (115), although some sources define the latter two as one suborder *Sorangineae* (19,102). Myxobacteria are typically rod-shaped, with a length of 3-15 µm and a diameter of 0.6-1.2 µm (101). Whereas most myxobacterial species live in normal soil, several strains have been isolated from more special habitats (115) such as marine (45) or halophilic (46) as well as acidic or alkaline environments (101). Myxobacteria are known for some outstanding biological features: On solid surfaces, they form swarms to gain access to nutrients (53,59), which are usually other microorganisms (25). Most myxobacteria secrete digestive enzymes to kill these microorganisms (124) and to decompose the released proteins, lipids and nucleic acids. In contrast to this, some members of the *Sorangiineae*, especially *Sorangium*, are cellulose degraders, enabling them to grow on cellulose in the presence of inorganic nitrogen sources (115).

Under starvation conditions, myxobacteria form multicellular aggregates called fruiting bodies (Figure 1), in which the cells differentiate into heat- and desiccation-resistant myxospores which are able to retransform into normal vegetative cells when nutrients become avaliable again (26). This type of multicellular organization is extraordinary among prokaryotes, and the morphology of fruiting bodies can vary from spheres to more complex tree-like structures (115). In contrast to this, myxobacterial cells grow independently in liquid media. Myxobacterial genomes are very large, for instance, the model organism *Myxococcus xanthus* DK1622 has a genome size of 9.1 Mbp (35), and the genome of

Sorangium cellulosum So ce56 (13 Mbp) is the largest bacterial genome known so far (113). Furthermore, myxobacteria are a source of natural products with promising lead structures for pharmaceutical and agricultural applications (9,34), the most prominent example being the tubuline-stabilizing epothilones from *S. cellulosum* (11), whose derivatives are predicted a high significance in cancer therapy because of their activity against multidrug resistant solid tumors (105).

The enormous size of myxobacterial genomes partially results from lineage-specific gene duplications (35,108,113), and in particular, genes encoding proteins with regulatory functions such as kinases/phosphatases, σ^{54} enhancer-binding proteins and extracytoplasmic σ-factors as well as two-component signal transduction systems are affected by these duplications (35,108,113). This shows that the myxobacterial lifestyle, including social behavior, gliding motility and fruiting body development (53) as well as predation (6) and even secondary metabolism (28,100), is governed by a large complex regulatory network, driven by chemotaxis (145) and intercellular signals (54,116). During the last decades, more and more insights were gained how these different aspects of the myxobacterial lifestyle are connected to each other, e.g. the coupling of motility and development (53) or the activities of certain secondary metabolites in the context of development (86,98). The following chapter will give a more detailed description of myxobacterial development, leading to the considerations about the role of primary metabolism for fruiting body formation and sporulation.

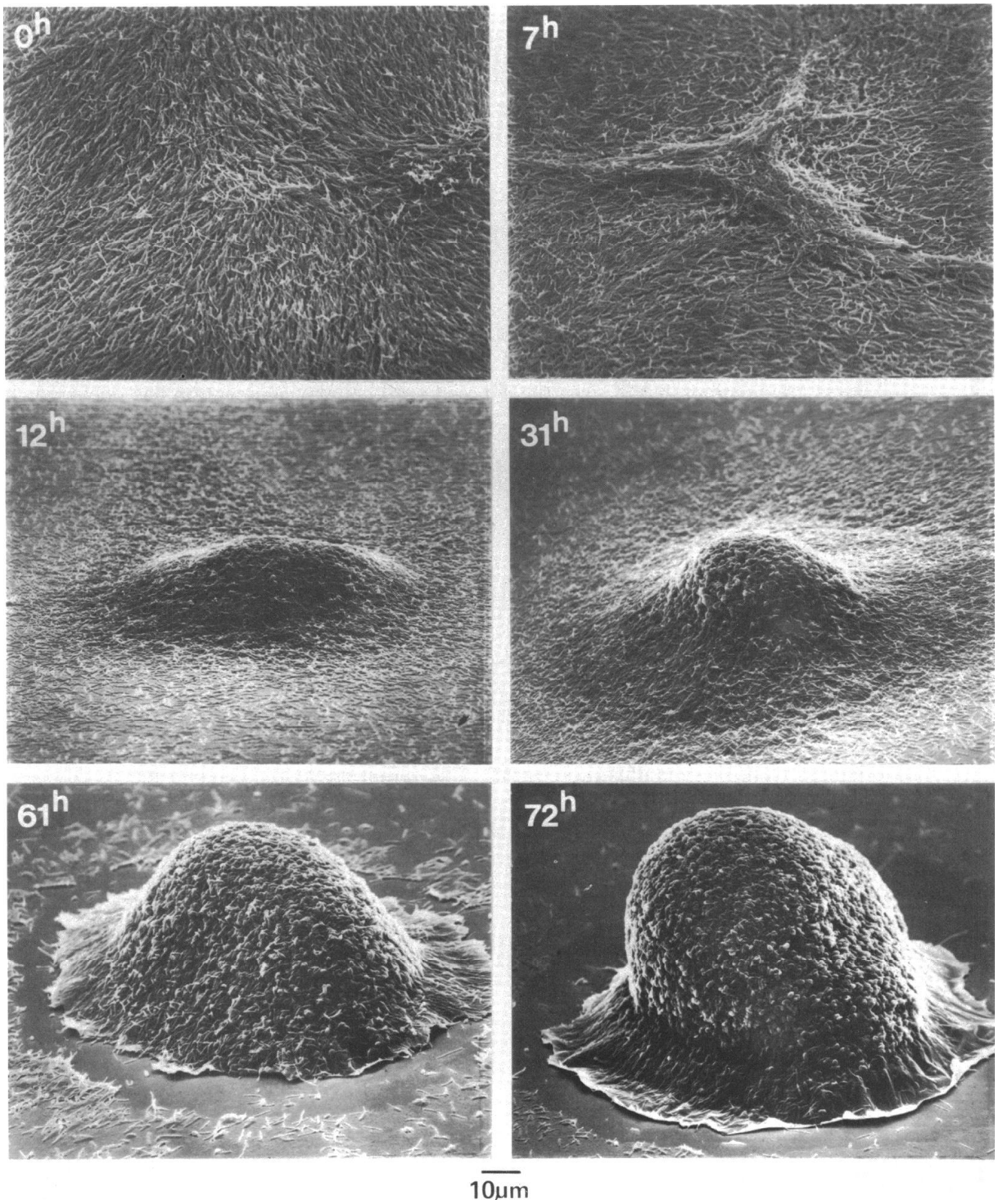


Figure 1. Fruiting body formation of *Myxococcus xanthus* DK1622.

Scanning electon micrographs of the developmental time course in submerged culture obtained by Kuner and Kaiser (64). Reprinted with permission.

1.2. Myxococcus xanthus – Motility and fruiting body development

The principles of myxobacterial motility and fruiting body development have been extensively studied in the model organism *Myxococcus xanthus* (53,54,116), as techniques for cultivation and subsequent induction of fruiting body formation have been established for this organism (13,43,63,64). Most experiments have been done with strain DK1622, which is fully motile on solid surfaces and grows dispersely in liquid culture (52). Furthermore, its genome has been sequenced (35), and protocols for targeted genetic manipulations are available (36,49), including recently developed autonomously replicating plasmids (144). Another model organism among myxobacteria which is worth mentioning and whose lipidome was also subject of the present study is *Stigmatella aurantiaca* DW4/3-1 (90,98).

Two engines have been described for the motility of *M. xanthus* (53), referred to as the A-engine and the S-engine. The A-engine (A for adventurous) accounts for the motility of single cells, and its underlying mechanism is supposed to be the production of slime at the cell pole pointing to the opposite direction of movement. The S-engine (S for social) is constituted by type IV pili which adhere to neighboring cells and thus enable movement towards these cells by retraction of the pilus. Mutants deficient in both motility engines are nonmotile and are also deficient in fruiting body formation (53).

Formation of fruiting bodies and subsequent differentiation of cells into myxospores (the whole process is referred to as myxobacterial development) is a highly complex procedure. Regarding the outward appearance, aggregates are visible 8-12 h after entering the starvation state, and fruiting body morphogenesis with around 10^5 cells per fruiting body is completed after 24-48 h (116) and followed by spore maturation. During formation of the fruiting body, some of the cells are killed by autolysis (26), which has been postulated to be a sacrifice to ensure survival of the colony, e.g. by improving the protein

biosynthesis capacity of the remaining cells, as the gene expression pattern changes severely during development (47). However, more recent comparisons have shown that the extent of autolysis varies between strains (145), and the requirement for sacrifice has been put into question by the finding that development is also induced by a step-down in nutrition rather than by sudden starvation (5). Most of the cells in the fruiting bodies transform into myxospores, which differ from vegetative cells by their round (*M. xanthus*) or short rod-shaped (*S. aurantiaca*) morphology (132), whereas 10 % of cells does not enter fruiting bodies but retains its rod shape and differentiates into so-called peripheral rods (see also Chapter 2.5), which are able to utilize low nutrient levels and are supposed to enable a faster re-formation of a swarm when nutrients become available again (92), in contrast to myxospores which have to re-differentiate into vegetative cells. Myxospores, however, are more resistant to environmental stress (123) and can survive long-term starvation (115). The above mentioned morphological transformation of a vegetative cell into a myxospore must be accompanied by biochemical changes in the cell, which is evident regarding the formation of a thick spore cortex and coat (48,125) and the accumulation of trehalose in myxospores (84). To obtain more information about the metabolic processes occurring during sporulation, alternatives to starvation on solid surfaces have been established for large-scale sporulation, such as glycerol-induced sporulation (27) and sporulation in starvation buffer (109). However, these spores differ from fruiting body spores already from the morphological point of view, e.g. glycerol spores do not have the typical spore coat, as the sporulation-specific protein S is not accumulated in glycerol spores (47), so the spores obtained by these practical simplifications are not fully comparable to fruiting body spores.

Several efforts have been made to characterize the mechanism by which myxobacterial cells coordinate the formation of fruiting bodies and sporulation. Information was gained by

the analysis of mutants unable to accomplish development. Among these mutants, five complementation groups have been identified (22,38), which are deficient in the formation of fruiting bodies and/or spores but whose deficiency can be overcome by co-development with wild-type cells or mutants from a different complementation group. Because of this observation, the presence of intercellular signals (A- to E-signal) was postulated, and the gene loci in which the respective mutations had occurred were designated *asg*, *bsg*, *csg*, *dsg* and *esg* (22,38).

A-signaling turned out to be a quorum sensing mechanism, relying on certain amino acids which are released at micromolar concentrations by starving cells (66) probably in the context of stringent response (120). The concentration of these amino acids directly correlates with cell density, and this enables *Myxococcus* cells to sense if there are sufficient cells for fruiting body formation nearby. On the other hand, amino acid concentrations supporting slow growth prevent induction of the developmental program. Thus, A-signaling is assumed to play a crucial role for the decision for or against development (54), which is supported by the fact that the release of A-signal occurs at a very early time point, usually 2 h after entering nutrient limitation conditions.

C-signaling is mediated by cell-to-cell contact, and the CsgA protein has been shown to be involved in this type of intercellular communication (117). CsgA is a 25 kDa cell surface protein (70) which is transformed into its 17 kDa active form by cleavage of the N-terminus via the secreted protease PopC (107). However, an overproduction of SocA, which is highly similar to CsgA (51 % similarity on the amino acid level), restores development (68). Both CsgA in its native 25 kDa form and SocA show similarities to short-chain alcohol dehydrogenases, and lysophosphatidylethanolamine has been identified as a substrate for SocA (3). These findings suggest that the C-signal is not the CsgA protein itself, but maybe the product of a reaction catalyzed by CsgA and/or SocA.

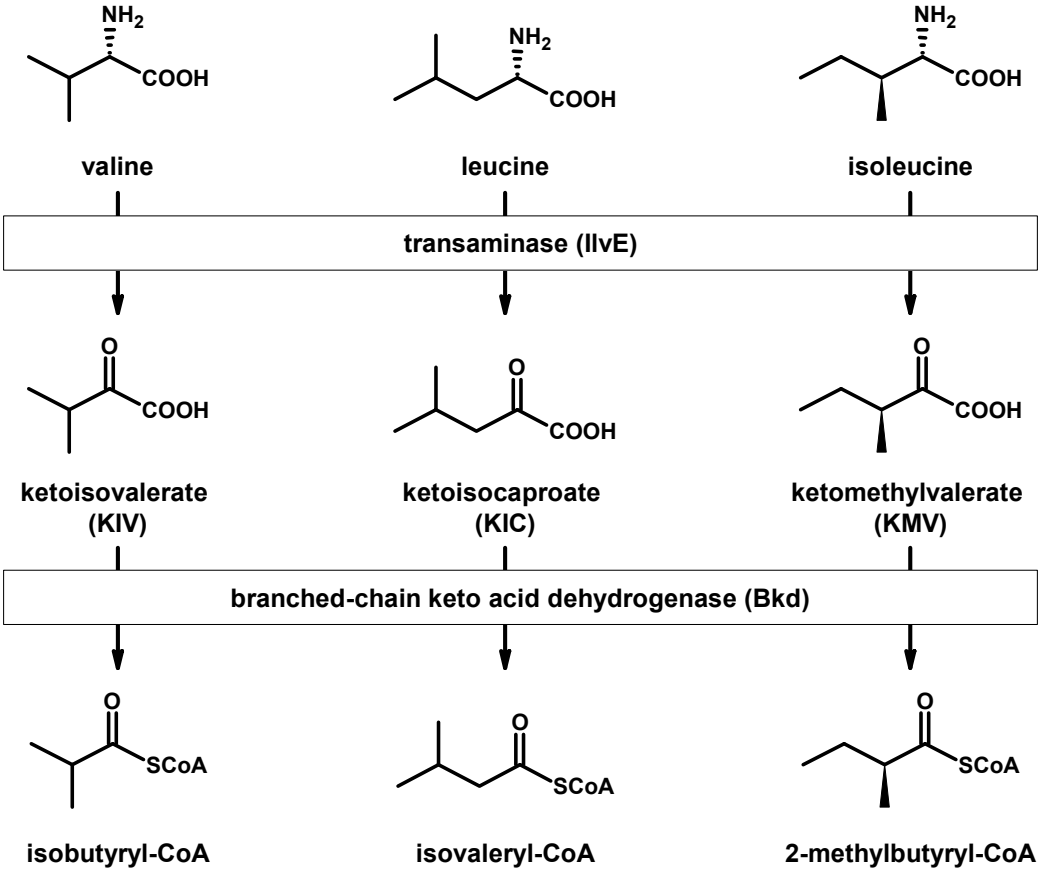


Figure 2. The first two biochemical steps of the branched-chain amino acid degradation pathway.

Valine, leucine and isoleucine are transaminated and then oxidatively decarboxylated to CoA esters.

The principles of B- and D-signaling have not been fully elucidated up till now. Although the *bsg* and *dsg* loci have been identified, it is still unclear whether signaling molecules or other mechanisms play a role (54).

The *esg* locus turned out to constitute the genes encoding the subunits of the branched-chain keto acid dehydrogenase Bkd (126), which is a key enzyme in the degradation pathway of branched-chain amino acids (82). Valine, leucine and isoleucine are transformed into 2-oxo acids by a transaminase (IlvE), and the following step is an oxidative decarboxylation catalyzed by Bkd (96), resulting in the coenzyme A (CoA)

thioesters isobutyryl-CoA, isovaleryl-CoA and 2-methylbutyryl-CoA, respectively (Figure 2). For a better understanding of the connections between this pathway and myxobacterial development, it is necessary to give an introduction about bacterial fatty acid biosynthesis.

1.3. Biosynthesis of fatty acids in myxobacteria

Fatty acid biosynthesis in bacteria is carried out via a group of highly conserved proteins (73,106,141). In contrast to the type I fatty acid synthase in most eukaryotes, which is a huge multifunctional enzyme, the type II fatty acid synthase system consists of several single proteins, each one catalyzing one step of the biosynthesis (Figure 3). The overall mechanism is the growth of an acyl chain bound to an acyl carrier protein (ACP). β-ketoacyl-ACP synthases (KAS, *syn.* 3-oxoacyl-ACP synthases) are responsible for elongation of the fatty acyl chain by two carbon atoms, and the underlying reaction is a Claisen-like condensation of a thioester with the active methylene group of malonyl-ACP, the latter resulting from carboxylation of acetyl-CoA and transfer of the malonyl residue to an ACP. In the first occurring reaction, which is catalyzed by KAS III (FabH), the thioester is a CoA ester, usually acetyl-CoA, giving rise to straight-chain fatty acids (SCFAs).

One type of fatty acid diversity often encountered in bacteria is caused by variation of the starter unit, i.e. the substitution of acetyl-CoA by other CoA esters. For instance, the use of propionyl-CoA results in an SCFA with an odd number of carbon atoms (42), whereas butyryl-CoA would not make a difference to acetyl-CoA at first sight. However, an interesting variation are branched-chain fatty acids (BCFAs), which are formed by employing isobutyryl-CoA, isovaleryl-CoA and 2-methylbutyryl-CoA as starter units of fatty acid biosynthesis (55). The substrate specificity of FabH is crucial for selection of the starter unit (15,40), and crystal structure data gave valuable information about correlations

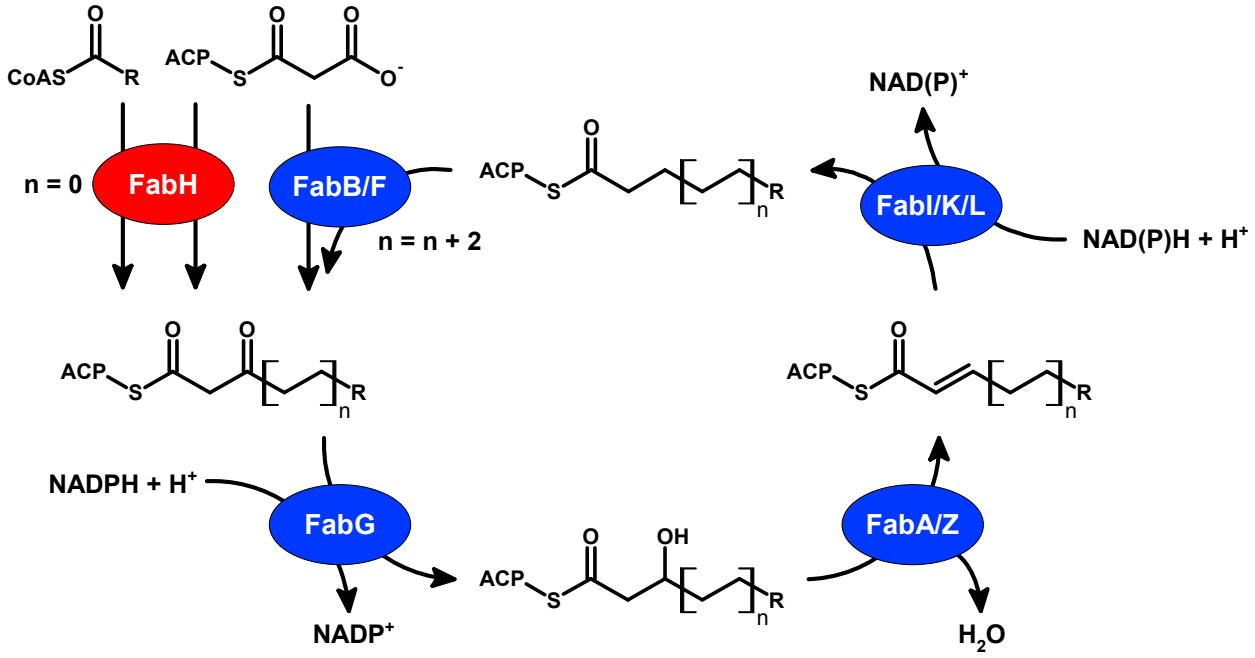


Figure 3. Fatty acid biosynthesis in bacteria.

The elongation unit malonyl-ACP is condensed with the starter CoA ester by FabH. The resulting 3-oxoacyl-ACP is then reduced in an NADPH-dependent reaction to a 3-hydroxyacyl-ACP by FabG, dehydrated by FabA or FabZ to a *trans*-2-enoyl-ACP, and the acyl chain is saturated by FabI. Subsequent codensations with further malonyl-ACP units are catalyzed by FabB or FabF.
For a complete list of gene and protein designations, see 4.2.

between substrate specificity and the size and structure of the acyl-CoA primer binding pocket of FabH enzymes (99,112).

The fatty acid profiles of myxobacteria (20,138) mainly consist of BCFAs, namely *iso*-fatty acids with an odd total number of carbons (*iso*-odd fatty acids). This type of fatty acid is biosynthesized by using isovaleryl-CoA as a starter unit (7,16,55), and as this metabolite is also an intermediate in leucine degradation, the pathways of branched-chain amino acid degradation and BCFA biosynthesis are directly coupled. The formation of *iso*-odd fatty acids is not only dependent on the substrate specificity of FabH, but also on precursor supply, for which Bkd is one of the essential enzymes. Consequently, myxobacterial

strains with a mutation in their *esg* locus (more appropriately: *bkd* locus) should be deficient in *iso*-fatty acid biosynthesis. However, in contrast to the *bkd* mutants of streptomycetes (17) or *Bacillus* (143), *bkd* mutants of *Myxococcus xanthus* and *Stigmatella aurantiaca* show a reduction, but not a total loss of *iso*-odd fatty acids, indicating that an alternative source of isovaleryl-CoA must be present. By feeding experiments, the existence of a shunt pathway branching from the mevalonate pathway has been proven (75,76).

Concerning the strategy of feeding experiments in this context, it has to be mentioned that isovaleryl-CoA (as well as isobutyryl-CoA and 2-methylbutyryl-CoA) also serves as a starter unit for polyketide synthesis, as shown for myxothiazol (119,128) or myxalamid (118), so these CoA ester primers connect primary and secondary metabolism. Thus, feeding of labeled substrates which are channeled into the isovaleryl-CoA pool will result in labeling of not only *iso*-odd fatty acids, but also compounds derived from *iso*-odd fatty acids and secondary metabolites originating from isovaleryl-CoA. On the one hand, this metabolic connection helps to distinguish *n*-odd fatty acids from *iso*-odd fatty acids during mass spectrometrical analysis, as the fragmentation patterns are identical in most cases but *iso*-odd fatty acids can be specifically labeled with leucine (see also Chapters 2.2 and 2.3). On the other hand, the different biochemical pathways leading to isovaleryl-CoA may be elucidated via analysis of the labeling pattern of *iso*-odd fatty acids and/or the appropriate secondary metabolites. The interface between primary and secondary metabolism is even extended by the shunt pathway mentioned above. The importance of different precursors on the mevalonate pathway can be determined by the analysis of secondary metabolites whose biosynthesis relies on the formation of isoprenoid structures. The most appropriate substance to be analyzed in this context is the degraded sesquiterpene geosmin (33), as it occurs in several myxobacterial strains and its

biosynthesis in myxobacteria has been elucidated (21,127). Other examples for natural products with isoprenoid structures in myxobacteria are aurachin (110) and leupyrrin (8,10).

1.4. Aims of the present study

The present work focuses on the lipidome of *Myxococcus xanthus* DK1622, with some comparative studies regarding other myxobacterial species, in particular *Stigmatella aurantiaca* DW4/3-1. The central aim was a further characterization of the biochemical changes occurring during development of myxobacterial cells. As mentioned above, trehalose (84) and protein S (48) accumulate in myxospores and can therefore be regarded as biomarkers for sporulation. The term "biomarker" in general refers to a characteristic which serves as an indicator of biological processes and is widely used in medicine (134), e.g. for substances in body fluids whose amount gives valuable information about the progress of a disease. Finding biomarkers is also an interesting field in microbiology, for measuring biomarker levels is a powerful tool to assess the physiological state of the cell without the need for time-consuming purely microbiological methods such as colony counting (62). Since finding and measuring biomarkers in microbiology usually implies transcriptomics (32,49,62), it would be interesting to find low molecular biomarkers, which may be more suitable for routine analysis.

In the context of myxobacterial development, substances which increase specifically during development and therefore can be used to monitor the progress of development can be regarded as biomarkers. For this reason, characterization of biochemical changes during development is directly linked to finding additional biomarkers for development. However, it has to be mentioned that the observation that a metabolite increases during development does not yield any information about whether the production of the

metabolite is a requirement or a consequence of development or if the metabolite is just a by-product of a developmentally upregulated reaction. If the biomarker is a protein or if the biosynthesis of the metabolite is known, inactivation of the responsible genes and phenotypical analysis of the resulting mutants will help to clarify the physiological role. In other cases, genes involved in the biosynthesis first have to be found.

During differentiation of the vegetative cell into a myxospore, the cell envelope undergoes a dramatical change regarding the shift from long rod-shaped to spherical or short rod-shaped morphology (132) and the formation of the spore coat (48). As a significant part of the cell envelope consists of membrane phospholipids, it is evident that the lipidome changes during sporulation and that additional biomarkers can be most likely found among cellular lipids. These considerations led to a more detailed characterization of the overall lipidome, i.e. the different classes of lipids. It has been shown that phosphatidylethanolamine (PE) is the major lipid class in *M. xanthus* (95), but there were no reports about the molecular species and the fatty acid composition of the distinct lipid classes. Consequently, there was no knowledge about shifts from one lipid class to another during development, nor about alterations of the molecular composition within one lipid class.

The connection between lipid metabolism and the postulated E-signal (see 1.3) brought up the aim to shed more light into the nature of the E-signal and the underlying mechanisms, which included gaining further insights into myxobacterial fatty acid biosynthesis via feeding experiments and analysis of mutants.

Finally, the generation of fatty acid diversity by structural modifications after the biosynthesis of the actual long-chain acyl residues was analyzed. Large amounts of fatty acids with double bonds at the unusual positions Δ^5 and Δ^{11} can be found in myxobacteria, with the otherwise rare fatty acid 16:1Δ^{11} (16:1ω5c) being the most abundant straight-chain

fatty acid in *M. xanthus* and *S. aurantiaca*. Besides double bonds, hydroxyl groups at the 2- or 3-position of fatty acids have been described (29). It was unknown how double bonds and hydroxyl groups are introduced into the fatty acyl chains in myxobacteria, and if or how these fatty acid families play a role in the myxobacterial life cycle.

To summarize, the overall aim of the present study was to find lipid biomarkers and other metabolites which are important in the context of the myxobacterial life cycle and to elucidate their biosynthetic pathways as well as their relevance for fruiting body formation and sporulation.

2. Publications of the results

2.1. Straight-chain fatty acids are dispensable in the myxobacterium Myxococcus xanthus for vegetative growth and fruiting body formation

Helge B. Bode*, Michael W. Ring, Dale Kaiser, Anna C. David,

Reiner M. Kroppenstedt and Gertrud Schwär

Journal of Bacteriology
Vol. 188 (15), pp. 5632-4, Aug. 2006

Abstract

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.

The full text of this article is available online at
http://jb.asm.org/cgi/content/full/188/15/5632

2.2. 3-Hydroxy-3-methylglutaryl-coenzyme A (CoA) synthase is involved in the biosynthesis of isovaleryl-CoA in the myxobacterium Myxococcus xanthus during fruiting body formation

Helge B. Bode*, Michael W. Ring, Gertrud Schwär, Reiner M. Kroppenstedt, Dale Kaiser and Rolf Müller*

Journal of Bacteriology
Vol. 188 (18), pp. 6524-8, Sept. 2006

Abstract

Isovaleryl-coenzyme A (IV-CoA) is the starting unit for some secondary metabolites and iso-odd fatty acids in several bacteria. According to textbook biochemistry, IV-CoA is derived from leucine degradation, but recently an alternative pathway that branches from the well-known mevalonate-dependent isoprenoid biosynthesis has been described for myxobacteria. A double mutant was constructed in *Myxococcus xanthus* by deletion of genes involved in leucine degradation and disruption of *mvaS* encoding the 3-hydroxy-3-methylglutarylcoenzyme A synthase. A dramatic decrease of IV-CoA-derived iso-odd fatty acids was observed for the mutant, confirming *mvaS* to be involved in the alternative pathway. Additional quantitative real-time reverse transcription-PCR experiments indicated that *mvaS* is transcriptionally regulated by isovalerate. Furthermore, feeding studies employing an intermediate specific for the alternative pathway revealed that this pathway is induced during fruiting body formation, which presumably increases the amount of IV-CoA available when leucine is limited.

The full text of this article is available online at
http://jb.asm.org/cgi/content/full/188/18/6524

2.3. Novel iso-branched ether lipids as specific markers of developmental sporulation in the myxobacterium Myxococcus xanthus

Michael W. Ring, Gertrud Schwär, Verena Thiel, Jeroen S. Dickschat, Reiner M. Kroppenstedt, Stefan Schulz and Helge B. Bode*

Journal of Biological Chemistry
Vol. 281 (48), pp. 36691-700, December 1, 2006

Abstract

Iso-fatty acids (FAs) are the dominant FA family in all myxobacteria analyzed. Furthermore, it was postulated that iso-FAs or compounds derived thereof are involved in fruiting body formation in *Myxococcus xanthus*, since mutants with a reduced level of iso-FA due to a reduced level of the precursor isovaleryl-CoA, are delayed in aggregation and produce only few myxospores. To elucidate the function of iso-FAs and their corresponding lipids we have analyzed the developmental phenotype of mutants having different levels of iso-FAs resulting in a clear correlation between the amount of iso-FAs and the delay of aggregation and reduction in spore yield. Addition of either isovalerate or 13-methyltetradecanoic acid resulted in restoration of the wild-type FA profile and normal development. Detailed analysis of the fatty acid (FA) profile during fruiting body formation in *Myxococcus xanthus* wild-type revealed the specific accumulation of 13-methyltetradecanal and 1-*O*-13-methyltetradecylglycerol which were produced specifically in the myxospores and which are derived from 1-*O*-(13-methyl-1-*Z*-tetradecenyl)-2-*O*-(13-methyltetradecanoyl)-glycero-3-phosphatidylethanolamine (VEPE) and 1,2-di-(13-methyltetradecanoyl)-3-(13-methyltetradecyl)glycerol (TG-1), respectively. The structures of these unusual ether

lipids have been determined by spectrometric methods and synthesis (for TG-1). Analysis of several mutants blocked at different stages of development indicated that the biosynthesis of TG-1 is developmentally regulated and that VEPE might be an intermediate in the TG-1 biosynthesis. Finally, addition of TG-1 to mutants blocked in the biosynthesis of isovaleryl-CoA could restore aggregation and sporulation emphasizing the important role of iso-branched lipids for myxobacterial development.

The full text of this article is available online at
http://www.jbc.org/cgi/content/full/281/48/36691

2.4. Identification of additional players in the alternative biosynthesis pathway to isovaleryl-CoA in the myxobacterium Myxococcus xanthus

Helge B. Bode, Michael W. Ring, Gertrud Schwär, Matthias O. Altmeyer, Carsten Kegler, Ivy R. Jose, Mitchell Singer and Rolf Müller*

ChemBioChem – A European Journal of Chemical Biology
Vol. 10 (1), pp. 128-140, January 5, 2009

Abstract

Isovaleryl-CoA (IV-CoA) is usually derived from the degradation of leucine by using the Bkd (branched-chain keto acid dehydrogenase) complex. We have previously identified an alternative pathway for IV-CoA formation in myxobacteria that branches from the well-known mevalonate-dependent isoprenoid biosynthesis pathway. We identified 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase (MvaS) to be involved in this pathway in *Myxococcus xanthus*, which is induced in mutants with impaired leucine degradation (e.g., *bkd*$^-$) or during myxobacterial fruiting-body formation. Here, we show that the proteins required for leucine degradation are also involved in the alternative IV-CoA biosynthesis pathway through the efficient catalysis of the reverse reactions. Moreover, we conducted a global gene-expression experiment and compared vegetative wild-type cells with *bkd* mutants, and identified a five-gene operon that is highly up-regulated in *bkd* mutants and contains *mvaS* and other genes that are directly involved in the alternative pathway. Based on our experiments, we assigned roles to the genes required for the formation of IV-CoA from HMG-CoA. Additionally, several genes involved in outer-membrane biosynthesis and a plethora of genes encoding regulatory proteins were decreased in expression levels in

the *bkd*⁻ mutant; this explains the complex phenotype of *bkd* mutants including a lack of adhesion in developmental submerse culture.

The full text of this article is available online at
http://www3.interscience.wiley.com/journal/121431880/abstract

2.5. Lipid body formation plays a central role in cell fate determination during developmental differentiation of *Myxococcus xanthus*

Egbert Hoiczyk*, Michael W. Ring, Colleen A. McHugh, Gertrud Schwär, Edna Bode, Daniel Krug, Matthias O. Altmeyer, Jeff Zhiqiang Lu and Helge B. Bode*

Molecular Microbiology
Vol. 74 (2), pp. 497-517, Oct. 2009

Summary

Cell differentiation is widespread during the development of multicellular organisms, but rarely observed in prokaryotes. One example of prokaryotic differentiation is the Gram-negative bacterium *Myxococcus xanthus*. In response to starvation, this gliding bacterium initiates a complex developmental programme that results in the formation of spore-filled fruiting bodies. How the cells metabolically support the necessary complex cellular differentiation from rod-shaped vegetative cells into spherical spores is unknown. Here, we present evidence that intracellular lipid bodies provide the necessary metabolic fuel for the development of spores. Formed at the onset of starvation, these lipid bodies gradually disappear until they are completely used up by the time the cells have become mature spores. Moreover, it appears that lipid body formation in *M. xanthus* is an important initial step indicating cell fate during differentiation. Upon starvation, two subpopulations of cells occur: cells that form lipid bodies invariably develop into spores, while cells that do not form lipid bodies end up becoming peripheral rods, which are cells that lack signs of morphological differentiation and stay in a vegetative-like state. These data indicate that

lipid bodies not only fuel cellular differentiation but that their formation represents the first known morphological sign indicating cell fate during differentiation.

The full text of this article is available online at
http://www3.interscience.wiley.com/journal/122612846/abstract

2.6. Functional analysis of desaturases from the myxobacterium Myxococcus xanthus

Michael W. Ring, Edna Bode, Gertrud Schwär and Helge B. Bode*

FEMS Microbiology Letters
Vol. 296 (1), pp. 124-130, Jul. 2009

Abstract

The fatty acid profiles of myxobacteria contain fatty acid species with double bonds at the Δ^5 and Δ^{11} positions, the latter being rather unusual among bacteria. Despite this knowledge, the mechanism for introduction of these double bonds has never been described before in myxobacteria. Searches for candidate genes in the genome of the model organism *Myxococcus xanthus* revealed 16 genes which have been annotated as fatty acid desaturases. However, due to redundant substrate specificity, functional analyses of these enzymes by construction of inactivation mutants did not lead to the identification of their function or substrate specificity. Therefore, we elucidated the regioselectivity of the desaturation reactions by heterologous expression of eight desaturases from *Myxococcus xanthus* in *Pseudomonas putida* and thus could prove five of them to be indeed active as desaturases with three (MXAN_1742, MXAN_3495 and MXAN_5461) and two (MXAN_0317 and MXAN_6306) acting as Δ^5 and Δ^{11} desaturases, respectively. This is the first report about heterologous expression and regioselectivity of fatty acid desaturases in myxobacteria.

The full text of this article is available online at
http://www3.interscience.wiley.com/journal/122368811/abstract

2.7. Biosynthesis of 2-hydroxy and iso-even fatty acids is connected to sphingolipid formation in myxobacteria

Michael W. Ring, Gertrud Schwär and Helge B. Bode*

ChemBioChem – A European Journal of Chemical Biology
Vol. 10 (12), pp. 2003-2010, August 17, 2009

Abstract

2-Hydroxy fatty acids can be found in several different organisms including bacteria. In this study, we have studied the biosynthesis of 2-hydroxy fatty acids in the myxobacteria *Myxococcus xanthus* and *Stigmatella aurantiaca* resulting in the identification of a family of stereospecific fatty acid α-hydroxylases. Although the stereospecificity of the hydroxylases differs between these two species, they share a common function in supporting fatty acid α-oxidation, i.e. the oxidative shortening of fatty acids. Whereas this process takes place during normal vegetative growth in *S. aurantiaca*, this process takes place only under developmental conditions in *M. xanthus*. We could also identify serine palmitoyltransferase encoding genes involved in sphingolipid biosynthesis and sphingolipids themselves in both myxobacteria and could show that the α-hydroxylation reaction is in fact dependent on the presence of fatty acids bound to sphingolipids.

The full text of this article is available online at
http://www3.interscience.wiley.com/journal/122477076/abstract

2.8. Statements of effort

ad 2.1

The author of this thesis performed all GC/MS analyses and their interpretation and established a protocol for the preparation of fatty acid picolinyl esters, enabling further structural characterization of fatty acids.

ad 2.2 and 2.4

The author of this thesis performed all lipid extraction as well as the GC/MS analyses and their interpretation. Developmental experiments and preparation of cell samples for lipid analysis were performed by Gertrud Schwär and the author.

ad 2.3

All analytical procedures (extraction and fractionation of lipids, analysis by GC/MS and HPLC/MS including interpretation), were done by the author of this thesis. Developmental experiments and preparation of cell samples were performed by Gertrud Schwär and the author. Verena Thiel and Jeroen S. Dickschat conducted the synthetic preparation of lipids.

ad 2.5

The author performed isolation and GC/MS analysis of lipids (including part of the developmental experiments and/or cultivations for this purpose) and data interpretation as well as the construction of the mutant strains MR1127, MR0412 and MR5582 and the heterologous *Pseudomonas putida* expression strain for MXAN_1127.

ad 2.6

GC/MS and comparative sequence analyses were done by the author of this thesis. The author also constructed the *P. putida* expression strain for MXAN_3495 (*desA*) and designed the primers for the construction of all other expression strains. These strains were constructed and cultivated by Edna Bode. Desaturase inactivation mutants were made by Gertrud Schwär and Helge B. Bode.

ad 2.7

This work was conducted completely by the author of this thesis, except for the construction of the STIAU_3334 (fah_{DW}) expression strains, which was done by Gertrud Schwär.

3. Discussion

3.1. Changes in the lipidome during development

Regarding the overall changes of the lipid profile of *M. xanthus* during development, four aspects have to be mentioned:

1. the accumulation of ether lipid structures (Chapter 2.3),
2. the formation and subsequent disappearance of triacylglycerol vesicles (Chapter 2.5), and
3. fatty acid α-oxidation (Chapter 2.7).

Figure 4 shows the main indicators for these changes in the lipidome: The ether lipid-derived structures *iso*-15:0 dimethyl acetal (DMA) and 1-*O*-alkylglycerol (OAG, Chapter 2.3), and *iso*-16:0 as a product of fatty acid α-oxidation (Chapter 2.7). The observation that these three substances increase during development was the starting point for most of the investigations carried out during this study. During sample preparation, *iso*-15:0 OAG is released from the triglyceride analogue TG-1, which is the major component in the lipid bodies (Chapter 2.5), so the increase of the OAG also indicated that triglyceride-like substances play a role during development. Although the amount of the OAG increases steadily, the amounts of TG-1 and the TAGs decrease after 24 h of development. This discrepancy will be discussed later.

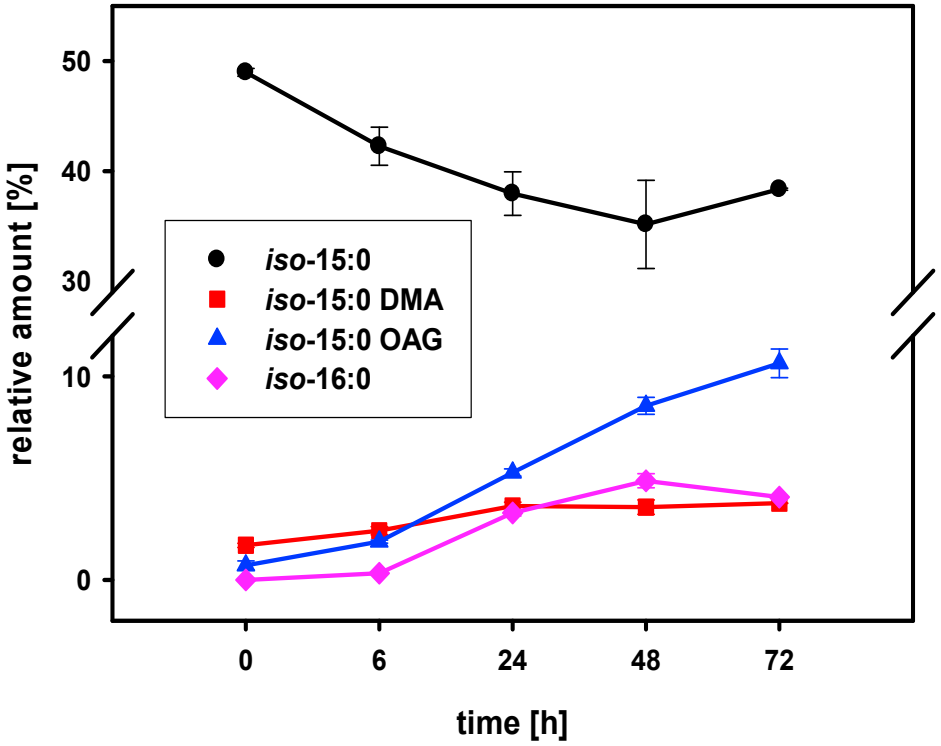
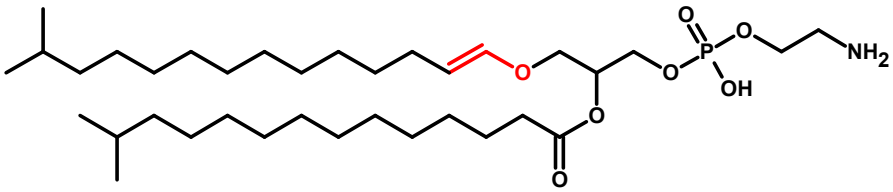
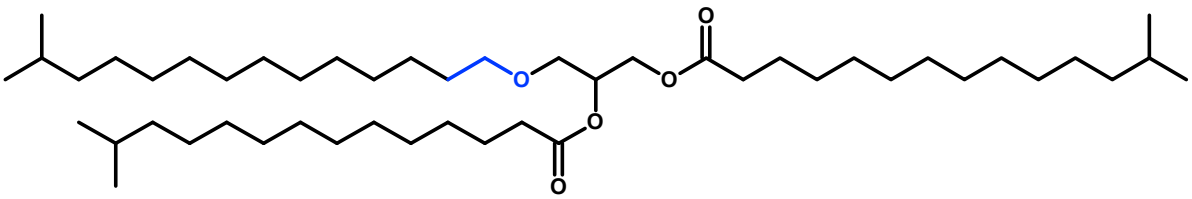


Figure 4. Fatty acids and ether lipid-derived structures during development.

These data were obtained by acidic methanolysis of cell samples and subsequent silylation. Relative amounts were calculated with respect to the total peak area of all FAMEs, DMAs and OAGs.



Vinyl ether phosphatidylethanolamine (VEPE)



1-*O*-Alkyl-2,3-*O*-diacylglycerol (TG-1)

Figure 5. Structures of both ether lipids from *M. xanthus* DK1622 identified during this study.

3.1.1. Ether lipids and triacylglycerols

The occurrence of ether phospholipids in myxobacteria has been described several decades ago (58). There are even hints that mono- and dialkyl phospholipids exist in *Stigmatella* (14), but these findings have never been confirmed by mass spectrometrical analysis. Later, a plasmalogen from *Myxococcus stipitatus* has been structurally characterized (122), and the same plasmalogen (VEPE) was purified from *M. xanthus* DK1622 during this study (Chapter 2.3 and Figure 5). Ether phospholipids can be found in several other bacterial species (50,130,133). However, the non-polar ether lipid TG-1 unites two unusual structural features in one molecule: alkyl ether lipid and triglyceride analogue. Despite the finding that ether lipids and TAGs are synthesized during development, the underlying biochemical pathways have not been elucidated so far. Albeit being the standard energy storage form in eukaryotes (89), the occurrence of TAGs in bacteria is restricted to certain taxonomic groups (2), but for some bacterial species in which TAGs have been found, a wax ester synthase/acyl-CoA:diacylglycerol acyltransferase (WS/DGAT) has been identified as the key enzyme for their formation (136). Unfortunately, a BlastP query against the genome of *M. xanthus* gave MXAN_1127 as a rather weak hit (45 % homology to the WS/DGAT from *Acinetobacter sp.* ADP-1, with 13 % gaps), and an inactivation of this gene had no influence on the presence of TAGs (Chapter 2.5). An alternative mechanism described for yeast and some plants employs a phospholipid:diacylglycerol acyltransferase (PDAT), using phospholipids as acyl donors (18), but a BlastP query gave no hit in bacteria at all.

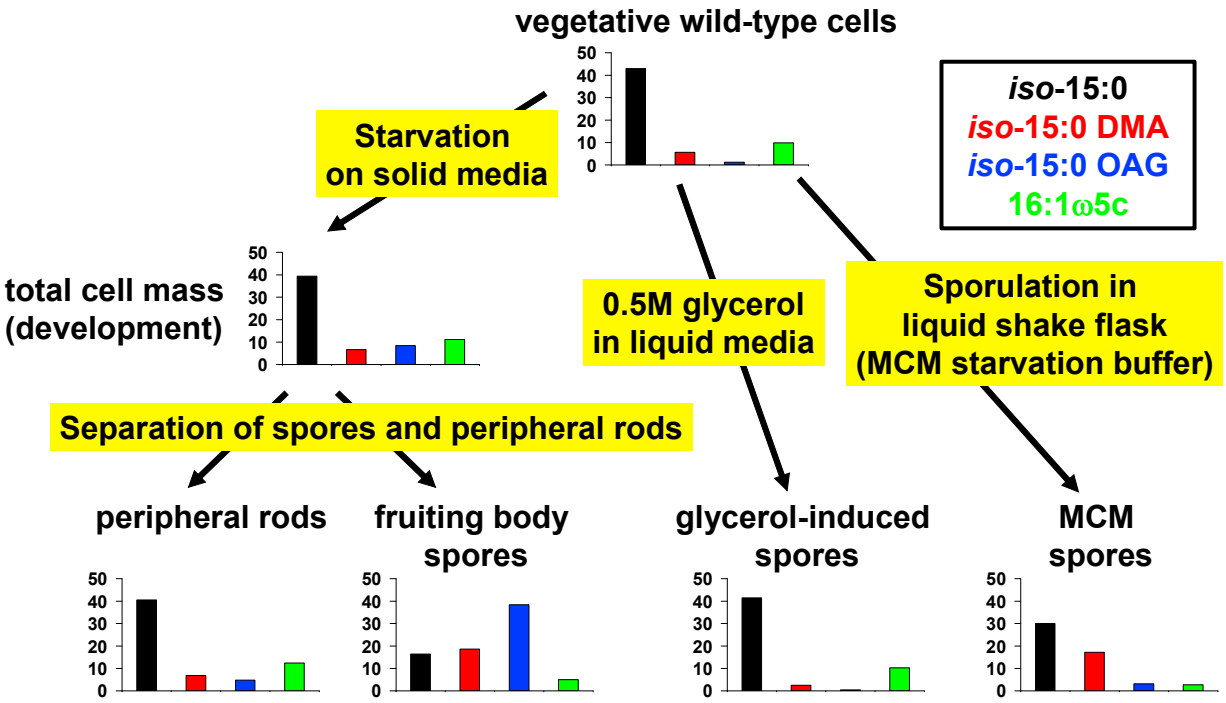


Figure 6. Relative amounts of fatty acids and ether lipid-derived structures in different cell types of *Myxococcus xanthus* DK1622.

Amounts are given in percent. FA, DMA and OAG amounts were measured after 72 h except for glycerol sporulation which reaches its final state after 6 h. The term "MCM spores" refers to spores obtained by shaking in starvation buffer (109).

Searches for further acyltransferases revealed the multi-domain protein MXAN_1528, whose architecture contains domains with predicted AMP-dependent acyl-CoA synthetase and acyltransferase activity, together with an NAD(P)-binding and a phosphopantetheine attachment site. Inactivation of this gene caused loss of ether lipids (W. Lorenzen, M. W. Ring, H. B. Bode, submitted). Interestingly, homologs of this type of enzyme regarding the full length of the amino acid sequence exist only in myxobacteria, as determined by BlastP searches. The most closely related enzymes, which belong to organisms from the classes of *Aquificae* and *Deltaproteobacteria* (in particular *Geobacter*), lack at least the NAD(P)-binding site.

However, the complete biosynthesis of ether lipids has not yet been fully clarified. The proposed pathway (Chapter 2.3) proceeds in two reduction steps from a PE with an *iso*-15:0 acyl residue at position *sn*-1 via VEPE to the corresponding plasmanylethanolamine (AEPE), which is then transformed into TG-1, probably by dephosphorylation and acyl transfer in analogy to TAG biosynthesis (2). This pathway is in accordance with the finding that the vinyl ether (represented by *iso*-15:0 DMA in Figure 6), but not the alkyl ether accumulates in spores from shaking cultures (109), indicating that certain biochemical reactions of the developmental program are carried out under these conditions, but the resulting metabolites are not processed further. Glycerol spores do not

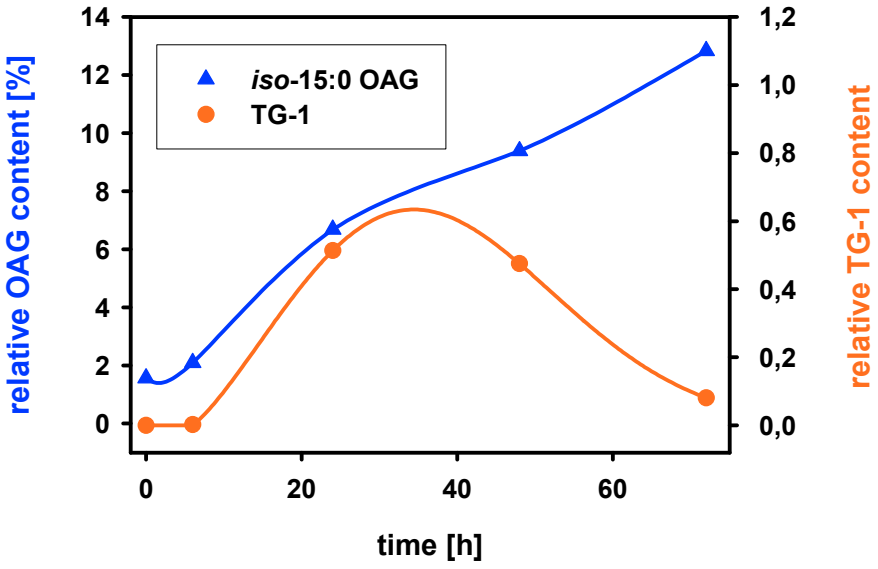


Figure 7. Kinetics of iso-15:0 OAG and TG-1 during development.

Although the amount of *iso*-15:0 OAG (determined by FAME analysis) increases steadily, the amount of the whole lipid TG-1 (determined by high-temperature GC/MS) reaches a maximum at 24 h and then declines.

The OAG content was calculated with respect to the total peak area of all FAMEs, DMAs and OAGs. TAG contents were calculated relative to the total lipid mass, by using the peak area of the internal standard cetyl palmitate (10 µg per 1 ml of total lipids, as described in Chapter 2.5) as a reference.

show any accumulation of either ether lipid (Figure 6), therefore, their differences from fruiting body spores (48,91) are also accompanied by a different lipid pattern.

Another highly interesting point is the discrepancy between the recoveries of *iso*-15:0 OAG from whole-cell methanolysis on the one hand and the amount of extractable TG-1 on the other hand (Figure 7). Whereas the former increases steadily during development, the latter reaches a maximum at about 24 h and then decreases again (Chapter 2.3 and 2.5). Thus, there is a transition of the glycerol ether structure from the extractible to the non-extractible fraction in the time course of development, and this observation leads to the assumption that the structure is being attached to the cell envelope by a covalent linkage. Posttranslational modification of proteins by attachment of a lipid structure is usually encountered in bacterial lipoproteins (111), where a diacylglyceryl moiety is transferred from phosphatidylglycerol to the prolipoprotein, more precisely to the sulfhydryl group of the cysteine residue at the N-terminus of the upcoming apolipoprotein, resulting in a thioether structure (111). However, thioethers are resistant to various hydrolysis conditions (104), and as acidic hydrolysis and methanolysis both liberated the OAG, it is unlikely that the glycerol ether structure is processed in the same manner in myxobacteria, but in general, a transfer of the glycerol ether to a protein or to the lipopolysaccharide or peptidoglycan fractions may be an explanation for the above mentioned transition. Theoretically, the chemical nature of this attachment may be an ester, e.g. a carboxy ester or a dialkyl phosphate or sulfate. For instance, the cell-surface glycoproteins of some halophilic archaea contain a diphytanylglyceryl phosphate moiety (56,61). Alkylacylglyceryl moieties have also been found in the glycosyl-phosphatidylinositol protein anchors in some parasitic protozoa, such as *Leishmania major* and *Trypanosoma cruzi*, as well as in mammals (85). Other examples for covalent bonds between lipid and amino acid structural elements are found in the surfactins from several *Bacillus* strains (97), where the carboxyl

group of the C-terminal amino acid of the peptide chain is esterified with a hydroxyl group of the lipid residue, and in the myxochromides from *S. aurantiaca* DW4/3-1 (139) and *M. xanthus* DK1050 (140), where a fatty acid is linked to the peptide backbone via an amide bond.

Another point, which is still unclear, is the physiological role of ether lipids. TG-1 has been shown to restore development in the double mutant DK5624 to a certain extent, with fruiting bodies forming preferentially around droplets of TG-1 (Chapter 2.3). Aggregation, but not the formation of viable spores, is also restored by TG-2 and TPG. A possible reason may be that the sporulation rescue is mediated by *iso*-15:0, which can be liberated from TG-1 by ester hydrolysis, whereas triglyceride-like molecules seem to trigger aggregation. This is supported by the observation that a disruption of the above mentioned acyltransferase genes and thus, a lack of ether lipids, causes a delay or even a strong defect of aggregation in *M. xanthus* and *S. aurantiaca*, respectively (W. Lorenzen, M. W. Ring, H. B. Bode, submitted).

In addition, the incorporation of the ether structure into the cell envelope brings up other physiological aspects and a comparison with archaea, whose polar lipids constituting the cell membrane are based on ether rather than on ester linkages (60). Together with bipolar tetraether lipids which span the whole membrane, leading to a mixture of phospholipid bi- and monolayers, these ether lipid membranes are important for the extremophilic lifestyle of archaea (129) due to their physicochemical properties. There may be an analogy to the resistance of myxospores against environmental stress factors such as heat, UV irradiation and desiccation (123). Furthermore, it would be interesting to analyze whether myxobacterial strains from halophilic (46), acidic or alkaline habitats (101) have a higher relative ether lipid content than mesophilic strains, which would also support the hypothesis that ether lipids play a role in stress tolerance in myxobacteria.

The occurrence of alkyldiacylglycerols has been described for some sharks such as the dogfish *Squalus acanthias* (77), where ether lipids may serve as storage lipids which are not metabolizable by predators and where they play a role in the determination of the specific buoyancy (78). Whereas the latter mechanism would rather not apply to bacteria, the former principle should be considered as one possible role of ether lipids in myxobacteria. As the alkyl ether structure is only found in myxospores (Figure 6), whose role is to ensure survival of the colony, the transformation of a storage lipid into a non-metabolizable form together with the incorporation into the cell envelope might result in a protection against enzymatic degradation.

Coming back to development, the increase of ether lipids and the formation of lipid bodies, in which TG-1 is a major component (Chapter 2.5), are restricted to cells undergoing differentiation into myxospores, at least in the context of development. The differentiation into peripheral rods (93) did not show this behavior (see also Figure 6), and this observation leads to the hypothesis that lipid body formation is an essential part of the early sporulation program.

However, lipid bodies have been shown to be formed in response to environmental stress factors other than starvation as well, such as anaerobiosis and kanamycin treatment (Chapter 2.5). This may be interpreted as building up a storage to survive these stress conditions, which is also supported by the finding that the intensity of lipid body formation correlates with the degree of stress (kanamycin concentration in this case). The observation that higher nutrient levels in the starvation agar lead to a higher portion of peripheral rods (92) fits into this model, as less starvation would be expected to cause less lipid body formation and thus less cells differentiating into spores. In contrast to this, a step-down in nutrition lead to lipid body formation in all cells (Chapter 2.5), but this experiment was performed in shaking culture, which does not allow normal development

requiring cell-to-cell contact (57), as sporulation in starvation buffer under shaking conditions (109) does not yield real fruiting body spores either (see also Chapter 2.3 and Figure 6). An interesting experiment would be to determine lipid body formation induced by a nutritional step-down on solid surfaces and/or in the presence of prey (5).

It should be mentioned that other bacteria modify the physicochemical properties of their membranes under stress conditions, in particular temperature stress, by alteration of the fatty acid unsaturation pattern (80,81,88). This type of stress response does not seem to occur in *M. xanthus* (Chapter 2.6).

Another point of significance is that the fate of cells seems to be sealed when the decision *pro* or *contra* lipid body formation is made, i.e. the differentiation into spores or peripheral rods, respectively. When this decision is caused artificially, e.g. by pretreatment with kanamycin, the cell population produces more spores under developmental conditions (Chapter 2.5). Cells already containing lipid bodies not only differentiate preferentially into spores, but also use their storage to build up a more resistant carbohydrate matrix around the fruiting body than non-pretreated cells do. Therefore, a stronger accumulation of lipids in response to drastic conditions does not only help the single cell to withstand the stress factors, but also serves to improve the protection of the whole colony.

3.1.2. Fatty acid α-oxidation and sphingolipids

Fatty acid α-oxidation is a phenomenon encountered in plants (39), humans (51) and bacteria (7,83). Although required for degradation of branched long-chain substrates with substituents preventing β-oxidation such as phytanic acid (137), an essential role of α-oxidation in myxobacterial biochemical pathways remains elusive. However, a family of fatty acid α-hydroxylases has been identified during this study (Chapter 2.7), with close sequence relationships to α-hydroxylases from other organisms such as yeast,

Arabidopsis (87) and humans (1). In the latter, the α-hydroxylase is predicted to be involved in α-oxidation (30), which would fit into the proposed myxobacterial model with the α-hydroxylation being the first step of α-oxidation. A connection to sphingolipid metabolism has been shown both for human (1) and yeast (24,37,87) hydroxylases. Whereas it is suggested that the fatty acid is hydroxylated prior to sphingolipid formation in humans (1), there is evidence that hydroxylation occurs after sphingolipid formation in yeast (37). It is quite interesting that these closely related enzymes exert different substrate specificities. Considering that the stereochemistry of α-hydroxylation differs between *M. xanthus* and *S. aurantiaca*, it might be that the substrate specificity differs as well, which might be an explanation why α-hydroxylation is completely shut down in the *spt* mutant strain *M. xanthus* MR3748, and in *S. aurantiaca* MR6449, a small amount of 2-hydroxy fatty acids still can be detected, although both strains are defective in sphingolipid biosynthesis (Chapter 2.7).

When regarding the overall mechanisms of fatty acid α-oxidation, it turns out that the plant pathway (39) employs a fatty acid α-dioxygenase rather than a fatty acid α-hydroxylase, although the latter type of enzyme has been found in *Arabidopsis* (87). In contrast, both human pathway models (30,51) are based on the formation of a 2-hydroxy fatty acid by the hydroxylase. All pathways mentioned have in common that they end up in an aldehyde which is oxidized to the fatty acid (summarized in Chapter 2.7). However, *iso*-16:0 aldehyde has never been found in the analyzed myxobacterial strains, but this may be due to the high reactivity and turnover of aldehydes.

Like with ether lipids, the physiological role of fatty acid α-oxidation remains unclear, and a role of α-oxidation and/or sphingolipids for development cannot be proposed from the obtained results. However, α-oxidation in *M. xanthus* takes place only under

developmental conditions, making its product *iso*-16:0 a biomarker for development (see 1.4). In contrast to this, *iso*-16:0 is present in *S. aurantiaca* even during vegetative growth (7). It is likely that the extent of α-oxidation remains at a basal level under vegetative conditions in both organisms and that the substantial amount of *iso*-16:0 in *S. aurantiaca* results from the high quantity of *iso*-17:0 2-OH in this strain. Further experiments regarding fruiting body formation are required to clarify if α-oxidation is also upregulated during *S. aurantiaca* development.

3.2. Fatty acid biosynthesis

As mentioned in the introduction (Chapter 1.3), the biosynthesis of fatty acids in bacteria is catalyzed by type II fatty acid synthases (73,141), which consist of several dissociable proteins (Figure 3). The major fatty acids in myxobacteria are BCFAs (20,138), and to understand the biosynthesis of this type of fatty acids, the research during this study focused on the formation and further processing of the starter units, mainly isovaleryl-CoA, which bring in the methyl group at the ω-end of the carbon chain.

3.2.1. Biosynthetic origin of the starter unit

The formation of the starter units of BCFAs is depicted in Figure 2. An inactivation of the genes encoding the subunits of the key enzyme Bkd leads to a decrease, but not a complete loss of BCFAs, as isovaleryl-CoA is synthesized from HMG-CoA via an alternative pathway (75,76). An alternative route to KIV, which employs the valine biosynthesis pathway, has been shown for *Staphylococcus carnosus* (4), however, the subsequent formation of isobutyryl-CoA still requires an active Bkd. In contrast to this, the myxobacterial pathway (Chapter 2.4 and Figure 8) consists of some biochemical steps corresponding to reverse reactions of the leucine degradation pathway (82). This alternative pathway is inactive in the wild-type under vegetative conditions, but it is upregulated under developmental conditions and in a Bkd^- background (Chapter 2.2). In the context of development, the alternative pathway can be interpreted as a compensatory mechanism to maintain biosynthesis of *iso*-fatty acids under leucine-limiting conditions. This theory is underlined by the fact that leucine is an essential amino acid for *M. xanthus* (13). Thus, the organism is incapable of leucine *de novo* biosynthesis, and amino acids are preferentially used for the biosynthesis of development-specific proteins (91).

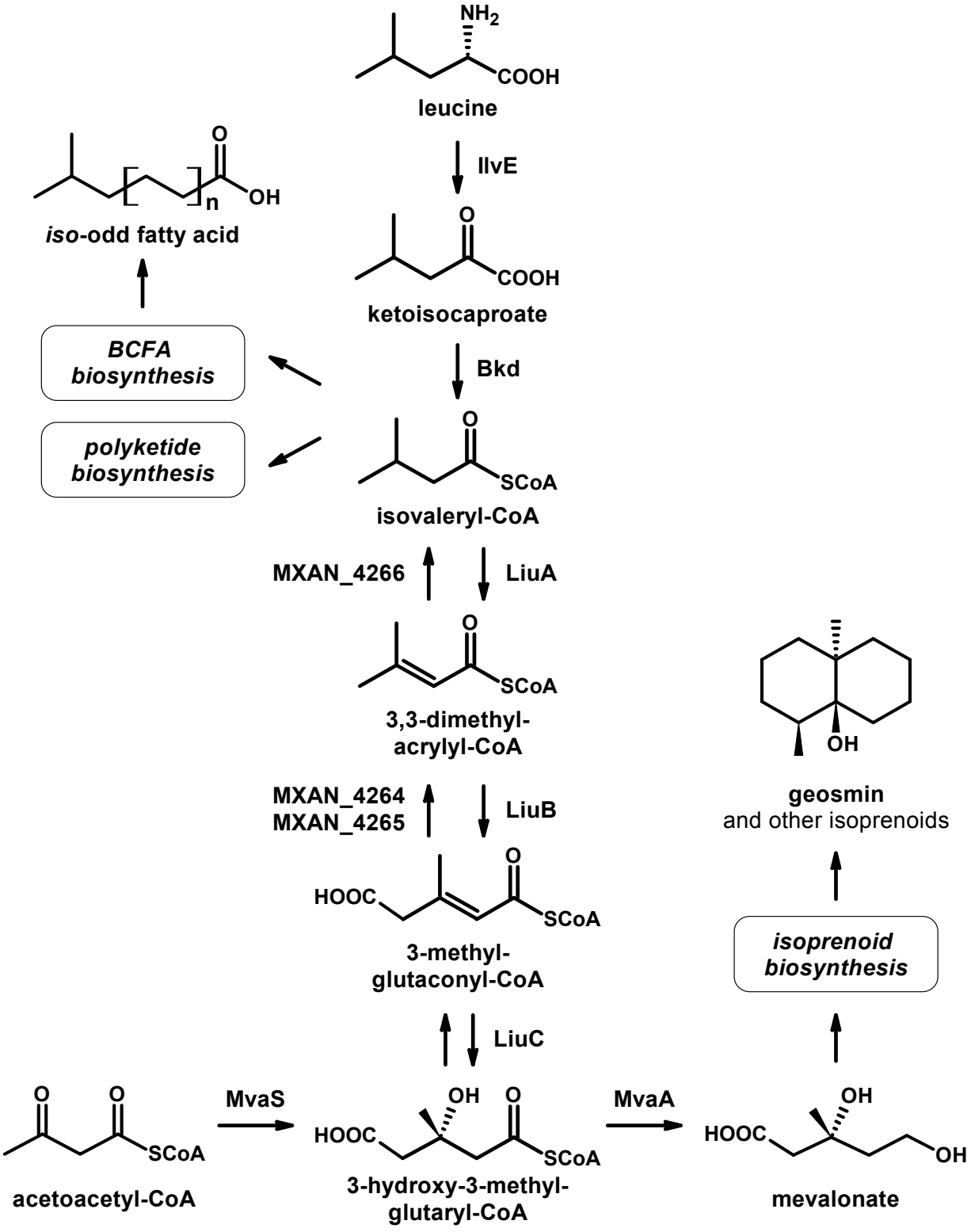


Figure 8. The interconnection of leucine degradation with BCFA, polyketide and isoprenoid biosynthesis.

Adapted from Chapter 2.4.

Further reactands and by-products were omitted for the sake of clarity.

Interestingly, the *bkd* mutant of *M. xanthus* contains small amounts of *iso*-16:0 even under vegetative conditions. It has been shown that the wild-type is capable of synthesizing *iso*-16:0 from valine (Chapter 2.7), and this is most likely the pathway leading to *iso*-16:0 in the *bkd* mutant, as the amount of this FA in the *bkd* mutant increases when feeding isobutyrate, but not isovalerate (7). Therefore, a second Bkd activity seems to be responsible for the formation of this *iso*-even fatty acid rather than the alternative pathway to isovaleryl-CoA and α-oxidation. This possibility is also discussed for *Staphylococcus aureus* (121), where a Bkd^- mutant still produces certain amounts of BCFAs, which is comparable to myxobacteria. The second Bkd activity might be exerted by other 2-oxo acid dehydrogenase complexes such as pyruvate dehydrogenase or α-ketoglutarate dehydrogenase; for instance, the pyruvate dehydrogenase from *Bacillus subtilis* also accepts branched-chain substrates (94). To investigate this hypothesis, the *poxB* genes MXAN_3654 and MXAN_3857, annotated as pyruvate dehydrogenases (35), were inactivated in the *bkd* mutant of *M. xanthus*, but feeding with labeled valine did not result in significantly different incorporation rates into *iso*-16:0 (not shown). This indicates that either both genes are able to complement each other or none of them is responsible for the second Bkd activity.

3.2.2. Incorporation of the starter unit

Although the biosynthetic pathways leading to isovaleryl-CoA are mostly clarified in *M. xanthus* (23,75,76)(Chapters 2.2 and 2.4), the corresponding FabH enzyme which is responsible for the incorporation of this starter unit into fatty acids has not been identified, whereas MXAN_0853 turned out to encode the FabH accepting acetyl-CoA as a substrate, as the respective mutant strain DK5614 showed loss of most straight-chain fatty acids (Chapter 2.1). A minor amount of straight-chain fatty acids can still be synthesized by

utilizing propionyl-CoA or butyryl-CoA, and a different FabH is thought to be involved in this biosynthesis.

Among bacteria, especially Gram-positive bacteria, FabHs with a substrate specificity for branched-chain starter units are known from different species (15,40,69,99). However, all efforts to generate a BCFA-negative mutant in *M. xanthus* DK1622 were unsuccessful, probably because the candidate genes MXAN_0215 and MXAN_7353 have complementary functions, a phenomenon often encountered in myxobacteria due to the lineage-specific gene duplications (35,108,113). If both genes are functional and essential for BCFA biosynthesis, a double inactivation may be lethal. Heterologous expression of myxobacterial FabHs in *Pseudomonas putida* KT2440 with leucine as the sole carbon source did not result in any phenotype (not shown), but this may be due to failure of interaction between the *Myxococcus* FabH and the *Pseudomonas* fatty acid biosynthesis machinery, as a proof of principle experiment with the FabH from *Streptomyces coelicolor* A3(2), whose functionality has been proven (103), did not work either. Furthermore, pseudomonads do not require FabH for their fatty acid biosynthesis (114), as shown by *in vitro* experiments with a mixture of the isolated enzymes (44). Thus, it is likely that the *Pseudomonas* ACP is not intended to interact with a FabH enzyme.

Some reports about *Streptomyces* strains (71,135) indicate that initiation of fatty acid biosynthesis can proceed by FabH-independent mechanisms via formation of a starter unit acyl-ACP. When both the starter and the elongation unit are present as acyl-ACP units, condensation can be catalyzed by FabB or FabF (Figure 3) instead of FabH, which needs an acyl-CoA for the starter unit. The alternative mechanism relies on an acetyl-CoA:ACP transacylase (ACAT), transferring the acetyl residue from coenzyme A to an ACP, or on acetyl-ACP formation by decarboxylation of malonyl-ACP (74). If, instead of an acetyl residue, an isovaleryl residue was linked to the ACP, the model would comply with

myxobacterial fatty acid profiles. Although there are some indications that distinct enzymes with ACAT activity exist in plants (41), this type of reaction has only been shown to be a side activity of other enzymes so far, such as a thiolase from *Streptomyces collinus* (71) or even FabH enzymes themselves (41,74). BlastP queries against the genomes of *M. xanthus* DK1622 and *S. aurantiaca* DW4/3-1 using the amino acid sequence of the *S. collinus* thiolase (71) gave MXAN_3791 and STIAU_6552 as hits, with 63 and 62 % homology, respectively. Both genes are annotated as acetyl-CoA acetyltransferases, so one would expect them to catalyze the formation of acetoacetyl-CoA for the mevalonate pathway (Chapter 2.4 and Figure 8), which corresponds to the reverse reaction of a thiolase. Due to these sequence and functional relationships, it is possible that the myxobacterial enzymes also exhibit transacylase activity.

If myxobacteria use a FabH-independent mechanism for fatty acid biosynthesis, inactivation of both *fabH* candidate genes in *M. xanthus* DK1622 at the same time would not be lethal, but the transacylase gene would still have to be found. Further investigations in this field will comprise additional *fabH* inactivation experiments in both *M. xanthus* and *S. aurantiaca*, as well as inactivations of the above mentioned candidate genes.

3.2.3. The bridge from fatty acid biosynthesis to leucine degradation and isoprenoid formation

Under vegetative conditions, leucine is not only used for protein and BCFA biosynthesis, but a significant amount is channeled into the mevalonate pathway (Chapter 2.4), as determined by feeding experiments and analysis of the incorporation into the isoprenoid geosmin (see also Chapter 1.3 and Figure 8). This connection between primary and secondary metabolism provides some metabolic versatility, in particular under developmental conditions: Fruiting body formation is not only induced under general starvation conditions, but the limitation of one amino acid (79) as well as an overall step-

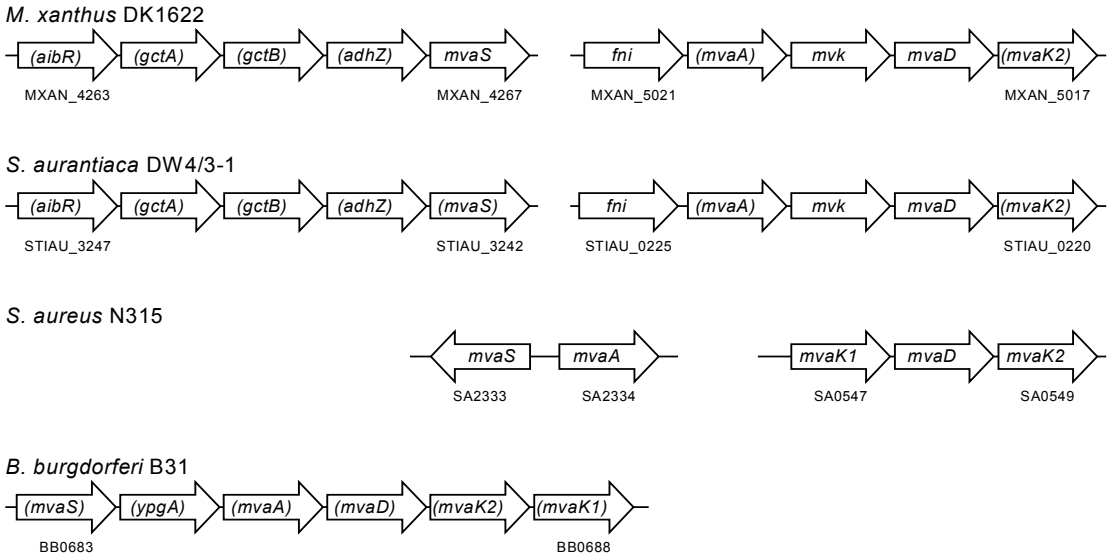


Figure 9. Genetic organization of the mevalonate pathway genes in different bacterial species.

The mevalonate pathway gene clusters of *M. xanthus* DK1622 (35), *S. aurantiaca* DW4/3-1 (108), *Staphylococcus aureus* N315 (65) and *Borrelia burgdorferi* B31 (31) are depicted. Clusters are arranged for comparison regardless of their position in the genome.
For an explanaition of gene designations see 4.2. Designations which have been assigned according to homologies to other genes, but which are not used in the genome annotations, are enclosed in brackets.

down in nutrition (5) may be sufficient. Under these conditions, which reflect the natural circumstances more than the usual laboratory conditions of sudden starvation (43), the organism is capable of utilizing acetyl-CoA from the degradation of the remaining nutrients to maintain the biosynthesis of isoprenoids and BCFAs in the absence of leucine. Evidence has come up from a recent study that not only BCFAs, but also isoprenoids are essential for development, as fruiting body formation in a mutant deficient in isovaleryl-CoA biosynthesis from both leucine and HMG-CoA was partially restored by the addition of mevalonolactone (72). Therefore, it is all the more important that isoprenoid biosynthesis is still supported under nutrient limitation and developmental conditions.

The fact that the genes encoding the enzymes of the alternative pathway for isovaleryl-CoA formation constitute an operon with *mvaS* (see Chapter 2.4 and Figure 9), which, in turn, is not transcriptionally linked to *mvaA*, brings up the hypothesis that the primary intended function of MvaS in *M. xanthus* is the support of this mechanism rather than isoprenoid biosynthesis. It has to be mentioned that, in most other bacteria, isoprenoids are synthesized by the "non-mevalonate" pathway via 1-deoxy-D-xylulose 5-phosphate (67). However, in bacteria using the mevalonate pathway (mainly Gram-positive cocci), *mvaA* and *mvaS* are either organized in one operon or are at least located in close proximity to each other in the genome (142). On the other hand, *mvaA* is in an operon with the other genes of the mevalonate pathway in *M. xanthus* and *S. aurantiaca* (Chapter 2.4 and Figure 9). It is suggested that the Gram-positive cocci have acquired these genes by horizontal gene transfer (142), which is also thought to be the background of *M. xanthus* secondary metabolite biosynthesis genes (35). If this was the case for the mevalonate pathway in *M. xanthus* as well, these genes would most likely originate from a different source due to the different gene organization.

3.3. Concluding remarks

It has been proposed that BCFAs or distinct BCFA species constitute the E-signal (23), but this model can be put into question regarding the data obtained during this study. BCFAs have to be considered a prerequisite for proper development, but the ability to restore development in DK5624 is not restricted to *iso*-fatty acids, but seems to be exerted by all substances acting as exogenous sources for *iso*-fatty acids or restoring *iso*-fatty acid biosynthesis. This may be an explanation why the formation of viable spores in DK5624 was not restored by TG-2 and TPG, but by TG-1 (Chapter 2.3), since this lipid contains *iso*-15:0 acyl residues which can be liberated by hydrolysis. However, all three lipids (TG-1, TG-2 and TPG) restored aggregation, but the underlying mechanism is still elusive. In addition, it cannot be ruled out from the presented data that BCFAs or substances derived from isovaleryl-CoA have signaling functions. It is still remarkable that a decreased level of *iso*-fatty acids causes developmental defects (Chapter 2.3) and several changes in the global expression pattern (Chapter 2.4).

Besides BCFAs, some SCFA-mediated phenomena have been described, such as the involvement of these fatty acids in autolysis (131) and chemotaxis (12). However, models which postulate essential roles of these phenomena for development should be reexamined regarding the observation that SCFAs are required neither for normal vegetative growth nor for developmental functions (Chapter 2.1). An explanation may be that the SCFA-deficient mutant strain uses alternative mechanisms to maintain proper developmental functions in the absence of SCFAs, like the *bkd* mutant produces isovaleryl-CoA via the alternative pathway (Chapters 2.2 and 2.4).

In summary, the present study gave many insights into the lipidomes of *Myxococcus xanthus* and *Stigmatella aurantiaca*, with the focus on lipids which are not ubiquitous among bacteria. These include ether lipids (Chapter 2.3), triacylglycerols (Chapter 2.5)

and sphingolipids (Chapter 2.7), and all three lipid classes are influenced by the developmental program of myxobacteria. Further investigations will have to concentrate on the roles of these lipids for proper function of the complex regulatory network directing the myxobacterial life cycle, and on the enzymes involved in lipid modification and transformation and in lipid-mediated developmental processes.

4. Appendix

4.1. Abbreviations

ACAT	acetyl-CoA:acyl-carrier protein transacylase
ACP	acyl-carrier protein
AEPE	alkyl ether phosphatidylethanolamine, in this context: 1-*O*-(13-methyltetradecyl)-2-*O*-(13-methyltetradecanoyl)-glycero-3-phosphatidylethanolamine
BCFA	branched-chain fatty acid
CoA	coenzyme A
DMA	dimethyl acetal
EC	enzyme commission
FA	fatty acid
FAME	fatty acid methyl ester
GC	gas chromatography
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
KAS	β-ketoacyl-ACP synthase
KIC	α-ketoisocaproate *syn.* 4-methyl-2-oxobutanoate
KIV	α-ketoisovalerate *syn.* 3-methyl-2-oxopropanoate
KMV	α–keto-β-methylvalerate *syn.* 3-methyl-2-oxobutanoate
MS	mass spectrometry

OAG	1-*O*-alkylglycerol
PE	phosphatidylethanolamine
SCFA	straight-chain fatty acid
TAG	triacylglycerol
TG-1	1,2-di-(13-methyltetradecanoyl)-3-(13-methyltetradecyl)glycerol
TG-2	1,2-di-(hexadecanoyl)-3-(13-methyltetradecyl)-glycerol
TPG	glycerol tripalmitate
UV	ultraviolet
VEPE	vinyl ether phosphatidylethanolamine, in this context: 1-*O*-(13-methyl-1-*Z*-tetradecenyl)-2-*O*-(13-methyl-tetradecanoyl)-glycero-3-phosphatidylethanolamine

4.2. Gene and locus designations

Designation	**Encoded protein**	**EC number**
adhZ	alcohol dehydrogenase, Zn-binding	1.1.1.1
aibR	alternative isovaleryl-CoA biosynthesis regulator	
bkd	branched-chain keto acid dehydrogenase	1.2.4.4 and 2.3.1.168
fabB or *fabF*	3-oxoacyl-ACP synthase (KAS) I or II	2.3.1.41 or 2.3.1.179
fabG	3-oxoacyl-ACP reductase	1.1.1.100

Designation	**Encoded protein**	**EC number**
fabH	3-oxoacyl-ACP synthase III	2.3.1.180
fabI, fabK or *fabL*	enoyl-ACP reductase	1.3.1.-
fabZ or *fabA*	3-hydroxyacyl-ACP dehydratase	4.2.1.-
fni	isopentenyl-diphosphate Δ-isomerase	5.3.3.2
gctA and *gctB*	glutaconyl-CoA transferase subunits A and B	
ilvE	branched-chain amino acid aminotransferase	2.6.1.42
liuA	isovaleryl-CoA dehydrogenase	1.3.99.10
liuB	3-methylcrotonyl-CoA carboxylase	6.4.1.4
	syn. 3,3-dimethylacrylyl-CoA carboxylase	
liuC	3-methylglutaconyl-CoA hydratase	4.2.1.18
mvaA	HMG-CoA reductase	1.1.1.34
mvaD	diphosphomevalonate decarboxylase	4.1.1.33
mvaK1 or *mvk*	mevalonate kinase	2.7.1.36
mvaK2	phosphomevalonate kinase	2.7.4.2
mvaS	HMG-CoA synthase	2.3.3.10
poxB	pyruvate dehydrogenase	1.2.2.2
spt	serine palmitoyltransferase	2.3.1.50
ypgA	carotenoid biosynthesis protein	

5. References

1. **Alderson, N. L., B. M. Rembiesa, M. D. Walla, A. Bielawska, J. Bielawski, and H. Hama**. 2004. The human FA2H gene encodes a fatty acid 2-hydroxylase. *J. Biol. Chem.* **279** (47), 48562-48568.
2. **Alvarez, H. M. and A. Steinbüchel**. 2002. Triacylglycerols in prokaryotic microorganisms. *Appl. Microbiol. Biotechnol.* **60** (4), 367-376.
3. **Avadhani, M., R. Geyer, D. C. White, and L. J. Shimkets**. 2006. Lysophosphatidylethanolamine is a substrate for the short-chain alcohol dehydrogenase SocA from *Myxococcus xanthus*. *J. Bacteriol.* **188** (24), 8543-8550.
4. **Beck, H. C.** 2005. Branched-chain fatty acid biosynthesis in a branched-chain amino acid aminotransferase mutant of *Staphylococcus carnosus*. *FEMS Microbiol. Lett.* **243** (1), 37-44.
5. **Berleman, J. E. and J. R. Kirby**. 2007. Multicellular development in *Myxococcus xanthus* is stimulated by predator-prey interactions. *J. Bacteriol.* **189** (15), 5675-5682.
6. **Berleman, J. E., J. Scott, T. Chumley, and J. R. Kirby**. 2008. Predataxis behavior in *Myxococcus xanthus*. *Proc. Natl. Acad. Sci. U. S. A* **105** (44), 17127-17132.
7. **Bode, H. B., J. S. Dickschat, R. M. Kroppenstedt, S. Schulz, and R. Müller**. 2005. Biosynthesis of iso-fatty acids in myxobacteria: iso-even fatty acids are derived by α-oxidation of iso-odd fatty acids. *J. Am. Chem. Soc.* **127** (2), 532-533.
8. **Bode, H. B., H. Irschik, S. C. Wenzel, H. Reichenbach, R. Müller, and G. Höfle**. 2003. The leupyrrins: a structurally unique family of secondary metabolites from the myxobacterium *Sorangium cellulosum*. *J. Nat. Prod.* **66** (9), 1203-1206.
9. **Bode, H. B. and R. Müller**. 2006. Analysis of myxobacterial secondary metabolism goes molecular. *J. Ind. Microbiol. Biotechnol.* **33** (7), 577-588.
10. **Bode, H. B., S. C. Wenzel, H. Irschik, G. Höfle, and R. Müller**. 2004. Unusual biosynthesis of leupyrrins in the myxobacterium *Sorangium cellulosum*. *Angew. Chem. Int. Ed.* **43** (32), 4163-4167.
11. **Bollag, D. M., P. A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides, and M. Woods**. 1995. Epothilones, a new class of microtubule-stabilizing agents with taxol-like mechanism of action. *Cancer Res.* **55** (11), 2325-2333.
12. **Bonner, P. J. and L. J. Shimkets**. 2006. Phospholipid directed motility of surface-motile bacteria. *Mol. Microbiol.* **61** (5), 1101-1109.
13. **Bretscher, A. P. and D. Kaiser**. 1978. Nutrition of *Myxococcus xanthus*, a fruiting myxobacterium. *J. Bacteriol.* **133** (2), 763-768.
14. **Caillon, E., B. Lubochinsky, and D. Rigomier**. 1983. Occurrence of dialkyl ether phospholipids in *Stigmatella aurantiaca* DW4. *J. Bacteriol.* **153** (3), 1348-1351.
15. **Choi, K. H., R. J. Heath, and C. O. Rock**. 2000. β-ketoacyl-acyl carrier protein synthase III (FabH) is a determining factor in branched-chain fatty acid biosynthesis. *J. Bacteriol.* **182** (2), 365-370.
16. **Conner, R. L., K. E. Koo, and J. R. Landrey**. 1974. Isovaleric acid as a precursor of odd numbered iso fatty acids in Tetrahymena. *Lipids* **9** (8), 554-559.
17. **Cropp, T. A., A. A. Smogowicz, E. W. Hafner, C. D. Denoya, H. A. McArthur, and K. A. Reynolds**. 2000. Fatty-acid biosynthesis in a branched-chain α-keto acid dehydrogenase mutant of *Streptomyces avermitilis*. *Can. J. Microbiol.* **46** (6), 506-514.

18. **Dahlqvist, A., U. Ståhl, M. Lenman, A. Banas, M. Lee, L. Sandager, H. Ronne, and S. Stymne**. 2000. Phospholipid:diacylglycerol acyltransferase: an enzyme that catalyzes the acyl-CoA-independent formation of triacylglycerol in yeast and plants. *Proc. Natl. Acad. Sci. U. S. A.* **97** (12), 6487-6492.
19. **Dawid, W.** 2000. Biology and global distribution of myxobacteria in soils. *FEMS Microbiol. Rev.* **24** (4), 403-427.
20. **Dickschat, J. S., H. B. Bode, R. M. Kroppenstedt, R. Müller, and S. Schulz**. 2005. Biosynthesis of iso-fatty acids in myxobacteria. *Org. Biomol. Chem.* **3** (15), 2824-2831.
21. **Dickschat, J. S., H. B. Bode, T. Mahmud, R. Müller, and S. Schulz**. 2005. A novel type of geosmin biosynthesis in myxobacteria. *J. Org. Chem.* **70** (13), 5174-5182.
22. **Downard, J., S. V. Ramaswamy, and K. S. Kil**. 1993. Identification of *esg*, a genetic locus involved in cell-cell signaling during *Myxococcus xanthus* development. *J. Bacteriol.* **175** (24), 7762-7770.
23. **Downard, J. and D. Toal**. 1995. Branched-chain fatty acids: the case for a novel form of cell-cell signalling during *Myxococcus xanthus* development. *Mol. Microbiol.* **16** (2), 171-175.
24. **Dunn, T. M., D. Haak, E. Monaghan, and T. J. Beeler**. 1998. Synthesis of monohydroxylated inositolphosphorylceramide (IPC-C) in *Saccharomyces cerevisiae* requires Scs7p, a protein with both a cytochrome b_5-like domain and a hydroxylase/desaturase domain. *Yeast* **14** (4), 311-321.
25. **Dworkin, M.** 1966. Biology of the myxobacteria. *Annu. Rev. Microbiol.* **20**, 75-106.
26. **Dworkin, M.** 1996. Recent advances in the social and developmental biology of the myxobacteria. *Microbiol. Rev.* **60** (1), 70-102.
27. **Dworkin, M. and W. Sadler**. 1966. Induction of cellular morphogenesis in *Myxococcus xanthus* .I. General description. *J. Bacteriol.* **91** (4), 1516-1519.
28. **Elias-Arnanz, M., M. Fontes, and S. Padmanabhan**. 2007. Carotenogenesis in *Myxococcus xanthus*: a complex regulatory network., p. 211-225. *In* D. Whitworth (ed.), *Myxobacteria: multicellularity and differentiation*. ASM Press, Chicago.
29. **Fautz, E., G. Rosenfelder, and L. Grotjahn**. 1979. Iso-branched 2- and 3-hydroxy fatty acids as characteristic lipid constituents of some gliding bacteria. *J. Bacteriol.* **140** (3), 852-858.
30. **Foulon, V., M. Sniekers, E. Huysmans, S. Asselberghs, V. Mahieu, G. P. Mannaerts, P. P. van Veldhoven, and M. Casteels**. 2005. Breakdown of 2-hydroxylated straight chain fatty acids via peroxisomal 2-hydroxyphytanoyl-CoA lyase. *J. Biol. Chem.* **280** (11), 9802-9812.
31. **Fraser, C. M., S. Casjens, W. M. Huang, G. G. Sutton, R. Clayton, R. Lathigra, O. White, K. A. Ketchum, R. Dodson, E. K. Hickey, M. Gwinn, B. Dougherty, J. F. Tomb, R. D. Fleischmann, D. Richardson, J. Peterson, A. R. Kerlavage, J. Quackenbush, S. Salzberg, M. Hanson, R. van Vugt, N. Palmer, M. D. Adams, J. Gocayne, J. Weidman, T. Utterback, L. Watthey, L. McDonald, P. Artiach, C. Bowman, S. Garland, C. Fujii, M. D. Cotton, K. Horst, K. Roberts, B. Hatch, H. O. Smith, and J. C. Venter**. 1997. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* **390** (6660), 580-586.
32. **Freiberg, C., N. Brunner, L. Macko, and H. P. Fischer**. 2006. Discovering antibiotic efficacy biomarkers: toward mechanism-specific high content compound screening. *Mol. Cell Proteomics* **5** (12), 2326-2335.

33. **Gerber, N. N.** 1968. Geosmin, from microorganisms, is trans-1,10-dimethyl-trans-9-decalol. *Tetrahedron Lett.* **9** (25), 2971-2974.
34. **Gerth, K., S. Pradella, O. Perlova, S. Beyer, and R. Müller**. 2003. Myxobacteria: proficient producers of novel natural products with various biological activities - past and future biotechnological aspects with the focus on the genus *Sorangium*. *J. Biotechnol.* **106** (2-3), 233-253.
35. **Goldman, B. S., W. C. Nierman, D. Kaiser, S. C. Slater, A. S. Durkin, J. Eisen, C. M. Ronning, W. B. Barbazuk, M. Blanchard, C. Field, C. Halling, G. Hinkle, O. Iartchuk, H. S. Kim, C. Mackenzie, R. Madupu, N. Miller, A. Shvartsbeyn, S. A. Sullivan, M. Vaudin, R. Wiegand, and H. B. Kaplan**. 2006. Evolution of sensory complexity recorded in a myxobacterial genome. *Proc. Natl. Acad. Sci. U. S. A.* **103** (41), 15200-15205.
36. **Gorski, L. and D. Kaiser**. 1998. Targeted mutagenesis of σ^{54} activator proteins in *Myxococcus xanthus*. *J. Bacteriol.* **180** (22), 5896-5905.
37. **Haak, D., K. Gable, T. Beeler, and T. Dunn**. 1997. Hydroxylation of *Saccharomyces cerevisiae* ceramides requires Sur2p and Scs7p. *J. Biol. Chem.* **272** (47), 29704-29710.
38. **Hagen, D. C., A. P. Bretscher, and D. Kaiser**. 1978. Synergism between morphogenetic mutants of *Myxococcus xanthus*. *Dev. Biol.* **64** (2), 284-296.
39. **Hamberg, M., I. P. de Leon, M. J. Rodriguez, and C. Castresana**. 2005. Fatty acid α-dioxygenases. *Biochem. Biophys. Res. Commun.* **338** (1), 169-174.
40. **Han, L., S. Lobo, and K. A. Reynolds**. 1998. Characterization of β-ketoacyl-acyl carrier protein synthase III from *Streptomyces glaucescens* and its role in initiation of fatty acid biosynthesis. *J. Bacteriol.* **180** (17), 4481-4486.
41. **Harwood, J. L.** 1996. Recent advances in the biosynthesis of plant fatty acids. *Biochim. Biophys. Acta* **1301** (1-2), 7-56.
42. **Heath, R. J. and C. O. Rock**. 1996. Inhibition of β-ketoacyl-acyl carrier protein synthase III (FabH) by acyl-acyl carrier protein in *Escherichia coli*. *J. Biol. Chem.* **271** (18), 10996-11000.
43. **Higgs, P. I. and J. P. Merlie, Jr.** 2007. *Myxococcus xanthus*: Cultivation, motility, and development, p. 465-478. *In* D. Whitworth (ed.), *Myxobacteria: multicellularity and differentiation*. ASM Press, Chicago.
44. **Hoang, T. T., S. A. Sullivan, J. K. Cusick, and H. P. Schweizer**. 2002. β-Ketoacyl acyl carrier protein reductase (FabG) activity of the fatty acid biosynthetic pathway is a determining factor of 3-oxo-homoserine lactone acyl chain lengths. *Microbiology* **148** (12), 3849-3856.
45. **Iizuka, T., Y. Jojima, R. Fudou, A. Hiraishi, J. W. Ahn, and S. Yamanaka**. 2003. *Plesiocystis pacifica* gen. nov., sp. nov., a marine myxobacterium that contains dihydrogenated menaquinone, isolated from the pacific coasts of Japan. *Int. J. Syst. Evol. Microbiol.* **53** (Pt 1), 189-195.
46. **Iizuka, T., Y. Jojima, R. Fudou, M. Tokura, A. Hiraishi, and S. Yamanaka**. 2003. *Enhygromyxa salina* gen. nov., sp. nov., a slightly halophilic myxobacterium isolated from the coastal areas of Japan. *Syst. Appl. Microbiol.* **26** (2), 189-196.
47. **Inouye, M., S. Inouye, and D. R. Zusman**. 1979. Gene expression during development of *Myxococcus xanthus*: pattern of protein synthesis. *Dev. Biol.* **68** (2), 579-591.
48. **Inouye, M., S. Inouye, and D. R. Zusman**. 1979. Biosynthesis and self-assembly of protein S, a development-specific protein of *Myxococcus xanthus*. *Proc. Natl. Acad. Sci. U. S. A.* **76** (1), 209-213.

49. **Jakobsen, J. S., L. Jelsbak, R. D. Welch, C. Cummings, B. Goldman, E. Stark, S. Slater, and D. Kaiser**. 2004. σ^{54} enhancer binding proteins and *Myxococcus xanthus* fruiting body development. *J. Bacteriol.* **186** (13), 4361-4368.
50. **Johnston, N. C. and H. Goldfine**. 1994. Isolation and characterization of new phosphatidylglycerol acetals of plasmalogens. A family of ether lipids in clostridia. *Eur. J. Biochem.* **223** (3), 957-963.
51. **Jones, J. M., J. C. Morrell, and S. J. Gould**. 2000. Identification and characterization of HAOX1, HAOX2, and HAOX3, three human peroxisomal 2-hydroxy acid oxidases. *J. Biol. Chem.* **275** (17), 12590-12597.
52. **Kaiser, D.** 1979. Social gliding is correlated with the presence of pili in *Myxococcus xanthus*. *Proc. Natl. Acad. Sci. U. S. A.* **76** (11), 5952-5956.
53. **Kaiser, D.** 2003. Coupling cell movement to multicellular development in myxobacteria. *Nat. Rev. Microbiol.* **1** (1), 45-54.
54. **Kaiser, D.** 2004. Signaling in myxobacteria. *Annu. Rev. Microbiol.* **58**, 75-98.
55. **Kaneda, T.** 1991. Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. *Microbiol. Rev.* **55** (2), 288-302.
56. **Kikuchi, A., H. Sagami, and K. Ogura**. 1999. Evidence for covalent attachment of diphytanylglyceryl phosphate to the cell-surface glycoprotein of *Halobacterium halobium*. *J. Biol. Chem.* **274** (25), 18011-18016.
57. **Kim, S. K. and D. Kaiser**. 1990. Cell alignment required in differentiation of *Myxococcus xanthus*. *Science* **249** (4971), 926-928.
58. **Kleinig, H.** 1972. Membranes from *Myxococcus fulvus* (myxobacterales) containing carotenoid glucosides: I. Isolation and composition. *Biochim. Biophys. Acta* **274** (2), 489-498.
59. **Koch, A. L. and D. White**. 1998. The social lifestyle of myxobacteria. *Bioessays* **20** (12), 1030-1038.
60. **Koga, Y. and H. Morii**. 2007. Biosynthesis of ether-type polar lipids in archaea and evolutionary considerations. *Microbiol. Mol. Biol. Rev.* **71** (1), 97-120.
61. **Konrad, Z. and J. Eichler**. 2002. Lipid modification of proteins in archaea: attachment of a mevalonic acid-based lipid moiety to the surface-layer glycoprotein of *Haloferax volcanii* follows protein translocation. *Biochem. J.* **366** (3), 959-964.
62. **Kort, R., B. Keijser, M. Caspers, F. Schuren, and R. Montijn**. 2008. Transcriptional activity around bacterial cell death reveals molecular biomarkers for cell viability. *BMC Genomics* **9** (1), 590.
63. **Kroos, L., A. Kuspa, and D. Kaiser**. 1986. A global analysis of developmentally regulated genes in *Myxococcus xanthus*. *Dev. Biol.* **117** (1), 252-266.
64. **Kuner, J. M. and D. Kaiser**. 1982. Fruiting body morphogenesis in submerged cultures of *Myxococcus xanthus*. *J. Bacteriol.* **151** (1), 458-461.
65. **Kuroda, M., T. Ohta, I. Uchiyama, T. Baba, H. Yuzawa, I. Kobayashi, L. Cui, A. Oguchi, K. i. Aoki, Y. Nagai, J. Lian, T. Ito, M. Kanamori, H. Matsumaru, A. Maruyama, H. Murakami, A. Hosoyama, Y. Mizutani-Ui, N. K. Takahashi, T. Sawano, R. i. Inoue, C. Kaito, K. Sekimizu, H. Hirakawa, S. Kuhara, S. Goto, J. Yabuzaki, M. Kanehisa, A. Yamashita, K. Oshima, K. Furuya, C. Yoshino, T. Shiba, M. Hattori, N. Ogasawara, H. Hayashi, and K. Hiramatsu**. 2001. Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. *Lancet* **357** (9264), 1225-1240.
66. **Kuspa, A., L. Plamann, and D. Kaiser**. 1992. A-signalling and the cell density requirement for *Myxococcus xanthus* development. *J. Bacteriol.* **174** (22), 7360-7369.

67. **Kuzuyama, T.** 2002. Mevalonate and nonmevalonate pathways for the biosynthesis of isoprene units. *Biosci. Biotechnol. Biochem.* **66** (8), 1619-1627.
68. **Lee, K. and L. J. Shimkets**. 1996. Suppression of a signaling defect during *Myxococcus xanthus* development. *J. Bacteriol.* **178** (4), 977-984.
69. **Li, Y., G. Florova, and K. A. Reynolds**. 2005. Alteration of the fatty acid profile of *Streptomyces coelicolor* by replacement of the initiation enzyme 3-ketoacyl acyl carrier protein synthase III (FabH). *J. Bacteriol.* **187** (11), 3795-3799.
70. **Lobedanz, S. and L. Søgaard-Andersen**. 2003. Identification of the C-signal, a contact-dependent morphogen coordinating multiple developmental responses in *Myxococcus xanthus*. *Genes Dev.* **17** (17), 2151-2161.
71. **Lobo, S., G. Florova, and K. A. Reynolds**. 2001. A *Streptomyces collinus* thiolase with novel acetyl-CoA:acyl carrier protein transacylase activity. *Biochemistry-US* **40** (39), 11955-11964.
72. **Lorenzen, W., M. W. Ring, G. Schwär, and H. B. Bode**. 2009. Isoprenoids are essential for fruiting body formation in *Myxococcus xanthus*. *J. Bacteriol.* **191** (18), 5849-5853.
73. **Lu, Y.-J., Y.-M. Zhang, and C. O. Rock**. 2004. Product diversity and regulation of type II fatty acid synthases. *Biochem. Cell Biol.* **82** (1), 145-155.
74. **Magnuson, K., S. Jackowski, C. O. Rock, and J. E. Cronan, Jr.** 1993. Regulation of fatty acid biosynthesis in *Escherichia coli*. *Microbiol. Rev.* **57** (3), 522-542.
75. **Mahmud, T., S. C. Wenzel, E. Wan, K. W. Wen, H. B. Bode, N. Gaitatzis, and R. Müller**. 2005. A novel biosynthetic pathway to isovaleryl-CoA in myxobacteria: the involvement of the mevalonate pathway. *Chembiochem* **6** (2), 322-330.
76. **Mahmud, T., H. B. Bode, B. Silakowski, R. M. Kroppenstedt, M. Xu, S. Nordhoff, G. Höfle, and R. Müller**. 2002. A novel biosynthetic pathway providing precursors for fatty acid biosynthesis and secondary metabolite formation in myxobacteria. *J. Biol. Chem.* **277** (36), 23768-32774.
77. **Malins, D. C.** 1968. Metabolism of glycerol ether-containing lipids in dog fish (*Squalus acanthias*). *J. Lipid Res.* **9** (6), 687-692.
78. **Malins, D. C. and A. Barone**. 1970. Glyceryl ether metabolism: regulation of buoyancy in dogfish *Squalus acanthias*. *Science* **167** (3914), 79-80.
79. **Manoil, C. and D. Kaiser**. 1980. Guanosine pentaphosphate and guanosine tetraphosphate accumulation and induction of *Myxococcus xanthus* fruiting body development. *J. Bacteriol.* **141** (1), 305-315.
80. **Mansilla, M. C., L. E. Cybulski, D. Albanesi, and D. de Mendoza**. 2004. Control of membrane lipid fluidity by molecular thermosensors. *J. Bacteriol.* **186** (20), 6681-6688.
81. **Mansilla, M. C. and D. de Mendoza**. 2005. The *Bacillus subtilis* desaturase: a model to understand phospholipid modification and temperature sensing. *Arch. Microbiol.* **183** (4), 229-235.
82. **Massey, L. K., J. R. Sokatch, and R. S. Conrad**. 1976. Branched-chain amino acid catabolism in bacteria. *Microbiol. Mol. Biol. Rev.* **40** (1), 42-54.
83. **Matsunaga, I., N. Yokotani, O. Gotoh, E. Kusunose, M. Yamada, and K. Ichihara**. 1997. Molecular cloning and expression of fatty acid α-hydroxylase from *Sphingomonas paucimobilis*. *J. Biol. Chem.* **272** (38), 23592-23596.
84. **McBride, M. J. and D. R. Zusman**. 1989. Trehalose accumulation in vegetative cells and spores of *Myxococcus xanthus*. *J. Bacteriol.* **171** (11), 6383-6386.

85. **McConville, M. J. and M. A. Ferguson**. 1993. The structure, biosynthesis and function of glycosylated phosphatidylinositols in the parasitic protozoa and higher eukaryotes. *Biochem. J.* **294** (Pt 2), 305-324.
86. **Meiser, P., H. B. Bode, and R. Müller**. 2006. The unique DKxanthene secondary metabolite family from the myxobacterium *Myxococcus xanthus* is required for developmental sporulation. *Proc. Natl. Acad. Sci. U. S. A.* **103** (50), 19128-19133.
87. **Mitchell, A. G. and C. E. Martin**. 1997. Fah1p, a *Saccharomyces cerevisiae* cytochrome b_5 fusion protein, and its *Arabidopsis thaliana* homolog that lacks the cytochrome b_5 domain both function in the α-hydroxylation of sphingolipid-associated very long chain fatty acids. *J. Biol. Chem.* **272** (45), 28281-28288.
88. **Murata, N. and H. Wada**. 1995. Acyl-lipid desaturases and their importance in the tolerance and acclimatization to cold of cyanobacteria. *Biochem. J.* **308** (Pt 1), 1-8.
89. **Murphy, D. J.** 2001. The biogenesis and functions of lipid bodies in animals, plants and microorganisms. *Prog. Lipid Res.* **40** (5), 325-438.
90. **Neumann, B., A. Pospiech, and H. U. Schairer**. 1992. Size and stability of the genomes of the myxobacteria *Stigmatella aurantiaca* and *Stigmatella erecta*. *J. Bacteriol.* **174** (19), 6307-6310.
91. **O'Connor, K. A. and D. R. Zusman**. 1991. Analysis of *Myxococcus xanthus* cell types by two-dimensional polyacrylamide gel electrophoresis. *J. Bacteriol.* **173** (11), 3334-3341.
92. **O'Connor, K. A. and D. R. Zusman**. 1991. Behavior of peripheral rods and their role in the life cycle of *Myxococcus xanthus*. *J. Bacteriol.* **173** (11), 3342-3355.
93. **O'Connor, K. A. and D. R. Zusman**. 1991. Development in *Myxococcus xanthus* involves differentiation into two cell types, peripheral rods and spores. *J. Bacteriol.* **173** (11), 3318-3333.
94. **Oku, H. and T. Kaneda**. 1988. Biosynthesis of branched-chain fatty acids in *Bacillus subtilis*. A decarboxylase is essential for branched-chain fatty acid synthetase. *J. Biol. Chem.* **263** (34), 18386-18396.
95. **Orndorff, P. E. and M. Dworkin**. 1980. Separation and properties of the cytoplasmic and outer membranes of vegetative cells of *Myxococcus xanthus*. *J. Bacteriol.* **141** (2), 914-927.
96. **Perham, R. N.** 1991. Domains, motifs, and linkers in 2-oxo acid dehydrogenase multienzyme complexes: a paradigm in the design of a multifunctional protein. *Biochemistry-US* **30** (35), 8501-8512.
97. **Peypoux, F., J. M. Bonmatin, and J. Wallach**. 1999. Recent trends in the biochemistry of surfactin. *Appl. Microbiol. Biotechnol.* **51** (5), 553-563.
98. **Plaga, W. and S. H. Ulrich**. 1999. Intercellular signalling in *Stigmatella aurantiaca*. *Curr. Opin. Microbiol.* **2** (6), 593-597.
99. **Qiu, X. Y., A. E. Choudhry, C. A. Janson, M. Grooms, R. A. Daines, J. T. Lonsdale, and S. S. Khandekar**. 2005. Crystal structure and substrate specificity of the β-ketoacyl-acyl carrier protein synthase III (FabH) from *Staphylococcus aureus*. *Protein Sci.* **14** (8), 2087-2094.
100. **Rachid, S., K. Gerth, I. Kochems, and R. Müller**. 2007. Deciphering regulatory mechanisms for secondary metabolite production in the myxobacterium *Sorangium cellulosum* So ce56. *Mol. Microbiol.* **63** (6), 1783-1796.
101. **Reichenbach, H.** 1999. The ecology of the myxobacteria. *Environ. Microbiol.* **1** (1), 15-21.
102. **Reichenbach, H. and G. Höfle**. 1993. Biologically active secondary metabolites from myxobacteria. *Biotechnol. Adv.* **11** (2), 219-277.

103. **Revill, W. P., M. J. Bibb, A. K. Scheu, H. J. Kieser, and D. A. Hopwood**. 2001. β-ketoacyl acyl carrier protein synthase III (FabH) is essential for fatty acid biosynthesis in *Streptomyces coelicolor* A3(2). *J. Bacteriol.* **183** (11), 3526-3530.
104. **Rilling, H. C., E. Bruenger, W. W. Epstein, and A. A. Kandutsch**. 1989. Prenylated proteins: demonstration of a thioether linkage to cysteine of proteins. *Biochem. Biophys. Res. Commun.* **163** (1), 143-148.
105. **Rivera, E., J. Lee, and A. Davies**. 2008. Clinical development of ixabepilone and other epothilones in patients with advanced solid tumors. *Oncologist.* **13** (12), 1207-1223.
106. **Rock, C. O. and S. Jackowski**. 2002. Forty years of bacterial fatty acid synthesis. *Biochem. Biophys. Res. Commun.* **292** (5), 1155-1166.
107. **Rolbetzki, A., M. Ammon, V. Jakovljevic, A. Konovalova, and L. Søgaard-Andersen**. 2008. Regulated secretion of a protease activates intercellular signaling during fruiting body formation in *M. xanthus*. *Dev. Cell.* **15** (4), 627-634.
108. **Ronning, C. M. and W. C. Nierman**. 2007. The genomes of *Myxococcus xanthus* and *Stigmatella aurantiaca*, p. 285-298. *In* D. Whitworth (ed.), *Myxobacteria: multicellularity and differentiation*. ASM Press, Chicago.
109. **Rosenbluh, A. and E. Rosenberg**. 1989. Sporulation of *Myxococcus xanthus* in liquid shake flask cultures. *J. Bacteriol.* **171** (8), 4521-4524.
110. **Sandmann, A., J. Dickschat, H. Jenke-Kodama, B. Kunze, E. Dittmann, and R. Müller**. 2007. A type II polyketide synthase from the Gram-negative bacterium *Stigmatella aurantiaca* is involved in aurachin alkaloid biosynthesis. *Angew. Chem. Int. Ed.* **46** (15), 2712-2716.
111. **Sankaran, K. and H. C. Wu**. 1994. Lipid modification of bacterial prolipoprotein. Transfer of diacylglyceryl moiety from phosphatidylglycerol. *J. Biol. Chem.* **269** (31), 19701-19706.
112. **Scarsdale, J. N., G. Kazanina, X. He, K. A. Reynolds, and H. T. Wright**. 2001. Crystal structure of the *Mycobacterium tuberculosis* β-ketoacyl-acyl carrier protein synthase III. *J. Biol. Chem.* **276** (23), 20516-20522.
113. **Schneiker, S., O. Perlova, O. Kaiser, K. Gerth, A. Alici, M. O. Altmeyer, D. Bartels, T. Bekel, S. Beyer, E. Bode, H. B. Bode, C. J. Bolten, J. V. Choudhuri, S. Doss, Y. A. Elnakady, B. Frank, L. Gaigalat, A. Goesmann, C. Groeger, F. Gross, L. Jelsbak, L. Jelsbak, J. Kalinowski, C. Kegler, T. Knauber, S. Konietzny, M. Kopp, L. Krause, D. Krug, B. Linke, T. Mahmud, R. Martinez-Arias, A. C. McHardy, M. Merai, F. Meyer, S. Mormann, J. Munoz-Dorado, J. Perez, S. Pradella, S. Rachid, G. Raddatz, F. Rosenau, C. Ruckert, F. Sasse, M. Scharfe, S. C. Schuster, G. Suen, A. Treuner-Lange, G. J. Velicer, F. J. Vorholter, K. J. Weissman, R. D. Welch, S. C. Wenzel, D. E. Whitworth, S. Wilhelm, C. Wittmann, H. Blocker, A. Puhler, and R. Muller**. 2007. Complete genome sequence of the myxobacterium *Sorangium cellulosum*. *Nat. Biotechnol.* **25** (11), 1281-1289.
114. **Schweizer, H. P.** 2004. Fatty acid biosynthesis and biologically significant acyl transfer reactions in pseudomonads, p. 83-110. *In* J.-L. Ramos (ed.), *Pseudomonas*, vol. 3. Kluwer Academic / Plenum Publishers, New York.
115. **Shimkets, L., M. Dworkin, and H. Reichenbach**. 2006. The myxobacteria, p. 31-115. *In* M. Dworkin (ed.), *The prokaryotes*, vol. 7. Springer, Berlin.
116. **Shimkets, L. J.** 1999. Intercellular signaling during fruiting-body development of *Myxococcus xanthus*. *Annu. Rev. Microbiol.* **53**, 525-549.

117. **Shimkets, L. J. and H. Rafiee**. 1990. CsgA, an extracellular protein essential for *Myxococcus xanthus* development. *J. Bacteriol.* **172** (9), 5299-5306.
118. **Silakowski, B., G. Nordsiek, B. Kunze, H. Blöcker, and R. Müller**. 2001. Novel features in a combined polyketide synthase/non-ribosomal peptide synthetase: the myxalamid biosynthetic gene cluster of the myxobacterium *Stigmatella aurantiaca* Sga15. *Chem. Biol.* **8** (1), 59-69.
119. **Silakowski, B., H. U. Schairer, H. Ehret, B. Kunze, S. Weinig, G. Nordsiek, P. Brandt, H. Blöcker, G. Höfle, S. Beyer, and R. Müller**. 1999. New lessons for combinatorial biosynthesis from myxobacteria: the myxothiazol biosynthetic gene cluster of *Stigmatella aurantiaca* DW4/3-1. *J. Biol. Chem.* **274** (52), 37391-37399.
120. **Singer, M. and D. Kaiser**. 1995. Ectopic production of guanosine penta- and tetraphosphate can initiate early developmental gene expression in *Myxococcus xanthus*. *Genes Dev.* **9** (13), 1633-1644.
121. **Singh, V. K., D. S. Hattangady, E. S. Giotis, A. K. Singh, N. R. Chamberlain, M. K. Stuart, and B. J. Wilkinson**. 2008. Insertional inactivation of branched-chain α-keto acid dehydrogenase in *Staphylococcus aureus* leads to decreased branched-chain membrane fatty acid content and increased susceptibility to certain stresses. *Appl. Environ. Microbiol.* **74** (19), 5882-5890.
122. **Stein, J. and H. Budzikiewicz**. 1987. 1-*O*-(13-Methyl-1-*Z*-tetradecenyl)-2-*O*-(13-methyltetradecanoyl)-glycero-3-phospho-ethanolamin, ein Plasmalogen aus *Myxococcus stipitatus*. *Z. Naturforsch.* **42b**, 1017-1020.
123. **Sudo, S. Z. and M. Dworkin**. 1969. Resistance of vegetative cells and microcysts of *Myxococcus xanthus*. *J. Bacteriol.* **98** (3), 883-887.
124. **Sudo, S. and M. Dworkin**. 1972. Bacteriolytic enzymes produced by *Myxococcus xanthus*. *J. Bacteriol.* **110** (1), 236-245.
125. **Tengra, F. K., J. L. Dahl, D. Dutton, N. B. Caberoy, L. Coyne, and A. G. Garza**. 2006. CbgA, a protein involved in cortex formation and stress resistance in *Myxococcus xanthus* spores. *J. Bacteriol.* **188** (23), 8299-8302.
126. **Toal, D. R., S. W. Clifton, B. A. Roe, and J. Downard**. 1995. The *esg* locus of *Myxococcus xanthus* encodes the E1α and E1β subunits of a branched-chain keto acid dehydrogenase. *Mol. Microbiol.* **16** (2), 177-189.
127. **Trowitzsch, W., L. Witte, and H. Reichenbach**. 1981. Geosmin from earthly smelling cultures of *Nannocystis exedens* (Myxobacterales). *FEMS Microbiol. Lett.* **12** (3), 257-260.
128. **Trowitzsch-Kienast, W., V. Wray, K. Gerth, H. Reichenbach, and G. Höfle**. 1986. Antibiotika aus gleitenden Bakterien, XXVIII: Biosynthese des Myxothiazols in *Myxococcus fulvus* Mx f16. *Liebigs Ann. Chem.* **1986** (1), 93-98.
129. **van de Vossenberg, J. L. C. M., A. J. M. Driessen, and W. N. Konings**. 1998. The essence of being extremophilic: the role of the unique archaeal membrane lipids. *Extremophiles* **2** (3), 163-170.
130. **van Golde, L. M., R. A. Prins, W. Franklin-Klein, and J. Akkermans-Kruyswijk**. 1973. Phosphatidylserine and its plasmalogen analogue as major lipid constituents in *Megasphaera elsdenii*. *Biochim. Biophys. Acta* **326** (3), 314-324.
131. **Varon, M., A. Tietz, and E. Rosenberg**. 1986. *Myxococcus xanthus* autocide AMI. *J. Bacteriol.* **167** (1), 356-361.
132. **Voelz, H. and H. Reichenbach**. 1969. Fine structure of fruiting bodies of *Stigmatella aurantiaca* (*Myxobacterales*). *J. Bacteriol.* **99** (3), 856-866.

133. **Wagner, F., S. Rottem, H. D. Held, S. Uhlig, and U. Zähringer**. 2000. Ether lipids in the cell membrane of *Mycoplasma fermentans*. *Eur. J. Biochem.* **267** (20), 6276-6286.
134. **Wagner, J. A.** 2008. Strategic approach to fit-for-purpose biomarkers in drug development. *Annu. Rev. Pharmacol. Toxicol.* **48** (1), 631-651.
135. **Wallace, K. K., S. Lobo, L. Han, H. A. McArthur, and K. A. Reynolds**. 1997. In vivo and in vitro effects of thiolactomycin on fatty acid biosynthesis in *Streptomyces collinus*. *J. Bacteriol.* **179** (12), 3884-3891.
136. **Wältermann, M., A. Hinz, H. Robenek, D. Troyer, R. Reichelt, U. Malkus, H. J. Galla, R. Kalscheuer, T. Stöveken, P. von Landenberg, and A. Steinbüchel**. 2005. Mechanism of lipid-body formation in prokaryotes: how bacteria fatten up. *Mol. Microbiol.* **55** (3), 750-763.
137. **Wanders, R. J. A., G. A. Jansen, and M. D. Lloyd**. 2003. Phytanic acid alpha-oxidation, new insights into an old problem: a review. *Biochim. Biophys. Acta* **1631** (2), 119-135.
138. **Ware, J. C. and M. Dworkin**. 1973. Fatty acids of *Myxococcus xanthus*. *J. Bacteriol.* **115** (1), 253-261.
139. **Wenzel, S. C., B. Kunze, G. Höfle, B. Silakowski, M. Scharfe, H. Blöcker, and R. Müller**. 2005. Structure and biosynthesis of myxochromides S1-3 in *Stigmatella aurantiaca*: evidence for an iterative bacterial type I polyketide synthase and for module skipping in nonribosomal peptide biosynthesis. *Chembiochem* **6** (2), 375-385.
140. **Wenzel, S. C., P. Meiser, T. Binz, T. Mahmud, and R. Müller**. 2006. Nonribosomal peptide biosynthesis: point mutations and module skipping lead to chemical diversity. *Angew. Chem. Int. Ed.* **45** (14), 2296-2301.
141. **White, S. W., J. Zheng, Y. M. Zhang, and C. O. Rock**. 2005. The structural biology of type II fatty acid biosynthesis. *Annu. Rev. Biochem.* **74** (1), 791-831.
142. **Wilding, E. I., J. R. Brown, A. P. Bryant, A. F. Chalker, D. J. Holmes, K. A. Ingraham, S. Iordanescu, C. Y. So, M. Rosenberg, and M. N. Gwynn**. 2000. Identification, evolution, and essentiality of the mevalonate pathway for isopentenyl diphosphate biosynthesis in Gram-positive cocci. *J. Bacteriol.* **182** (15), 4319-4327.
143. **Willecke, K. and A. B. Pardee**. 1971. Fatty acid-requiring mutant of *Bacillus subtilis* defective in branched chain α-keto acid dehydrogenase. *J. Biol. Chem.* **246** (17), 5264-5272.
144. **Zhao, J. Y., L. Zhong, M. J. Shen, Z. J. Xia, Q. X. Cheng, X. Sun, G. P. Zhao, Y. Z. Li, and Z. J. Qin**. 2008. Discovery of an autonomously-replicating plasmid pMF1 from *Myxococcus fulvus* and the development of a gene cloning system in *Myxococcus xanthus*. *Appl. Environ. Microbiol.* **74** (7), 1980-1987.
145. **Zusman, D. R., A. E. Scott, Z. Yang, and J. R. Kirby**. 2007. Chemosensory pathways, motility and development in *Myxococcus xanthus*. *Nat. Rev. Microbiol.* **5** (11), 862-872.

Danksagung

Mein größter Dank gilt meinem Doktorvater Prof. Dr. Helge B. Bode, nicht nur für die Aufnahme in seine Gruppe und das interessante Thema, sondern auch für das gute Arbeitsklima im Büro und im Labor und für die fruchtbare Zusammenarbeit, ohne die diese Arbeit nicht möglich gewesen wäre. Ihm und den Mitgliedern seines Arbeitskreises wünsche ich viel Erfolg für ihre Forschung in Frankfurt und alles Gute für die Zukunft.

Bei Prof. Dr. Rita Bernhardt und Prof. Dr. Jörn Piel bedanke ich mich für die Übernahme des Zweit- und Drittgutachtens, außerdem bei Prof. Dr. Johann Jauch für den Prüfungsvorsitz.

Ebenso möchte ich mich bei Prof. Dr. Rolf Müller bedanken für die Aufnahme an sein Institut und die fortwährende Unterstützung meiner Tätigkeit in Forschung und Lehre. In diese Danksagung möchte ich auch seine ehemaligen und aktuellen Mitarbeiterinnen und Mitarbeiter einschließen, insbesondere Dr. Peter Meiser, Matthias Altmeyer, Dr. Tina Binz und Thorsten Klefisch sowie Dr. Silke Wenzel, der ich auch für die weitere Besetzung der Prüfungskommission danken möchte.

Besonders herausheben möchte ich an dieser Stelle das Engagement von Gertrud Schwär, die durch ihre Arbeit wesentlich zu den Ergebnissen dieser Dissertation beigetragen hat. Darüber hinaus danke ich ihr und ihrem Mann Eric für die netten Abende.

Ich danke außerdem Edna Bode für ihre Mitarbeit bei diversen Experimenten in der Endphase meiner Arbeit. Meinen Wahlpflichtpraktikantinnen Anne Flauder und Christine Lang möchte ich für ihre praktische Unterstützung im Labor danken.

Der Fachschaft und dem Dozentenkreis der Pharmazie danke ich für die gute Zusammenarbeit und dafür, dass sie mir trotz meiner Tätigkeit als Studienkoordinator Gelegenheit zur Vollendung meiner Dissertation gegeben haben.

Meinen Eltern und Großeltern danke ich für die seelische und moralische, oft genug auch finanzielle Unterstützung während der Zeit meiner Promotion. Besonders denke ich dabei an meinen Opa Werner Ring, der den Abschluss leider nicht mehr miterleben durfte.
Nicht zuletzt danke ich Anton Linnenbrink, Nadine Offeringa, Stephan Ley sowie Kerstin und André Zimmer für ihre freundschaftliche Verbundenheit.