Catabolic Pathways and Enzymes Involved in the Anaerobic Degradation of Terpenes

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Contents

Abstract

Monoterpenes are ubiquitous and, in contrast to other terpenes, their anaerobic mineralization has been studied, especially in denitrifying betaproteobacteria. *Castellaniella defragrans* has a degradation pathway for cyclic monoterpenes, with a limonene dehydrogenase and a ring-cleavage reaction known from anoxygenic phototrophic bacteria. Toxic monoterpene alcohols are transformed in the periplasm by the linalool dehydratase/isomerase (Ldi) to the less toxic myrcene. *Thauera linaloolentis* degrades linalool with a membrane-anchored linalool isomerase and the enzymes of the Atu/Liu pathways for acyclic mono-

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terpenes. The development of a genetic system for *Castellaniella defragrans* together with physiological and biochemical studies have provided an excellent toolbox to study the monoterpene metabolism. On the horizon, *Pseudomonas aeruginosa* and other gammaproteobacterial pseudomonads are waiting for a thorough exploration of their monoterpene metabolism.

1 Introduction

Isoprenoids are derived from isoprene (2-methyl-buta-1,3-diene) and comprise an extreme variety of natural compounds. A large number of reference books describe the chemical features of these compounds (reviewed in Harder 2009a). The biosynthesis of isoprenoids occurs via the mevalonate or the 2-*C*-methyl-D-erythritol 4-phosphate pathway and provides molecular structures like quinones, hopanoids, steroids, and ether-type membrane lipids, as well as photosynthetic pigments (rhodopsins, carotenoids, and chlorophylls), and a large variety of secondary metabolites (reviewed in Boronat and Rodríguez-Concepción 2015; Schmidt-Dannert 2015; Tholl 2015). The biological mineralization of all these diverse structures has been studied only to a limited extent. Most studies so far reported the transformation of monoterpenes and sequiterpenes by aerobic organisms (Noma and Asakawa 2016; Asakawa and Noma 2016). The biochemical and genetic characterization of pseudomonads and Gram-positive bacteria has elucidated degradation pathways for a number of monoterpenes, including limonene, pinene, geraniol, citronellol, myrcene, and camphor (reviewed in Marmulla and Harder 2014; for chemical structures, see Fig. 1). In this contribution, recent insights into the anaerobic degradation of monoterpenes are reviewed, updating the earlier reviews of Hylemon and Harder (1998) and Harder (2009b). This chapter is complemented by the next ▶ Chap. 7, "Anaerobic Biodegradation of Steroids" (Chiang and Ismail 2017).

Isoprenoids as electron donor and carbon source for anaerobic bacteria were first studied under oxygen-free, nitrate-reducing conditions with monoterpenes (Harder and Probian 1995) and cholesterol (Harder and Probian 1997). Betaproteobacterial strains of *Castellaniella* (ex *Alcaligenes*) *defragrans* were obtained from enrichment cultures grown on single monoterpenes as sole organic carbon source: the strains 51Men, 54Pin^T, 63Car, and 65Phen were isolated on the monocyclic monoterpenes menthene, α-pinene, 2-carene, and α-phellandrene, respectively (Foß et al. 1998) (Fig. 1). The complete genome of strain 65Phen has 3,952,282 bp and includes a genetic island for monoterpene utilization (Petasch et al. 2014). In contrast, oxygen-functionalized monoterpenes as substrate in enrichment cultures yielded denitrifying strains of the betaproteobacterial genus *Thauera*: *T. linaloolentis* 47Lol^T on linalool, *T. terpenica* 58Eu^T on eucalyptol, and *T. terpenica* 21Mol on menthol (Foß and Harder 1998). Draft genomes of *T. linaloolentis* 47Lol^T and *T. terpenica* 58Eu^T are available (Liu et al. 2013).

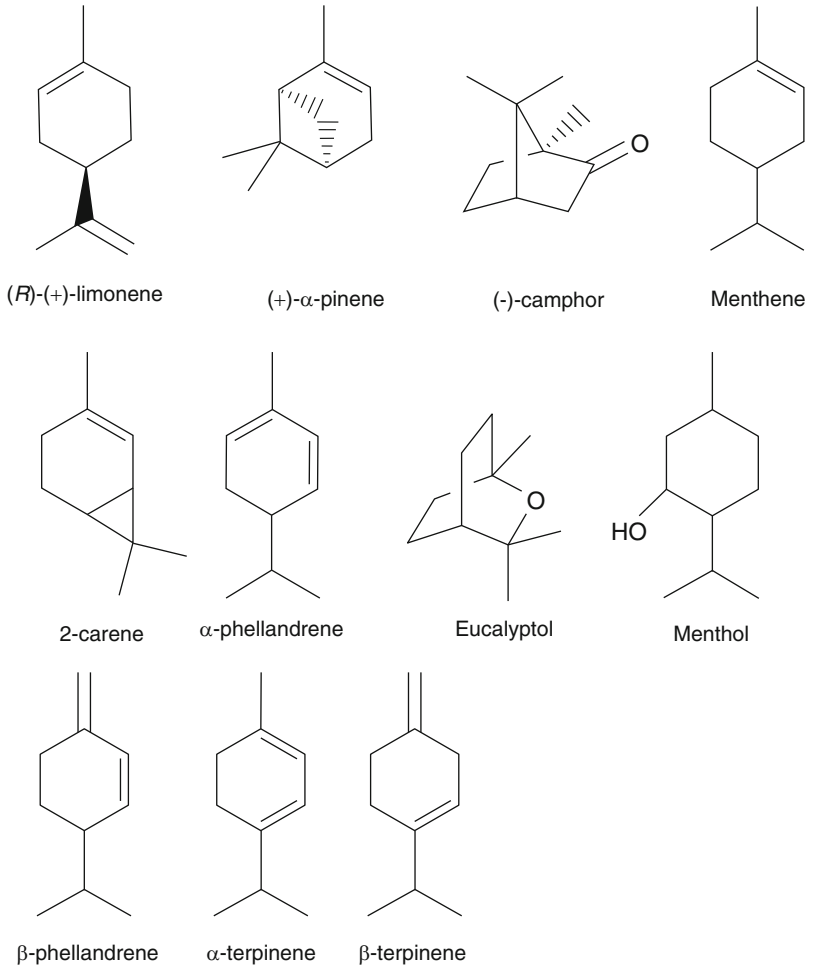

Fig. 1 Monoterpene structures

2 Degradation of Acyclic Monoterpenes: From Myrcene to Geranic Acid

β-myrcene (7-methyl-3-methylene-1,6-octadiene) is an acyclic monoterpene with a buta-1,3-diene structural motif. Some strains of the betaproteobacterium *C. defragrans* use myrcene as growth substrate (Heyen and Harder 2000). The detection of geranic acid as metabolite in cells and culture medium guided the identification of myrcene as growth substrate and precursor of geranic acid (Heyen and Harder 2000). The transformation is catalyzed by a linalool dehydratase/

isomerase (Ldi) yielding (*S*)-linalool ((*S*)-3,7-dimethyl-octa-1,6-dien-3-ol) and geraniol ((*trans*)-3,7-dimethyl-octa-2,6-dien-1-ol) (Brodkorb et al. 2010; Lüddeke and Harder 2011) and by two dehydrogenases acting on geraniol and geranial ((*trans*)-3,7-dimethyl-octa-2,6-dienal) (GeoA and GeoB) (Lüddeke et al. 2012b).

2.1 Linalool Dehydratase/Isomerase (Ldi) of *C. defragrans* 65Phen

In the thermodynamically favorable direction, the periplasmic enzyme reversibly catalyzes the isomerization from the primary alkenol geraniol into the tertiary alkenol (*S*)-linalool and its dehydration to β-myrcene (Fig. 2). Activity occurs *in vitro* in oxygen-free conditions in the presence of a reductant (dithiothreitol). Two years after the first publication (Brodkorb et al. 2010), the first patent applications reported the use of the dehydration reaction of Ldi or genetically engineered variants thereof for the production of butadiene and isoprene (reviewed in Weidenweber et al. 2015). The enzyme may become a key enzyme in the biotechnological production of these precursors for many polymers (e.g., nylon, polyester, polyisoprene).

The gene *ldi* of *C. defragrans* 65Phen encodes a preprotein with an N-terminal signal peptide for transport into the periplasmatic space. Expression of the gene in *E. coli* yielded a protein lacking the predicted signal peptide. Crystals of this Ldi preparation contained a homopentameric holoenzyme (PDB:5HLR), and incubation experiments with linalool as substrate yielded crystals with either myrcene or geraniol in the active site of the monomers (PDB:5HSS) (Weidenweber et al. 2015). A third crystal structure of the mature Ldi was published 2 months later, in June 2016 (PDB:5I3T).

Ldi is a homopentameric protein complex in the form of a planar rosette with a central hole. The monomers have a classical $(\alpha,\alpha)_6$ barrel fold (Fig. 3). This basket structure is well known from many monomeric terpene-transforming enzymes and glycosyl epimerases and hydrolases. An unprecedented structure is the pentameric complex with narrow channels between two adjacent monomers that allows the hydrophobic substrate to enter the active site. The neighboring monomer provides a loop that closes the in $(\alpha,\alpha)_6$ barrel fold-enzymes usually polar and accessible binding site. The peptide segment 36′–52′ presents a stable ram build on three legs by the peptide backbone of the loop and an essential intrasubunit disulfide bond between Cys48′ and Cys101′. The electron density in the linalool-soaked crystals

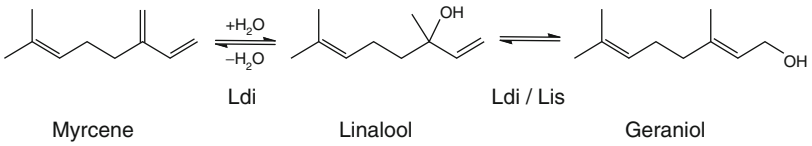

Fig. 2 Myrcene to geraniol conversion

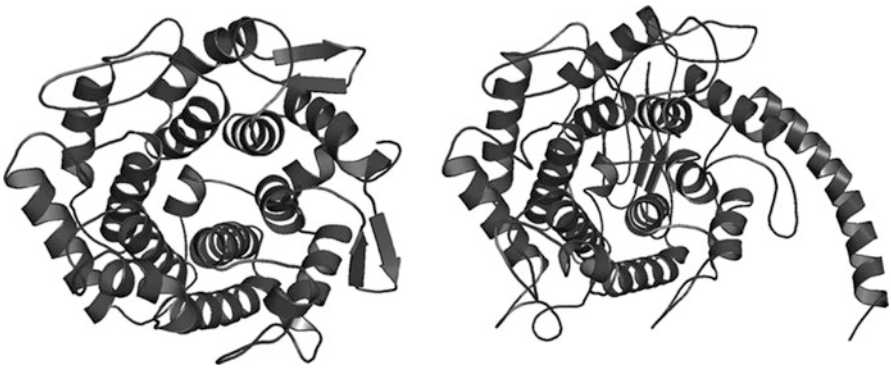

Fig. 3 The linalool dehydratase/isomerase structure (*left*) and the linalool isomerase model (*right*)

was best fitted with β-myrcene or geraniol, thus suggesting that linalool binding was less preferred and the enzymes in the crystals were catalytically active.

The unusual pentameric structure has led to a reaction hypothesis in which the pentamer plays its own role. Overall, the LDI structure evokes associations with a high pressure press. Linalool is less favorably bound in what can be considered to be an anvil at the bottom of a channel. In it, a ram press is moving up and down which is the adjacent loop with the amino acid residues Asp38′ and Tyr44′ on top that interact with the substrate. Thus, the movement of one monomer into the active site of the adjacent monomer is enforcing the catalysis. If no linalool is present, the collision of the monomers invokes an energy transfer and sends the "anvil" monomer as ram press in the direction of the next monomer in the ring. Thus, all monomers are active one after the other orbiting around the center of the complex. The system can be seen as molecular pentagonal Klemperer rosette – a gravitational system of equal bodies rotating around a barycenter (Klemperer 1962).

Ldi of *C. defragrans* 65Phen is still a unique protein as bioinformatic searches currently reveal no related protein with an *E*-value below 10^{-20}. However, linalool is also the growth substrate of *Thauera linaloolentis* 47Lol^T, and we identified in this bacterium a linalool isomerase (Lis) that shares in the catalytic domain structural similarities to Ldi.

2.2 Linalool Isomerase (Lis) of *Thauera linaloolentis* 47Lol^T

Metabolite formation in cultures of *Thauera linaloolentis* 47Lol^T with an excess of the electron donor and carbon source linalool over the electron acceptor nitrate suggested the presence of a 3,1-hydroxyl-Δ^1-Δ^2-mutase activity transforming linalool into geraniol (Foß and Harder 1997). The corresponding protein was enriched as geraniol isomerase yielding linalool and was located in the inner membrane (Marmulla et al. 2016b). Subcellular fractionation and a twofold sucrose gradient centrifugation yielded a highly enriched geraniol isomerase fraction containing

NCBI:ENO87364 as dominant protein. This protein (644 aa) has a predicted N-terminal membrane domain consisting of four transmembrane helices within the first 139 aa and a C-terminal, cytosolic domain of 505 aa that aligned with the Ldi (371 aa) with 62 identical and totally 132 positive amino acids (blastp). The secondary structure prediction using JPred (Drozdetskiy et al. 2015) matched the helices of the Ldi crystal structure and predicted a similar number of helices in the Lis. This information was incorporated in a sequence alignment using the Clustal Omega tool (Sievers et al. 2011) and with I-Tasser (Yang et al. 2015) the soluble domain of Lis was modeled into the Ldi structure. The resulting model showed that the Lis protein can fold into a highly similar $(\alpha,\alpha)_6$ structure (Fig. 3). The active site is in both enzymes a hydrophobic cavity with four conserved amino acids (Ldi: Y65, M124, C170, E171, C179; Lis: Y220, M287, C349, E350, C358) and three similar amino acids (Ldi: F69, H128, F176; Lis: H224, W246, Y355) that matched in the sequence alignment and in the superimposed structures (Fig. 4). Several other amino acids are conserved in the same structural position (Marmulla 2015).

The superimposition of the Ldi structure and the Lis model suggests similarities in the isomerase reaction. The lack of the dehydratase reaction in the Lis is likely due to the missing loop of an adjacent monomer. It may also explain why the Ldi turns over only (*S*)-linalool, whereas the Lis acts on both (*R*) and (*S*) isomers of linalool.

The cellular location of Lis inside of the inner membrane offers the product geraniol to cytosolic enzymes that oxidizes the alcohol with NAD. In contrast, the periplasmatic location of Ldi suggests a contribution to the defense: monoterpene alcohols arriving at the periplasm are to a large extent transformed into the less toxic monoterpene myrcene. Still, a small pool of monoterpene alcohols is present and enters the cytoplasm either by passive diffusion or by active transport.

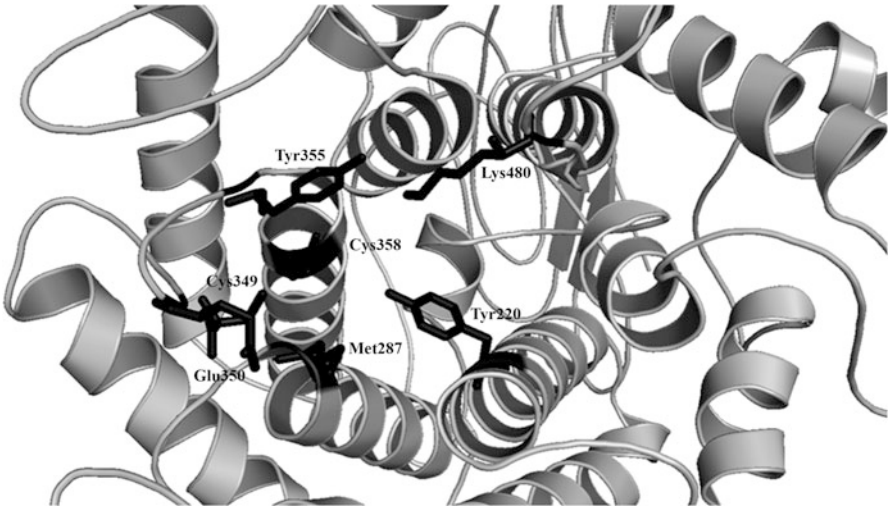

Fig. 4 Active site of the structure model of the linalool isomerase, with the conserved Tyr220, Met287, Cys349, Glu350, Tyr355, Cys358, and Lys480

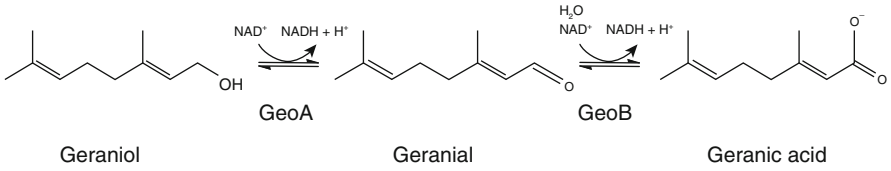

Fig. 5 Enzymatic reaction sequence from geraniol to geranic acid

2.3 Geraniol and Geranial Dehydrogenases

Allyl and benzyl alcohols both have sp^2-hybridized C2- and C3-atoms next to the carbon carrying the alcohol group. Hence, allyl alcohol dehydrogenases have an activity on benzyl alcohol and vice versa. The geraniol dehydrogenase GeoA was purified from *C. defragrans* 65Phen grown on monocyclic monoterpenes (limonene or α-phellandrene) (Lüddeke et al. 2012b). The homodimeric enzyme affiliating with zinc-containing benzyl alcohol dehydrogenases in the medium-chain dehydrogenases/reductases (MDR) superfamily was highly expressed (Petasch et al. 2014) and catalyzed the oxidation of perillyl alcohol more efficient than the one of geraniol (Lüddeke et al. 2012b). The oxidation product geranial is further oxidized by the highly expressed GeoB protein, a member of the aldehyde dehydrogenase superfamily (Lüddeke et al. 2012b) (Fig. 5).

The physiological relevance of GeoA was studied in a *geoA*-deletion mutant of *C. defragrans* 65Phen: growth rate and biomass yield were reduced on limonene, α-phellandrene, or β-myrcene as sole carbon and energy source in the culture, indicating a role of the enzyme in the acyclic and in the monocyclic monoterpene degradation (Lüddeke et al. 2012a). Extracts still contained geraniol dehydrogenase activity (Lüddeke et al. 2012a), and the purification of the enzyme identified the protein NCBI:CDM24151: an aryl alcohol dehydrogenase of the cinnamyl alcohol dehydrogenase family (Koska et al. unpublished) that was not among the proteins induced by α-phellandrene as substrate in the wild-type strain (Petasch et al. 2014).

Two proteins with a high similarity to GeoA and GeoB (>70% sequence identities) were found to be expressed during the mineralization of *p*-cymene in the betaproteobacterial strain pCyN1: their suggested function is the oxidation of 4-isopropylbenzyl alcohol to the corresponding aldehyde and acid, yielding 4-isopropylbenzoate (Strijkstra et al. 2014). Future studies on these enzymes and on GeoA and GeoB may provide an understanding on the substrate specialization for allylic and benzylic substrates.

3 The Acyclic Terpene Utilization Pathway (Atu/Liu)

In aerobic pseudomonads, Atu enzymes together with the leucine degradation enzymes (*liu*) channel the carbon atoms of the acyclic monoterpene alcohols and acids into the tricarboxylic acid cycle (reviewed in Marmulla and Harder 2014; Poudel et al. 2015) (Fig. 6).

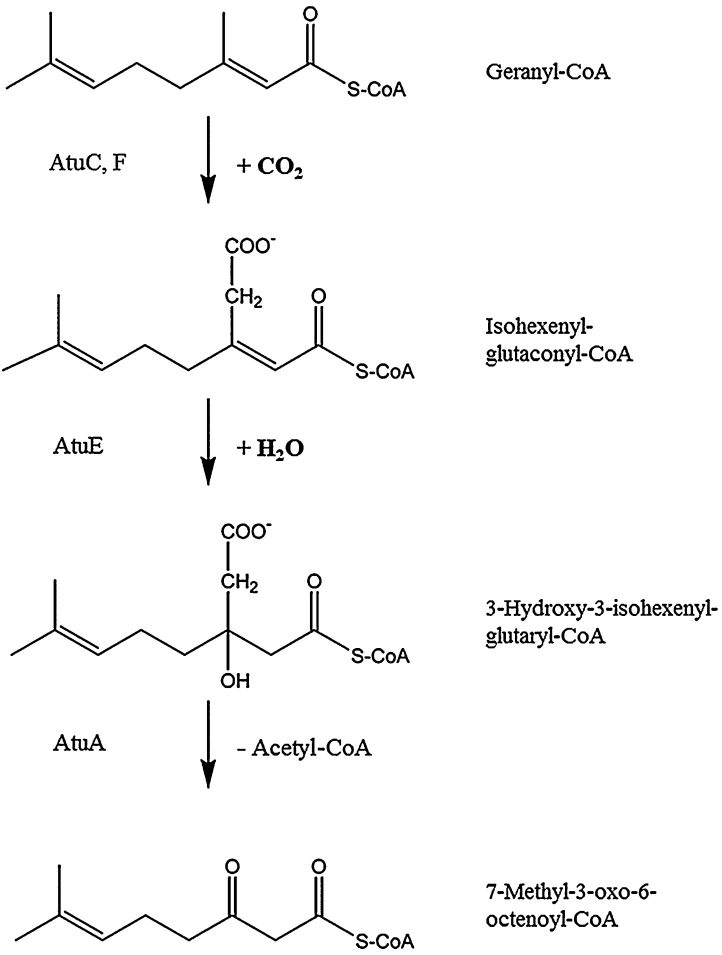

Fig. 6 Removal of a methyl group in β-position via carboxylation as demonstrated for geranyl-CoA

The presence of Atu and Liu enzymes was recently demonstrated in denitrifying *Thauera linaloolentis* 47Lol^T cells grown on linalool (Marmulla et al. 2016a). All subunits of the geranyl-CoA carboxylase (AtuCF) and 3-methylcrotonyl-CoA carboxylase (LiuBD) were identified by Maldi-Tof, together with Lis, GeoA, and the 3-hydroxy-3-isohexenylglutaryl-CoA:acetate lyase (AtuA). The corresponding genes were present in two contigs, generated from the available draft genomes (Marmulla et al. 2016a). *AtuCFE* and *lis* seem to be one transcriptional unit, and the *liu* cluster has been enlarged by a membrane protein with at least four transmembrane helices (NCBI:ENO88224, annotated as DUF4216) and a protein with a periplasmatic N-terminal domain of 200 aa, a membrane-spanning linker and a C-terminal fatty acid-CoA ligase (NCBI:ENO88222). These findings demonstrate

the presence of the Atu/Liu pathway for the degradation of acyclic monoterpenes in anaerobic bacteria. Key enzymes are the carboxylases that transform the methyl group in β-position to a CoA-thioester into a removable carboxymethylene group. This principle of branched-chain degradation pathway may also be operative for larger acyclic terpenes.

Although *C. defragrans* 65Phen grows on or metabolizes the acyclic compounds myrcene, linalool, geraniol, nerol, geranial, neral, and geranic acid, the closed genome of *C. defragrans* 65Phen did not contain the expected pathway for further mineralization for the acyclic monoterpene utilization, the *atu* genes. Transposon mutants in the degradation pathway of monocyclic monoterpenes also lacked the capacity to grow on β-myrcene (Petasch et al. 2014). This is evidence for an enzymatic formation of monocyclic monoterpenes from acyclic monoterpenes, although the genome does not code for classical monoterpene synthases.

4 Degradation of Cyclic Monoterpenes

Our knowledge on the degradation of cyclic monoterpenes originates from the genome of *C. defragrans* 65Phen and the corresponding proteome of cells grown on α-phellandrene in comparison to the proteome of cells grown on acetate (Petasch et al. 2014). Cyclic monoterpenes have a water solubility of ~50 μM, and growth experiments showed the simultaneous consumption of several monoterpenes (Harder et al. unpublished; Foß et al. 1998; Harder et al. 2000). Initial transformations may involve a ring-opening reaction of bicyclic monoterpenes to monocyclic monoterpenes, as suggested by the formation of metabolites in cultures growing on 2-carene, 3-carene, eucalyptol, or α-pinene (Harder and Probian 1995). The initial enzyme of the monocyclic monoterpenes seems to be a limonene dehydrogenase (Fig. 7), and the product perillyl alcohol is oxidized by GeoA and GeoB to perillic acid. A ligase seems to synthesize the perillyl-CoA thioester that undergoes ring cleavage reactions similar to the ring opening reactions of cyclohexanecarboxyl- and benzoyl-CoA thioesters (Fig. 8). The gene clusters involved were named *ctm* for cyclic terpene metabolism and *mrc* for monoterpene ring cleavage-associated genes (Petasch et al. 2014).

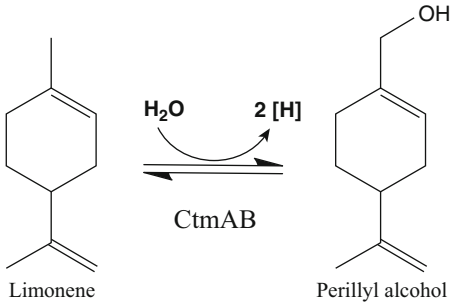

Fig. 7 Limonene oxidation to perillyl alcohol

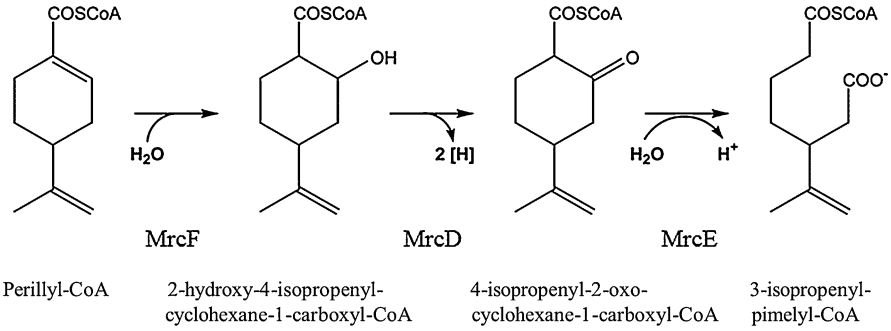

Fig. 8 From perillyl-CoA to 3-isopropenyl-pimelyl-CoA

4.1 Limonene Dehydrogenase

A collection of transposon mutants of *C. defragrans* 65Phen with a loss of denitrifying growth on limonene was dominated by mutants with an inactivation of *ctmA*: 45 of 72 mutant strains had insertions of the transposon in 22 different positions (Petasch et al. 2014). The *ctm* gene cluster was also inactivated in three mutants in *ctmB* and two mutants in *ctmE*. Transposon mutants in *ctmA*, *ctmB*, or *ctmE* did grow on perillyl alcohol. Growth studies with the in-frame deletion mutant *C. defragrans* 65Phen *ΔgeoB* revealed the *in vivo* formation of perillyl alcohol from limonene. These experiments assigned a function as limonene dehydrogenase to the *ctm* gene cluster. The proteomic study identified only the two FAD-dependent oxidoreductases (CtmA and CtmB) and an electron transfer system consisting of a 2Fe-2S ferredoxin (CtmE) and a NADH:ferredoxin oxidoreductase (CtmF) as proteins induced by α-phellandrene, but not the other genes in the *ctm* gene cluster: three hypothetical genes (*ctmCD*, *ctmG*) and a putative transcriptional regulator of the luxR family.

CtmA and CtmB belong to COG1233, a group of proteins related to phytoene dehydrogenase. In the biosynthesis of conjugated systems in vitamins and carotenoids, these enzymes oxidize a hexa-1,5-diene into a hexa-1,3,5-triene moiety, oxidizing the two allylic methylene groups located between the isolated alkene bonds. The use of this protein class for the oxidative introduction of an alcohol group is unprecedented (Petasch et al. 2014). The fate of the electrons is likely a participation in the reduction of nitrate. Electrons coming from NADH via the NADH:ferredoxin oxidoreductase CtmF may eventually join the electrons coming from CtmAB on the ferredoxin CtmE and together may be channeled into the respiratory chain at a specific complex. A candidate for this is an electron transfer flavoprotein:ubiquinone oxidoreductase (NCBI: CDM23589) that was identified as essential for the cyclic monoterpene degradation by transposon mutagenesis (Petasch et al. 2014).

4.2 Perillyl Alcohol Oxidation

The proteomic study (Petasch et al. 2014) identified the aforementioned GeoA and GeoB as candidates for the oxidation of perillyl alcohol to perillic acid. This is supported by the verified broad substrate spectrum of GeoA (Lüddeke et al. 2012b) and the cometabolic formation of perillyl alcohol and perillyl aldehyde *in vivo* by the in-frame deletion mutant *C. defragrans* 65Phen *ΔgeoB*. An induced protein annotated as ATP-dependent ligase (NCBI:CDM25265) with a gene location next but one to *geoA* is currently considered as perillic acid-CoA ligase preparing the substrate for the ring cleavage pathway (Petasch et al. 2014). The protein shares conserved domains with long-chain-fatty-acid-CoA and benzoate-CoA ligases.

4.3 Ring Opening Reactions of Cyclic Terpenes

The degradation of perillic acid-CoA seems to resemble the degradation pathways of cyclohexane carboxylate or benzoate in the phototrophic *Rhodopseudomonas palustris* (Pelletier and Harwood 2000), with cyclohex-1-ene-carboxyl-CoA as intermediate. In all other facultative and obligate anaerobes, an alternative benzoyl-CoA pathway exists with 6-hydroxy-cyclohex-1-ene-carboxyl-CoA as intermediate and *bamA* as hydrolase. The latter was suggested to be widely used as functional marker in environmental analyses (Kuntze et al. 2008). A copy of *bamA* is absent in *C. defragrans* 65Phen.

The monoterpene ring cleavage-associated gene cluster *mrcABCDEFGH* is part of the genetic island in *C. defragrans* 65Phen that contains most genes for the monoterpene metabolism (Petasch et al. 2014). The proteins MrcABCDEF were highly expressed during growth on α-phellandrene. Like the transposon mutants in *ctmA*, *ctmB*, and *ctmE*, transposon mutants in *mrcC* and *mrcF* are unable to grow on limonene, α-phellandrene, and β-myrcene (Petasch et al. 2014). The degradation of perillyl-CoA likely starts with a hydration catalyzed by MrcF. Oxidation of the product 2-hydroxy-4-isopropenylcyclohexane-1-carboxyl-CoA by MrcD may form 4-isopropenyl-2-oxocyclohexane-1-carboxyl-CoA. A hydrolysis by MrcE is assumed to form 4-isopropenylpimelyl-CoA. This is paralogous to the BadK, BadH, and BadI enzymes of the anaerobic benzoate catabolism in *Rhodopseudomonas palustris* strains that catalyze the oxidation of cyclohexenecarboxyl-CoA to pimelyl-CoA. *MrcC* codes for a 2,4-dienoyl reductase and presumably the substrates are the cyclohexadienyl CoA esters that may result from the methyl group-oxidation of the monocyclic menthadienes. The product of the ring cleavage reactions, 4-isopropenylpimelyl-CoA, is suitable for β-oxidation. Besides acetyl-CoA, methacrylyl-CoA may be produced and further undergoes decarboxylation to propionyl-CoA which may be metabolized via the methylcitrate cycle fueling the citric acid cycle. This suggestion is supported by the increased expression of several enzymes of the valine degradation pathway in cells grown on α-phellandrene. Two transposon

mutants in 2-methylcitrate synthase and 2-methylcitrate dehydratase supported the participation of the methyl citrate cycle in anaerobic monoterpene mineralization (Petasch et al. 2014).

5 Difficulties to Use Monoterpenes as Only Carbon Source

The application of transposon mutagenesis for the identification of catabolic genes involved in the monoterpene degradation has yielded a large number of noncatabolic genes that are essential for growth on monoterpenes, but not on acetate (Petasch et al. 2014; Marmulla et al. 2016a). This included transport systems and biosynthetic pathways for membrane components, an indication for a change in the composition of the cellular membranes in response to the presence of monoterpenes. Additional experimental evidence for an adaption to the toxicity of monoterpenes came from the analysis of membrane fatty acids and from proteomic studies (Foß et al. 1998; Petasch et al. 2014).

The toxicity of hydrocarbons is well understood (Sikkema 1995). Monoterpenes have a low absolute solubility in water – below 100 μM, resulting in a low diffusive flux – and a partition coefficient of logP(octanol/water) of 4.46 (pinene, limonene); thus in equilibrium, one molecule stays in the aqueous phase and 16,893 molecules are located in the hydrophobic solvent octanol (as proxy for the hydrophobic membrane interior). In contrast, monoterpenoids (oxygen-functionalized monoterpenes like geraniol and geranial) are soluble in millimolar amounts and have a lower logP value, e.g., 3.56 for geraniol. *C. defragrans* 65Phen grows in the presence of a pure limonene phase (Heyen and Harder 2000), but already 5 μM geraniol in the aqueous phase are toxic for the cells (Lüddeke et al. 2012b). As a consequence, biomass on monoterpene alcohols is only obtained either in the presence of a large organic carrier phase (2,2,4,4,6,8,8-heptamethylnonane) or in multi-fed batch cultures. Also, growth failure of cultures on monoterpenes can often be attributed to aging of monoterpenes by air, likely the accumulation of monoterpenoids. The diffusive flux of volatile hydrocarbon substrates through the gas phase to a colony on a plate is more efficient than the diffusive flux through an aqueous phase (Harms 1996). This coincides with our observation that several transposon mutants isolated on plates did not show the phenotype “lack of growth” in liquid cultures (Petasch et al. 2014; Marmulla et al. 2016a).

6 Research Needs

This review on the anaerobic degradation of terpenes focuses on monoterpenes because knowledge on isoprene and larger terpenes is nonexistent. The last decade has envisioned the first enzymes in the anaerobic monoterpene metabolism. The linalool dehydratase/isomerase or genetic variants thereof are already in consideration for the biotechnological production of butadiene. The characterization of the limonene dehydrogenase will provide access to the hydroxylation of allylic methylene groups. Physiological observations depicted the existence of more unusual enzymes. For *Castellaniella*, genetic methods like transposon mutagenesis and in-frame deletion mutant constructions were established and allowed together with

well-designed physiological studies of in-frame deletion mutants insights into the *in vivo* metabolism. In a broader view, little research has been performed on the aerobic monoterpene metabolism of the opportunistic pathogen *Pseudomonas aeruginosa*, a facultative anaerobe capable of denitrification. Nowadays, over 100 complete and over 2000 draft genomes of *Pseudomonas* strains await an exploration for aerobic and anaerobic pathways of monoterpene mineralization and this may contribute to a better understanding of the health benefits of essential oils.

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