Isoprene and monoterpenes constitute a significant fraction of new plant biomass. Emission rates into the atmosphere alone are estimated to be over 500 Tg per year. These natural hydrocarbons are mineralized annually in similar quantities. In the atmosphere, abiotic photochemical processes cause lifetimes of minutes to hours. Microorganisms encounter isoprene, monoterpenes, and other volatiles of plant origin while living in and on plants, in the soil and in aquatic habitats. Below toxic concentrations, the compounds can serve as carbon and energy source for aerobic and anaerobic microorganisms. Besides these catabolic reactions, transformations may occur as part of detoxification processes. Initial transformations of monoterpenes involve the introduction of functional groups, oxidation reactions, and molecular rearrangements catalyzed by various enzymes. *Pseudomonas* and *Rhodococcus* strains and members of the genera *Castellaniella* and *Thauera* have become model organisms for the elucidation of biochemical pathways. We review here the enzymes and their genes together with microorganisms known for a monoterpenes metabolism, with a strong focus on microorganisms that are taxonomically validly described and currently available from culture collections. Metagenomes of microbiomes with a monoterpenes-rich diet confirmed the ecological relevance of monoterpenes metabolism and raised concerns on the quality of our insights based on the limited biochemical knowledge.

**Keywords:** isoprenoids, acyclic monoterpene utilization, camphor, pinene, limonene, linalool, myrcene, eucalyptol

**INTRODUCTION**

Annually about 120 Pg of carbon dioxide are assimilated by plants. A part is transformed into chemically complex molecules and released into the environment by emission or excretion (Ghirardo et al., 2011). Volatile organic compounds (VOCs) comprise a large number of molecules, including various hydrocarbons, single carbon compounds (e.g. methane), isoprene and terpenes (e.g. mono- and sesquiterpenes). The atmosphere is loaded with an estimated VOC emission rate of about 1150 Tg C yr\(^{-1}\) (Stotzky and Schenck, 1976; Guenther et al., 1995; Atkinson and Arey, 2003). These estimates included only non-methane VOCs of biogenic origin (BVOCs); a second source are anthropogenic VOCs. Among the BVOCs, isoprene and monoterpenes dominate with estimated emission rates of about 500 Tg C yr\(^{-1}\) and 127 Tg C yr\(^{-1}\), respectively (Guenther et al., 1995). Monoterpenes (C\(_{10}\)H\(_{16}\)) consist of two linked isoprene (C\(_{5}\)H\(_{8}\)) units and include in the strict sense only hydrocarbons. Often the term monoterpane is applied including monoterpeneoids which are characterized by oxygen-containing functional groups. Structural isomers—acyclic, mono-, and bicyclic monoterpenes—, stereoisomers as well as a variety of substitutions result in a large diversity of molecules. Today, more than 55,000 different isoprenoids are known (Ajikumar et al., 2008). Monoterpenes are not only emitted as cooling substances (Sharkey et al., 2008), but can also be stored intracellularly serving mainly as deterrent or infochemical (Dudareva et al., 2013). Wood plants mainly accumulate pinene and other pure hydrocarbon monoterpenes as constituents of their resins, whereas citrus plants are the major source of limonene. Flowers, however, produce and emit a variety of oxygenated monoterpenes (e.g. linalool) (Kesselmeier and Staudt, 1999 and references therein, Sharkey and Yeh, 2001; Bicas et al., 2009).

In the atmosphere, monoterpenes are transformed in purely chemical reactions within hours. Photolysis and reactions with molecular oxygen, ozone, hydroxyl radicals, NO\(_x\) species, and chloride atoms result in carbonyls, alcohols, esters, halogenated hydrocarbons, and peroxynitrates. These products condense and lead to the formation of secondary aerosols. Rain or precipitation transports them to soils (Atkinson and Arey, 2003; Fu et al., 2009; Ziemann and Atkinson, 2012). Monoterpenes reach the surface layers of soils by leaf fall and excreted resins. Also roots emit monoterpenes into the rhizosphere (Wilt et al., 1993; Kainulainen and Holopainen, 2002). Deeper soil layers do contain significant less monoterpenes than the surface soil layer. Emission into the atmosphere and biotransformations in the surface layer mainly by microorganisms are the major sinks. An alternative, abiotic photo-reactions like in the atmosphere, is limited by light availability in soil (Kainulainen and Holopainen, 2002; Insam and Seewald, 2010).

Bacteria encountering monoterpenes have to deal with their toxic effects (reviewed by Bakkali et al., 2008). In order to prevent the accumulation of monoterpenes in the cell and cytoplasmatic membrane, bacteria modify their membrane lipids, transform monoterpenes and use active transport by efflux pumps (Papadopoulos et al., 2008; Martinez et al., 2009). Below toxic concentrations monoterpenes are used by microorganisms.
as sole carbon and energy source. The mineralization of the hydrocarbons requires the introduction of functional groups to access beta-oxidation like fragmentation reactions yielding central metabolites, e.g. acetyl-CoA. In many aerobic microorganisms molecular oxygen serves as reactive agent to functionalize the monoterpenes (Figure 1). Strains of *Pseudomonas* and *Rhodococcus* have become model organisms for the elucidation of pathways in aerobic bacteria. Nearly 40 years after the first reports on aerobic mineralization (Seubert, 1960; Seubert and Fass, 1964; Dhavalikar and Bhattacharyya, 1966; Dhavalikar et al., 1966), the mineralization of monoterpenes in denitrifying bacteria and methanogenic communities was discovered (Harder and Probian, 1995; Harder and Foss, 1999). Betaproteobacterial strains of the genera *Castellaniella* and *Thauera* are the study objects for the elucidation of anaerobic pathways. All these bacteria were obtained in single-fed batch enrichments with high substrate concentrations (mmol L\(^{-1}\)), in contrast to low concentrations in nature (\(\mu\)mol L\(^{-1}\)). Consequently, in batch enrichments isolated strains exhibit often a solvent tolerance; they grow in the presence of a pure monoterpene phase. Cultivation was rarely attempted by physical separation followed by single-fed batch cultivations. Such dilution-to-extinction series performed in replicates—also known as most-probable-number (MPN) method—revealed a frequent presence of the degradative capacities in natural populations: denitrifying communities in sewage sludge and forest soil yielded \(10^8\)–\(10^9\) monoterpene-utilizing cells ml\(^{-1}\), representing 0.7–100% of the total cultivable nitrate-reducing microorganisms (Harder et al., 2000). MPN cultivations for aerobic bacteria have not been reported so far, and for both cases the highly abundant bacteria with the capacity to grow on monoterpenes have not been identified.

Over the last 50 years, many monoterpene transformations have been reported for microbial cultures, but the biochemical pathways were rarely disclosed. More important for the maintenance of our knowledge, only a small portion of the investigated strains were deposited in culture collections. Without detailed knowledge of genes or the availability of strains, the observations of biotransformation experiments are of limited value for future studies. Therefore, this review on the transformation of monoterpenes focusses on enzymes for which the gene and protein sequences are available in public databases as well as on microorganisms that at least have been deposited in a public culture collection and ideally are validly described (Table 1). A broad overview on microbial biotransformations is also provided by a number of older review articles (Trudgill, 1990, 1994; van der Werf et al., 1997; Hylemon and Harder, 1998; Duetz et al., 2003; Ishida, 2005; Li et al., 2006; Bicas et al., 2009; Li and Lan, 2011; Schewe et al., 2011; Tong, 2013). KEGG and MetaCyc, two widely used reference datasets of metabolic pathways (reviewed by Altman et al., 2013), include degradation pathways of limonene, pinene, geraniol, and citronellol. Single reactions of \(p\)-cymene and \(p\)-cumate degradation are covered. MetaCyc additionally covers the metabolism of myrcene, camphor, eucalyptol, and carvnel.

**BICYCLIC MONOTERPENES**

(+)Camphor [1, Figure 2] (C\(_{10}\)H\(_{16}\)O) is the substrate of one of the first and best described monoterpene transforming enzymes, a specific cytochrome P450 monooxygenase (cam-ABC, P450cam, EC 1.14.15.1) from *Pseudomonas putida* (ATCC 17453). Initially, (+)camphor is hydroxylated. The resulting 5-exo-hydroxycamphor [2] is oxidized by a NAD-reducing dehydrogenase (EC 1.1.1.327) which gene *camD* is part of the operon *camDCAB*. The diketone is oxidized in a Baeyer–Villiger like oxidation to a lactone, either by a 2,5-diketocamphane 1,2-monooxygenase or a 3,6-diketocamphane 1,6-monooxygenase.
(camE<sub>25-1</sub>camE<sub>36</sub> or camE<sub>36</sub>, EC 1.14.13.162). The lactone spontaneously hydrolyses to 2-oxo-Δ<sup>3</sup>-4,5,5-trimethylcyclopentenyl-acetic acid which is activated as coenzyme A thioester by a specific synthase (camF<sub>1.2</sub>, EC 6.2.1.38). This CoA-ester serves as substrate for another specific monoxygenase (camG, EC 1.14.13.160), which initiates the cleavage of the second ring by formation of a lactone. After hydrolysis of the lactone, the linear product is oxidized to isobutanoyl-CoA and three acetyl-CoA. All corresponding genes (camABCDEFG) have been identified on a linear plasmid (Ougham et al., 1983; Taylor and Trudgill, 1986; Aramaki et al., 1993; Kadow et al., 2012; Leisch et al., 2012; Iwaki et al., 2013).

The most abundant bicyclic monoterpane is pinene with the isomers α-pinene [3] and β-pinene [4] (<sub>10</sub>H<sub>16</sub>), a main constituent of wood resins (e.g. conifers). Pseudomonas rhode-siae (CIP 107491) and P. fluorescens (NCIMB 11671) grew on α-pinene as sole carbon source. α-pinene is oxidized to α-pinene oxide [5] by a NADH-dependent α-pinene oxygenase (EC 1.14.12.155) and undergoes ring cleavage by action of a specific α-pinene oxide lyase (EC 5.5.1.10), forming apparently isonovalal as first product which is isomerized to novalal (Best et al., 1987; Bicas et al., 2008; Linares et al., 2009). The cleavage reaction of α-pinene oxide was also described for a Nocardiopsis sp. strain P18.3 (Griffiths et al., 1987; Trudgill, 1990, 1994).

An alternative route for pinene degradation via a monocyclic p-menthene derivat has been described for Pseudomonas sp. strain PIN (Yoo and Day, 2002). Bacillus pallidus BR425 degrades α- and β-pinene apparently via limonene [6] and pinocarveol. While α-pinene is transformed into limonene and pinocarveol, β-pinene yields pinocarveol only. Both intermediates may be further transformed into carveol [7] and carvone. The activity of a specific monoxygenases has been suggested, but experimental evidence is lacking (Savithiry et al., 1998). Serratia marcescens uses α-pinene as sole carbon source. Trans-verbenol [8] was a detectable metabolite. In glucose and nitrogen supplemented medium, this strain formed α-terpineol [9]. The two oxidation products were considered to be dead-end products as they accumulated in cultures (Wright et al., 1986). A general precaution has to be mentioned for many biotransformation studies: monoterpenes contain often impurities and oxidation products which may be utilized as substrates resulting in traces of monoterpen and monoterpeneoid transformation products that are not further metabolized. Stoichiometric experiments have to show that the amount of metabolite is larger than the amount of impurity in the substrate. Only such careful stoichiometric experiments, mutants in functional genes or the characterization of enzymes in vitro can provide a proof of the presence of a biotransformation.

Eucalyptol, the bicyclic monoterpane 1,8-cineole [10] (<sub>10</sub>H<sub>18</sub>O), is transformed in several pathways. Novosphingobium subterranea converts 1,8-cineole initially into 2-endohydroxycineole, 2,2-oxo-cineole, and 2-exo-hydroxycineole. Acidic products from ring cleavages have been identified in situ (Rasmussen et al., 2005). Hydroxy-cineole formation occurred in 1,8-cineole-grown cultures of Pseudomonas flava (Carman et al., 1986). A cytochrome P450 monoxygenase from Bacillus cereus UI-1477 catalyzes the hydroxylation of 1,8-cineole, yielding either 2R-endo- or 2R-exo-hydroxy-1,8-cineole [11] (Liu and Rosazza, 1990, 1993). Another 1,8-cineole-specific P450 monoxygenase (EC 1.14.13.156) has been purified and characterized from Citrobacter braakii, which yielded 2-endo-hydroxy-1,8-cineole only. Further oxidation and lactonization were followed by a spontaneous lactone ring hydrolysis (Hawkes et al., 2002). Biotransformation in Rhodococcus sp. C1 involves an initial hydroxylation to 6-endo-hydroxycineole [12] and further oxidation to 6-oxocineole by a 6-endo-hydroxycineole dehydrogenase (EC 1.1.1.241). A 6-oxocineole monoxygenase (EC 1.14.13.51) converts the ketone into an unstable lactone. Spontaneous decomposition results in (R)-5,5-dimethyl-4-(3′-oxobutyl)-4,5-dihydrofuran-2(3H)-one. An initial monoxygenase activity has not been detected in cell-free systems, while the dehydrogenase and oxygenase activities have been measured in crude cell extracts (Williams et al., 1989).

**MONOCYCLIC MONOTERPENES**

Limonene [6, Figure 3] (<sub>10</sub>H<sub>16</sub>) is the most abundant monocyclic monoterpane, besides toluene the second most abundant VOC indoors (Brown et al., 1994). It represents the main component of essential oils from citrus plants, e.g. lemon and orange. Rhodococcus erythropolis DCL14 transforms (R/S)-limonene via limonene-1,2-epoxide into limonene-1,2-diol [13, Figure 5], applying a limonene-1,2 monoxygenase (EC 1.14.13.107) and a limonene-1,2-epoxide hydrolase (EC 3.3.2.8),
respectively. A specific dehydrogenase (EC 1.1.1.297) forms the ketone, 1-hydroxy-2-oxolimonene, which is oxidized to a lactone by a 1-hydroxy-2-oxolimonene 1,2-monooxygenase (EC 1.14.13.105). Enzyme activities were only detected in limonene-induced cells, suggesting a tight regulation of the limonene degradation. R. erythropolis DCL14 harbors a second pathway for limonene degradation. Initially, \((R)-\text{limonene}\) is hydroxylated by a NADPH-dependent limonene 6-monooxygenase (EC 1.14.13.48) to \(\text{trans-carveol}\) [7]. Subsequently, \(\text{trans-carveol}\) is oxidized to carvone and dihydrocarvone by a carvone dehydrogenase (EC 1.1.1.243) and carvone reductase (EC 1.3.99.25), respectively. A monocyclic monoterpen ketone monoxygenase (EC 1.14.13.105) inserts an oxygen atom, forming isopropenyl-7-methyl-2-oxo-oxepanone [14, Figure 6]. This lactone is cleaved by a specific \(\varepsilon\)-lactone hydrolase (EC 3.1.1.83) yielding hydroxyl-3-isopropenyl-heptanoate. Oxidation and activation as coenzyme A thioester enable a further degradation in accordance to the beta-oxidation (van der Werf et al., 1999b; van der Werf and Boot, 2000). \(R. \text{opacus PWD4}\) uses \((R)\)-limonene on the same pathway. Biomass from a glucose-toluene chemostat culture transformed limonene into trans-carveol, which was further oxidized to carvone by a \(\text{trans-carveol}\) dehydrogenase (EC 1.1.1.275) (Duetz et al., 2001).

Studies on the limonene metabolism in \(P. \text{gladioli}\) identified \(\alpha\)-terpineol [9, Figure 4] and perillyl alcohol [15] as major metabolites. However, none of the involved enzymes has been purified or further characterized (Cadwallader et al., 1989). A \(\alpha\)-terpineol dehydratase from \(P. \text{gladioli}\) was isolated and partially purified. The hydration reaction to the isopropenyl double bond of \(\text{(4R)}\)-\(\text{limonene}\) resulted in \((\text{4S})\)-\(\text{limonene}\) as only product (Cadwallader et al., 1992).

\(\text{Geobacillus stearothermophilus}\) (ex \(Bacillus\)) showed growth on limonene as sole carbon source. The main limonene transformation product was perillyl alcohol, while \(\alpha\)-terpineol and perillyl aldehyde were found in minor concentrations. After heterologous expression of a putative limonene degradation pathway in \(E. \text{coli}\), \(\alpha\)-terpineol was identified as major product of the biotransformation. Other studies reported a limonene hydroxylation on the methyl group yielding perillyl alcohol, which underwent further oxidation to perillic acid (Chang and Oriel, 1994; Chang et al., 1995). Additional studies on the recombinant limonene hydroxylase confirmed the production of perillyl alcohol from limonene but revealed in addition the formation of carvone. The limonene hydroxylase showed dependency on molecular oxygen and NADH as cofactors and was suggested to belong to the \((S)\)-limonene 7-monoxygenase family (EC 1.14.13.49) (Cheong and Oriel, 2000).

\(\text{Enterobacter agglomerans} 6L\) and \(Kosakonia cowani}\) 6L (ex \(\text{Enterobacter cowani}\)) transformed \((R)-\text{limonene}\) [6]. The main metabolites detected in ether extracts of \(E. \text{agglomerans} 6L\) cultures were \(\gamma\)-valerolactone and cryptone [16]. In assays using four recombinant expressed limonene-transforming enzymes from \(K. \text{cowani} 6L\), \(\text{linalool}\) [17, Figure 8] was identified as main product besides smaller amounts of dihydrolinalool. It was proposed that the potential limonene hydroxylase converts limonene into linalool, perillyl alcohol, \(\alpha\)-terpineol and \(\gamma\)-terpineol [18] (Park et al., 2003; Yang et al., 2007).

\(\text{Pseudomonas putida}\) (MTCC 1072) converts limonene to \(p\)-menth-1-ene-6,8-diol [19] and perillyl alcohol (Chatterjee and Bhattacharyya, 2001). No sequence information was found in public databases. Two other strains of \(Pseudomonas putida\) (F1 and GS1) have been found to convert \((\pm)\)-limonene to perillic
acid in co-substrate fed-batch cultures (Speelmans et al., 1998). Experimental results indicated the participation of the p-cymene pathway (CYM) (Mars et al., 2001). Castellaniella defragrans grows anaerobically on cyclic monoterpenes as sole carbon and energy source under denitrifying conditions (Foss et al., 1998). Recent experiments suggested an oxygen-independent hydroxylation on the methyl group of limonene to perillyl alcohol as the initial activation step, followed by subsequent oxidation to perilllic acid (Petasch et al., 2014).

P-cymene [20, Figure 7] (C10H14) is an aromatic monoterpene (p-isopropyl-toluene). Pseudomonas putida F1 (ATCC 700007) degrades p-cymene to p-cumate [21] via the CYM-pathway (cymBCAaAbDE). A two-component p-cymene monooxygenase (cymAaAb, EC 1.14.13.-) introduces a hydroxyl group on the methyl group of p-cymene. The resulting p-cumic alcohol is oxidized to the corresponding carboxylic acid by an alcohol and an aldehyde dehydrogenase (cymB and cymC, EC 1.1.1.- and EC 1.2.1.-). The genes cymD and cymE encode for a putative outer membrane protein and an acetyl coenzyme A synthetase, respectively. However, their role in the pathway remains unclear (Eaton, 1997). Upstream of the cym-operon, the genes for the further degradation of p-cumate are located. They are organized in another operon and comprise eight genes (cmtABCDEFGH). P. putida F1 has been shown to use p-cumate as sole carbon source. It is hydroxylated by a ferredoxin dependent p-cumate 2,3-dioxygenase. The genes cmtAaAd encode a ferredoxin reductase and a ferredoxin, and cmtAbAc encode the large and the small subunits of the dioxygenase (EC 1.14.12.-). The resulting cis-2,3-dihydroxy-2,3-dihydro-p-cumate is oxidized and ring cleavage occurs by introduction of another oxygen molecule. The responsible enzymes are a specific dehydrogenase (cmtB, EC 1.3.1.58) and a 2,3-dihydroxy-p-cumate dioxygenase (cmtC, EC 1.13.11.-), respectively. Further degradation is accomplished by a decarboxylation and elimination of an isobutyrate molecule, catalyzed by a 2-hydroxy-3-carboxy-6-oxo-7-methyllocta-2,4-dienoate decarboxylase (cmtD, EC 4.1.1.-) and a 2-hydroxy-6-oxo-7-methyllocta-2,4-dienoate hydrolase (cmtE, EC 3.7.1.-). The product, 2-hydroxypenta-2,4-dienoate, undergoes a water addition by a specific hydratase (cmtF, EC 4.2.1.80). Then, a carbon-carbon lyase reaction yields pyruvate and acetate, catalyzed by 2-hydroxyvalerate aldolase (cmtG, EC 4.1.3.39). Acetate is oxidized and enters as acetyl-CoA the citrate cycle (Eaton, 1996).

Thauera terpenica 21 Mol utilizes menthol [22] as sole carbon source. The proposed degradation mechanism involves two initial oxidation reactions leading to menth-2-enone, followed by a hydration and an additional oxidation step. Finally, ring cleavage may occur and the molecule is attached to coenzyme A to yield 3,7-dimethyl-5-oxo-octyl-CoA (Foss and Harder, 1998; Hylemon and Harder, 1998).

ACYCLIC MONOTERPENES

First studies on acyclic monoterpenoids in the early sixties by Seubert and colleagues described the degradation of citronellol [23], geraniol [24], and nerol via an oxidation of the alcohol to an acid, followed by the formation of a CoA-thioester and subsequent beta-oxidation in Pseudomonas citronellolis (ATCC 13674) (Seubert, 1960; Seubert and Remberger, 1963; Seubert et al., 1963; Seubert and Fass, 1964). This knowledge has been extended toward other Pseudomonas strains (Cantwell et al., 1978). The complete degradation pathway has been classified as the acyclic terpene utilization and leucine utilization (ATU/LIU) pathway involving the genes atuABCDEFGH and...
The acyclic monoterpene β-myrcene [25] (C_{10}H_{16}) is transformed by *Pseudomonas aeruginosa* (PTCC 1074) into dihydrodinalool, 2,6-dimethyloctane and α-terpineol. Limonene has been proposed as possible intermediate in α-terpineol formation but was not detected in the culture broth (Esmaeili and Hashemi, 2011). *Pseudomonas* sp. M1 accomplishes degradation by hydroxylation on the C8 position to myrcene-8-ol, which is further oxidized, linked to coenzyme A and metabolized in a beta-oxidation like manner (Iurescia et al., 1999). The formation of geraniol from β-myrcene has been observed with resting cells of *Rhodococcus erythropolis* MT1, regardless of the presence of a cytochrome P450 inhibitor. The reaction was dependent on aerobic conditions, however it remains unclear if a monooxygenase or lyase system is involved (Thompson et al., 2010).

The tertiary alcohol linalool is also transformed at the C8 position. A linalool monooxygenase (EC 1.14.13.151) has been described in *P. putida* Pp2777 and *Novosphingobium aromaticivorans* (ATCC 700278D-5) (Ullah et al., 1990; Bell et al., 2010). In the absence of molecular oxygen, *Castellaniella defragrans* 65Phen has an unique enzyme for the linalool transformation, the linalool dehydratase-isomerase (Brodkorb et al., 2010). *Castellaniella* and *Thaueria* strains were the first anaerobic microorganisms shown to anaerobically degrade and mineralize monoterpenes (Harder and Probian, 1995; Harder et al., 2000). The linalool dehydratase-isomerase (EC 4.2.1.127 and 5.4.4.4) of *C. defragrans* 65Phen catalyzes a regio- and stereo-specific hydration of β-myrcene yielding the tertiary alcohol (S)-(1)-linalool [17] and the isomerization to the primary alcohol geraniol (Brodkorb et al., 2010; Lueddeke and Harder, 2011). Geraniol and geraniol dehydrogenases formed geranic acid (Heyen and Harder, 2000; Lueddeke et al., 2012). *T. linaloenum* 47LoL grows on linalool as sole carbon and energy source. A similar isomerization of linalool to geraniol with subsequent oxidation of geraniol to geranial has been observed in cultures (Foss and Harder, 1997).

**MONOTERPENE TRANSFORMATION BY FUNGI**

Fungi excrete laccases which are copper-containing oxidases. Utilizing molecular oxygen as a cosubstrate, an unspecific oxidation of organic molecules is initiated by these enzymes. Additionally, fungi express a variety of cytochrome P450 mono- and di-oxygenases. Thus, several fungi were described to transform monoterpenes during growth in rich medium (reviewed by Farooq et al., 2004). Species with a reported capacity to transform monoterpenes are *Aspergillus niger*, *Botrytis cinerea*, *Diplodia gossypina*, *Macr cirrinelloides*, *Penicillium italicum*, *Penicillium digitatum*, *Corynespora cassiicola*, and *Glomerella cingulata*. For a long time, no species have been described to use monoterpenes as sole carbon and energy source for growth (Trudgill, 1994 and references therein). Recently, *Grosmannia clavigera*, a bark beetle-associated fungal pathogen of pine trees, was shown to grow on a mono- and diterpene mixture, containing α/β-pinene and 3-carene (Diguistini et al., 2011). ABC efflux transporter and cytochrome P450 enzymes confer a monoterpenes resistance to the blue-stain fungi (Lah et al., 2013; Wang et al., 2013).

**MONOTERPENES IN THE CARBON CYCLE**

Habitats with a dense vegetation of wood and flowers are expected to contain larger populations of monoterpenes transforming microorganisms. Whereas coniferous forests emit up to 6.7 g carbon m\(^{-2}\)yr\(^{-1}\), broadleaf evergreen forest and grassland emit only 3.5 and 2.5 g carbon m\(^{-2}\)yr\(^{-1}\), respectively (Tanaka et al., 2012). Monoterpene emission rates between 0.3 and 7 g carbon m\(^{-2}\)yr\(^{-1}\) for the United States—mainly α- and β-pinene, limonene and β-myrcene (Geron et al., 2000)—can support the aerobic growth of 0.15–3.5 g bacteria m\(^{-2}\)yr\(^{-1}\), assuming 50% of carbon incorporated into biomass. This is a significant potential, considering the presence of around 10 g microbial biomass in the top centimeter of soil per square meter.

In marine systems, isoprene and monoterpenes (mainly α-pinene) are produced by phytoplankton and algae and partially emitted into the atmosphere (reviewed by Yassaa et al., 2008; Shaw et al., 2010). Isoprene emission was estimated to 0.2–1.2 Tg carbon\(^{-1}\)yr\(^{-1}\) (Palmer and Shaw, 2005; Gantt et al., 2009; Shaw et al., 2010). For the ocean surface area this results in an emission rate of 0.0025 g carbon m\(^{-2}\)yr\(^{-1}\). Current uncertainties in the size of emission based on shipborne measurements in comparison to satellite data (Luo and Yu, 2010) may be resolved by incorporating an export from the continental atmosphere to the oceanic atmosphere (Hu et al., 2013). Isoprene-amended samples from marine habitats were enriched in bacteria affiliated with *Actinobacteria*, *Alphaproteobacteria*, and *Bacteroidetes* and first strains were shown to degrade isoprene and aliphatic hydrocarbons (Acuña Alvarez et al., 2009).

In summary, these findings indicate a higher abundance of monoterpenes transforming and mineralizing bacteria in soils than in the ocean. Indeed, most monoterpenes transforming bacteria have been enriched or isolated from soil and freshwater samples in habitats with monoterpenes emitting vegetation.
Table 1 | Summary table of monoterpene transforming enzymes in validly described species of Bacteria.

| EC number | Enzyme name | Organism | Substrate | Co-substrate | Product | Co-product | References |
|-----------|-------------|----------|-----------|--------------|---------|------------|------------|
| 1.14.13.155 | α-pinene monooxygenase | Pseudomonas fluorescens NCIMB 11671 | α-pinene | Oxygen, NADH | α-pinene oxide | Water, NAD⁺ | Best et al., 1987 |
| 5.5.1.10 | α-pinene oxide lyase | Pseudomonas fluorescens NCIMB 11671 | α-pinene oxide |  | (E)-2,6-dimethyl-5-methyldiene-hept-2-enal (iso-novalal) | | Best et al., 1987 |
| 1.14.13.156 | 1,8-cineole 2-endo-monoxygenase | Citrobacter braakii | 1,8-cineole | Oxygen, NADPH | 2-endo-hydroxy-1,8-cineole | Water, NADP⁺ | Hawkes et al., 2002 |
| 1.14.13.105 | Monocyclic monoterpene ketone monooxygenase | Rhodococcus erythropolis | (-)-menthone | Oxygen, NADPH | 1-hydroxy-p-menth-8-en-2-one | Water, NAD(P)⁺ | van der Werf et al., 1999b |
| 1.1.1.297 | Limonene 1,2-diol dehydrogenase | Rhodococcus erythropolis DCL 14 | Limonene 1,2-diol | NAD⁺ | 1-hydroxy-p-menth-8-en-2-one | NADH | van der Werf et al., 1999b |
| 1.14.13.107 | Limonene 1,2-monooxygenase | Rhodococcus erythropolis DCL 14 | (R)-limonene | Oxygen, NAD(P)H | 1,2-epoxy-p-menth-8-ene | Water, NAD(P)⁺ | van der Werf et al., 1999b |
| 1.1.1.243 | Carveol dehydrogenase | Rhodococcus erythropolis DCL 14 | (-)-trans-carveol | NAD⁺ | (-)-carvone | NADPH | van der Werf et al., 1999b |
| 1.3.99.25 | Carvone reductase | Rhodococcus erythropolis DCL 14 | (+)-dihydrocarvone | Oxidized electron acceptor | (-)-carvone | Reduced electron acceptor | van der Werf et al., 1999b |
| 1.1.1.275 | Trans-carveol dehydrogenase | Rhodococcus opacus PWD4 (DSM 44313) | (+)-trans-carveol | NAD⁺ | (+)-carvone | NADH | Duetz et al., 2001 |
| 3.1.1.83 | Monoterpene ε-lactone hydrolase | Rhodococcus erythropolis DCL 14 | (4S,7R)-7-methyl-4-prop-1-en-2-yl-oxepan-2-one | Water | 6-hydroxy-3-prop-1-en-2-yl-heptanoate | | van der Werf et al., 1999a |
| 3.3.2.8 | (4R)-limonene-1,2-epoxide hydrolase | Rhodococcus erythropolis DCL 14 | 1,2-epoxy-p-menth-8-ene | Water | Menth-8-en-1,2-diol | | van der Werf et al., 1999a |
| 1.1.1.297 | (1S,2S,4R)-limonene-1,2-diol dehydrogenase | Rhodococcus erythropolis DCL 14 | Menth-8-en-1,2-diol | NAD⁺ | 1-hydroxy-p-menth-8-en-2-one | NADH | van der Werf et al., 1999a |
| 1.14.13.49 | (S)-limonene 7-monooxygenase | Geobacillus stearothermophilus (ex Bacillus strain BR388) | (S)-limonene | Oxygen, NADPH | (−)-perillyl alcohol | Water, NAD⁺ | Cheong and Oriel, 2000 |
| 1.14.13.161 | Linalool 8-monooxygenase | Novosphingobium aromaticivorans ATCC 700278D-5 Pseudomonas putida PpG777 | Linalool | 2 oxygen, 2 NADH | (6E)-8-oxolinalool | 3 Water, 2 NAD⁺ | Bell et al., 2010 |
|           |           |           |           |           |           |           | Ullah et al., 1990 |
Table 1 | Continued

| EC number | Enzyme name | Organism | Substrate | Co-substrate | Product | Co-product | References |
|-----------|-------------|----------|-----------|--------------|---------|------------|------------|
| 4.2.1.127 | Linalool dehydratase (-isomerase) | Castellaniella defragrans 65Phen (DSM 12143) | β-myrcene | Water (S)-(-)-linalool | (S)(-)-linalool | | Brodkorb et al., 2010 |
| 5.4.4.4   | Linalool (dehydratase)-isomerase | Castellaniella defragrans 65Phen (DSM 12143) | (S)(-)-linalool | Geraniol | | Brodkorb et al., 2010 |
| 1.1.1.347 | Geraniol dehydrogenase | Castellaniella defragrans 65Phen (DSM 12143) | Geraniol | NAD⁺ | Geranial | NADH | Lueddeke et al., 2012 |
| 1.2.1.86  | Geraniol dehydrogenase | Castellaniella defragrans 65Phen (DSM 12143) | Geranial | Water, NAD⁺ | Geranic acid | NADH | Lueddeke et al., 2012 |

**Cym PATHWAY**

| 1.14.13 | p-cymene monooxygenase, hydroxylase subunit (CymAa) | Pseudomonas putida F1 (ATCC 700007) | p-cymene | Oxygen, NADH | p-cumic alcohol | Water, NAD⁺ | Eaton, 1997 |
| 1.14.13 | p-cymene monooxygenase, reductase subunit (CymAb) | Pseudomonas putida F1 (ATCC 700007) | p-cymene | Oxygen, NADH | p-cumic alcohol | Water, NAD⁺ | Eaton, 1997 |
| 1.1.1 | p-cumic alcohol dehydrogenase (CymB) | Pseudomonas putida F1 (ATCC 700007) | p-cumic alcohol | NAD⁺ | p-cumic aldehyde | NADH | Eaton, 1996 |
| 1.2.1 | p-cumic aldehyde dehydrogenase (CymC) | Pseudomonas putida F1 (ATCC 700007) | p-cumic aldehyde | Water, NAD⁺ | p-cumic acid | NADH | Eaton, 1996 |
| -.-.- | Putative outer membrane protein, unknown function (CymD) | Pseudomonas putida F1 (ATCC 700007) | | | | | Eaton, 1996 |

| 6.2.1.1 | Acetyl-CoA synthetase (CymE) | Pseudomonas putida F1 (ATCC 700007) | Acetate | CoA, ATP | Acetyl-CoA | Diphosphate, AMP | Eaton, 1996 |

**Cmt PATHWAY**

| 1.14.12 | p-cumate 2,3-dioxygenase (CmtAaAbAcAd) | Pseudomonas putida F1 (ATCC 700007) | p-cumate | Oxygen, NADH | cis-2,3-dihydroxy-2,3-dihydro-p-cumate | NAD⁺ | Eaton, 1996 |
| 1.3.1.98 | 2,3-dihydroxy-2,3-dihydro-p-cumate dehydrogenase (CmtB) | Pseudomonas putida F1 (ATCC 700007) | cis-2,3-dihydroxy-2,3-dihydro-p-cumate | NAD⁺ | 2,3-dihydroxy-p-cumate | NADH | Eaton, 1996 |
| 1.13.11 | 2,3-dihydroxy-p-cumate dioxygenase (CmtC) | Pseudomonas putida F1 (ATCC 700007) | 2,3-dihydroxy-p-cumate | Oxygen | 2-hydroxy-3-carboxy-6-oxo-7-methylocta-2,4-dienoate | | Eaton, 1996 |
| 4.1.1 | 2-hydroxy-3-carboxy-6-oxo-7-methylocta-2,4-dienoate decarboxylase (CmtD) | Pseudomonas putida F1 (ATCC 700007) | 2-hydroxy-3-carboxy-6-oxo-7-methylocta-2,4-dienoate | | 2-hydroxy-6-oxo-7-methylocta-2,4-dienoate | Carbon dioxide | Eaton, 1996 |

(Continued)
| EC number | Enzyme name | Organism | Substrate | Co-substrate | Product | Co-product | References |
|-----------|-------------|----------|-----------|--------------|---------|------------|------------|
| 3.7.1.2   | 2-hydroxy-6-oxo-7-methylocta-2,4-dienoate hydrolase (CmtE) | *Pseudomonas putida* F1 (ATCC 700007) | 2-hydroxy-6-oxo-7-methylocta-2,4-dienoate | Water | 2-hydroxypenta-2,4-dienoate | Isobutyrate | Eaton, 1996 |
| 4.2.1.80  | 2-hydroxypenta-2,4-dienoate hydratase (CmtF) | *Pseudomonas putida* MT2 (ATCC 33015) | 2-hydroxy-penta-2,4-dienoate | Water | 2-oxo-4-hydroxy-pentanoate | | Harayama et al., 1989 |
| 4.1.3.39  | 2-oxo-4-hydroxyvalerate aldolase (CmtG) | *Pseudomonas putida* PG (DSM 8368) | 2-oxo-4-hydroxy-pentanoate | Acetaldehyde | Pyruvate | | Platt et al., 1995 |
| 1.2.1.10  | Acetaldehyde dehydrogenase (CmtH) | *Pseudomonas citronellolis* (ATCC 13674) | Acetaldehyde | NAD\(^+\), CoA | Acetyl-CoA | NADH | Platt et al., 1995 |

### Atu PATHWAY

#### 1.199.2/1.2.99.2
- Citronellol/citronellal dehydrogenase (AtuB; AtuG)
  - *Pseudomonas citronellolis* (ATCC 13674)
  - Citronellol/citronellal
  - Water, oxidized electron acceptor
  - Citronellol/citronellate
  - Reduced electron acceptor
  - Förster-Fromme et al., 2006

#### 6.2.1.1
- Putative citronellyl-CoA synthetase (AtuH)
  - *Pseudomonas citronellolis* (ATCC 13674)
  - Citronellate
  - CoA, ATP
  - Citronellyl-CoA
  - Diphosphate, AMP
  - Förster-Fromme et al., 2006

#### 1.3.99.2
- Putative citronellyl-CoA desaturase (AtuD)
  - *Pseudomonas citronellolis* (ATCC 13674)
  - Citronellyl-CoA
  - Oxidized electron acceptor
  - Cis-geranyl-CoA
  - Reduced electron acceptor
  - Förster-Fromme et al., 2006

#### 6.4.1.5
- Geranyl-CoA carboxylase, carboxylase alpha-subunit (AtuF)
  - *Pseudomonas citronellolis* (ATCC 13674)
  - Cis-geranyl-CoA
  - Bicarbonate, ATP
  - Isohexenyl-glutaconyl-CoA
  - ADP, phosphate
  - Förster-Fromme et al., 2006

#### 6.4.1.5
- Geranyl-CoA carboxylase, carboxylase beta-subunit (AtuC, AtuF)
  - *Pseudomonas mendocina* (ATCC 25411)
  - Pseudomonas mendocina (ATCC 25411)
  - Cis-geranyl-CoA
  - Bicarbonate, ATP
  - Isohexenyl-glutaconyl-CoA
  - ADP, phosphate
  - Fall and Hector, 1977; Förster-Fromme et al., 2006; Cantwell et al., 1978; Diaz-Pérez et al., 2004; Höschle et al., 2005

#### 4.2.1.57
- Isohexenyl-glutaconyl-CoA hydratase (AtuE)
  - *Pseudomonas citronellolis* (ATCC 13674)
  - Isohexenyl-glutaconyl-CoA
  - Water
  - 3-hydroxy-3-isohexenyl-glutaryl-CoA
  - Förster-Fromme et al., 2006
  - Diaz-Pérez et al., 2004; Höschle et al., 2005; Cantwell et al., 1978

#### 4.1.3.26
- 3-hydroxy-3-isohexenyl-glutaryl-CoA:acetate lyase (LiuE)
  - *Pseudomonas citronellolis* (ATCC 13674)
  - 3-hydroxy-3-isohexenyl-glutaryl-CoA
  - 7-methyl-3-oxo-6-octenoyl-CoA
  - Acetate
  - Förster-Fromme et al., 2006
  - Chávez-Avilés et al., 2010

(Continued)
Table 1 | Continued

| EC number | Enzyme name | Organism | Substrate | Co-substrate | Product | Co-product | References |
|-----------|-------------|----------|-----------|--------------|---------|------------|------------|
| LiuE      | Pseudomonas aerugenosa PAO1 (ATCC 15692) |           |           |              |         |            | Chávez-Avilies et al., 2010 |

Cam PATHWAY

1.14.15.1 Camphor 5-monoxygenase (CamABC)

| EC number | Enzyme name | Organism | Substrate | Co-substrate | Product | Co-product | References |
|-----------|-------------|----------|-----------|--------------|---------|------------|------------|
| 1.14.15.1 | Camphor 5-monoxygenase (CamABC) | Pseudomonas putida (ATCC 29607) | (+)-camphor | Oxygen, reduced putidaredoxin | 5-oxo-hydroxy-camphor | Water, oxidized putidaredoxin | Poulos et al., 1985 |
|           |             | Novosphingobium aromaticivorans (ATCC 700278D-5) |            |              |         |            | (Iwaki et al., 2013, and references therein) |

1.1.1.327 5-exo-hydroxycamphor dehydrogenase (CamD)

| EC number | Enzyme name | Organism | Substrate | Co-substrate | Product | Co-product | References |
|-----------|-------------|----------|-----------|--------------|---------|------------|------------|
| 1.1.1.327 | 5-exo-hydroxycamphor dehydrogenase (CamD) | Pseudomonas putida (ATCC 17453) | 5-oxo-hydroxy-camphor | NAD+ | 2,5-diketocamphane/3,6-diketocamphane | NADH | Aramaki et al., 1993 |

1.14.13.162 2,5-diketocamphane 1,2-monooxygenase (CamE25-1, CamE25-2, CamE36)

| EC number | Enzyme name | Organism | Substrate | Co-substrate | Product | Co-product | References |
|-----------|-------------|----------|-----------|--------------|---------|------------|------------|
| 1.14.13.162 | 2,5-diketocamphane 1,2-monooxygenase (CamE25-1, CamE25-2, CamE36) | Pseudomonas putida (ATCC 17453) | 2,5-diketocamphane | Oxygen, NADH | (+)-5-oxo-1,2-campholide | Water, NAD+ | Taylor and Trudgill, 1986 |

1.14.13.162 3,6-diketocamphane 1,6-monooxygenase (CamE36)

| EC number | Enzyme name | Organism | Substrate | Co-substrate | Product | Co-product | References |
|-----------|-------------|----------|-----------|--------------|---------|------------|------------|
| 1.14.13.162 | 3,6-diketocamphane 1,6-monooxygenase (CamE36) | Pseudomonas putida (ATCC 17453) | 3,6-diketocamphane | Oxygen, NADH | (−)-5-oxo-1,2-campholide | Water, NAD+ | Taylor and Trudgill, 1986 |

6.2.1.38 (2,2,3-trimethyl-5-oxocyclopent-3-enyl) acetyl-CoA synthase (CamF1, CamF2)

| EC number | Enzyme name | Organism | Substrate | Co-substrate | Product | Co-product | References |
|-----------|-------------|----------|-----------|--------------|---------|------------|------------|
| 6.2.1.38  | (2,2,3-trimethyl-5-oxocyclopent-3-enyl) acetyl-CoA synthase (CamF1, CamF2) | Pseudomonas putida (ATCC 17453) | (1R)-2,2,3-trimethyl-5-oxocyclopent-3-enyl acetate | ATP, CoA | (1R)-2,2,3-trimethyl-5-oxocyclopent-3-enyl acetyl-CoA | Diphosphate, AMP | Oughham et al., 1983 |

1.14.13.160 2-oxo-Δ2-4,5,5-trimethyl cyclopentenyl acetyl-CoA 1,2-monooxygenase (CamG)

| EC number | Enzyme name | Organism | Substrate | Co-substrate | Product | Co-product | References |
|-----------|-------------|----------|-----------|--------------|---------|------------|------------|
| 1.14.13.160 | 2-oxo-Δ2-4,5,5-trimethyl cyclopentenyl acetyl-CoA 1,2-monooxygenase (CamG) | Pseudomonas putida (ATCC 17453) | (1R)-2,2,3-trimethyl-5-oxocyclopent-3-enyl acetyl-CoA | Oxygen, NADPH | (2R)-3,3,4,trimethyl-6-oxo-3,6-dihydro-1H-pyran-2-yl acetyl-CoA | Water, NADP+ | Oughham et al., 1983; Leisch et al., 2012 |

DATABASES FOR PATHWAY ANALYSIS AND A LOOK AT METAGENOMES

Databases are nowadays available for the analysis of enzymatic reactions and metabolic pathways in metagenomic and genomic sequence datasets. The most relevant are the Kyoto Encyclopedia of Genes and Genomes (KEGG), MetaCyc and the Biocatalysis/Biodegradation database of the University of Minnesota.

First studies used KEGG to identify monoterpene-related genes in metagenomes of microbiomes in insects and nematodes feeding on a monoterpene-rich diet. Pine beetles encounter the high terpenoid concentrations of conifers and may take advantage of detoxification processes catalyzed by their symbionts/microbiomes (Adams et al., 2013). The KEGG pathway for limonene and pinene degradation (ko00903) was used to identify genes encoding enzymes putatively involved in monoterpene degradation. Five enzymes were present and more abundant in the metagenomes than in a combined metagenomic set of plant biomass-degrading communities. These enzymes were an aldehyde dehydrogenase, an oxidoreductase, an enoyl-CoA hydratase and two hydratases/epimerases. Whether these genes are truly involved in monoterpene metabolism or the degradation of cyclic
compounds, e.g. related aromatic lignin monomers, is an open question. Taxonomically, these genes affiliated with the genera *Pseudomonas, Rahmella, Serratia*, and *Stenotrophomonas*. The pinewood nematode *Bursaphelenchus xylophilus* transcribes cytochrome P450 genes as main metabolic pathway for xenobiotics detoxification, but not all enzymes needed for terpenoid metabolism were detected by transcriptomic analysis. Metagenomic data of nematode bacterial symbionts included the complete α-pinene degradation pathway (Cheng et al., 2013). Annotation based on KEGG revealed that the degradation pathways for limonene and pinene (map00903) and for geran-iol (map00281) accounted for 2.5% of mapped metagenomes. The majority of these genes affiliated to *Pseudomonas, Achromobacter*, and *Agrobacterium*. Strains isolated from the nematode and capable of growth on α-pinene affiliated to *Pseudomonas, Achromobacter, Agrobacterium, Cytophaga, Herbaspirillum*, and *Stenotrophomonas*.

**CONCLUSION**

The synthesis and transformation of BVOCs, especially terpenoids, by plants is well studied (Kesselmeier and Staudt, 1999). Corresponding pathways have been elucidated and a variety of corresponding enzymes have been isolated and characterized (Mahmoud and Croteau, 2002; Yu and Utsumi, 2009). In contrast, the exploration of the microbial transformation and mineralization of monoterpenes has accumulated a small coverage of the field. Simply, over the last 50 years, research on bacterial monoterpenic metabolism had only found the interest of very few principal investigators. Now, large sequence datasets of organisms and biological communities provide an unprecedented insight into the diversity of pathways and provide us with challenging hypotheses. However, the basis for the annotation is the biochemical characterization of enzymes which is only available for few monoterpenes. Only three pathways are completely known on the genetic and enzymatic level: the ones for camphor (CAM), *p*-cymene (CYM/CMT), and citronellol/geraniol (ATU/LIU). For pinene, the gene for a key enzyme, the α-pinene oxide lyase (EC 5.5.1.10), is still unknown. The lack of such a key enzyme sequence for a KEGG pathway (map00903) illustrates our uncertainty in the interpretation of metagenomic and genomic datasets. Progress in proteomic and metabolomic analyses in the last years support now biochemical and genetic experiments which will swiftly reveal the desired identification of key enzymes in the monoterpenic metabolism.

**REFERENCES**

Aguilar, J. A., Zavala, A. N., Díaz-Pérez, C., Cervantes, C., Díaz-Pérez, A. L., and Campos-García, J. (2006). The *atu* and *liu* clusters are involved in the catabolic pathways for aromatic monoterpenes and leucine in *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol*. 72, 2070–2079. doi: 10.1128/AEM.72.3.2070-2079.2006

Ajikumar, P. K., Tyo, K., Carlsen, S., Mucha, O., Phon, T. H., and Stephanopoulos, G. (2008). Terpenoids: opportunities for biosynthesis of natural product drugs using engineered microorganisms. *Mol. Pharm*. 5, 167–190. doi: 10.1021/mp700151b

Alfrman, T., Travers, M., Kothari, A., Caspi, R., and Karp, P. D. (2013). A systematic comparison of the MetaCyc and KEGG pathway databases. *BMC Bioinformatics* 14:112. doi: 10.1186/1471-2105-14-112

Aramaki, H., Koga, H., Sagara, Y., Hosoi, M., and Horiiuchi, T. (1993). Complete nucleotide-sequence of the 5-exo-hydroxycamphor dehydrogenase gene on the CAM plasmid of *Pseudomonas putida* (ATCC-17453). *Biochem. Biophys. Acta* 1174, 91–94. doi: 10.1016/0167-4781(93)90098-X

Atkinson, R., and Arey, J. (2003). Atmospheric degradation of volatile organic compounds. *Chem. Rev.** 103, 4605–4638. doi: 10.1021/cr0206420

Bicas, J. L., Averbek, S., Averbek, D., and Woosam, M. (2008). Biological effects of essential oils - a review. *Food Chem. Toxicol*. 46, 446–475. doi: 10.1016/j.fct.2007.09.106

Bell, S. G., Dale, A., Rees, N. H., and Wong, L. L. (2010). A cytochrome P450 class I electron transfer system from *Novosphingobium aromaticivorans*. *Appl. Microbiol. Biotechnol*. 86, 163–175. doi: 10.1007/s00253-009-2234-y

Best, D. J., Floyd, N. C., Magalhaes, A., Burfield, A., and Rhodes, P. M. (1987). Initial enzymatic steps in the degradation of alpha-pinene by *Pseudomonas fluorescens* NCimb 11671. *Bicatal. Biotransf*. 1, 147–159. doi: 10.3109/2042428709040139

Bicas, J. L., Dionisio, A. P., and Pastore, G. M. (2009). Bio-oxidation of terpenes: an approach for the flavor industry. *Chem. Rev*. 109, 4518–4531. doi: 10.1021/cr800190y

Bicas, J. L., Fontanille, P., Pastore, G. M., and Larroche, C. (2008). Characterization of monoterpene biotransformation in two pseudomonads. *App. Microbiol*. 105, 1991–2001. doi: 10.1136/1365-2672.2008.03923.x

Brodkorb, D., Gottschall, M., Marmulla, R., Lueddeke, F., and Harder, J. (2010). Linalool dehydratase-isomerase, a bifunctional enzyme in the anaerobic degradation of monoterpenes. *J. Biol. Chem*. 285, 30436–30442. doi: 10.1074/jbc.M109.084244

Brown, S. K., Sim, M. R., Abramson, M. J., and Gray, C. N. (1994). Concentrations of volatile organic compounds in indoor air - a review. *Indoor Air*, 4, 123–134.

Cadwallader, K. R., Braddick, R. J., and Parish, M. E. (1992). Isolation of alpha-pinene dehydrogenase from *Pseudomonas gladioli*. *J. Food Sci*. 57, 241. doi: 10.1111/j.1365-2621.1992.tb05464.x

Cadwallader, K. R., Braddick, R. J., Parish, M. E., and Higgins, D. P. (1989). Bioconversion of (+)-limonene by *Pseudomonas gladioli*. *J. Food Sci*. 54, 1241–1245. doi: 10.1111/j.1365-2621.1989.tb05964.x

Cantwell, S. G., Lau, E. P., Watt, D. S., and Fall, R. R. (1978). Biodegradation of acyclic isoepinoids by *Pseudomonas species*. *J. Bacteriol*. 135, 324–333.

Carman, R. M., Macrea, I. C., and Perkins, M. V. (1986). The oxidation of 1,8-cineole by *Pseudomonas flava*. *Aust. J. Chem*. 39, 1739–1746. doi: 10.1071/CH961739

Chang, H. C., Gage, D. A., and Oriel, P. J. (1995). Cloning and expression of a limonene degradation pathway from *Bacillus stearothermophilus* in *Escherichia coli*. *J. Food Sci*. 60, 551–553. doi: 10.1111/j.1365-2621.1995.tb09824.x

Chang, H. C., and Oriel, P. J. (1994). Bioproduction of perillyl alcohol and related monoterpenes by isolates of *Bacillus stearothermophilus*. *J. Food Sci*. 59, 660. doi: 10.1111/j.1365-2621.1994.tb05588.x

Chatterjee, T., and Bhattacharyya, D. K. (2001). Biotransformation of limonene by *Pseudomonas putida*. *Appl. Microbiol. Biotechnol*. 55, 541–546. doi: 10.1007/s002530000358

Cheang, Y. C., Tian, X. L., Wang, Y. S., Lin, R. M., Mao, Z. C., Chen, N. S., et al. (2013). Metagenomic analysis of the pinewood nematode microbiome reveals a symbiotic relationship critical for xenobiotics degradation. *Sci. Rep*. 3:1869. doi: 10.1038/srep01869

Cheong, T. K., and Oriel, P. J. (2000). Cloning and expression of the limonene hydroxylase of *Bacillus stearothermophilus* BR388 and utilization in two-phase limonene conversions. *Appl. Biochem. Biotechnol*. 84–86, 903–915. doi: 10.1385/ABAB:84-86:1-9:903
Dhavalikar, R., and Bhattacharyya, P. (1966). Microbiological transformation of terpenes. 8. Fermentation of limonene by a soil Pseudomonad. Indian J. Biochem. 3, 144–157.

Dhavalikar, R., Ranagchari, P., and Bhattacharyya, P. (1966). Microbiological transformations of terpenes. 9. Pathways of degradation of limonene in a soil Pseudomonad. Indian J. Biochem. 3, 158–164.

Diaz-Perez, A. L., Zavala-Hernandez, A. N., Cervantes, C., and Campos-Garcia, J. (2004). The gnyRDBHAL cluster is involved in acyclic isoprenoid degradation in Pseudomonas aeruginosa. Appl. Environ. Microbiol. 70, 5102–5110. doi: 10.1128/AEM.70.9.5102-5110.2004

Diguistini, S., Wang, Y., Liao, N. Y., Taylor, G., Tanguay, P., Feau, N., et al. (2011). Genome and transcriptome analyses of the mountain pine beetle-fungal symbiont Grosmannia clavigera, a lodgepole pine pathogen. Proc. Natl. Acad. Sci. U.S.A. 108, 2504–2509. doi: 10.1073/pnas.1012891010

Dudareva, N., Klemptien, A., Muhlemann, J. K., and Kaplan, I. (2013). Biosynthesis, function and metabolic engineering of plant volatile organic compounds. New Phytol. 198, 16–32. doi: 10.1111/np.12145

Duetz, W. A., Bouwmeester, H., Van Beilen, J. B., and Witholt, B. (2003). Analysis and cloning and characterization of DNA encoding conversion of -cymene to -cumate. Appl. Environ. Microbiol. 69, 2829–2832. doi: 10.1128/AEM.69.6.2829-2832.2003

Duetz, W. A., Fjallman, A. H. M., Ren, S. Y., Jourdat, C., and Witholt, B. (2001). Pseudomonas putida PF1: a new and efficient biocatalyst for production of isonovalal from -cumate. J. Bacteriol. 173, 1351–1362.

Eaton, R. W. (1997). Microbial transformation of terpenes. 8. Fermentation of limonene by a soil Pseudomonad. Indian J. Biochem. 32, 227–233. doi: 10.1143/SP.32.227

Esmaeili, A., and Hashemi, E. (2011). Biotransformation of myrcene by Pseudomonas aeruginosa. Chem. Cent. J. 5:26. doi: 10.1186/1752-153X-5-26

Farooq, A., Atta-Ur-Rahman, and Choudhary, M. I. (2004). Fungal transformation in plant volatile organic compounds. Biocatal. Biotransformation 20, 413–421. doi: 10.1080/1024242021000058702

Fu, P. Q., Kawamura, K., Chen, J., and Barrie, L. A. (2009). Isoprene, monoterpenes, and sesquiterpene oxidation products in the high arctic aerosol during late winter to early summer. Environ. Sci. Technol. 43, 4022–4028. doi: 10.1021/es803666v

Gantt, B., Meshkide, N., and Kamyskowki, D. (2009). A new physically-based quantification of marine isoprene and primary organic aerosol emissions. Atmos. Chem. Phys. 9, 4915–4927. doi: 10.5194/acp-9-4915-2009

Geron, C., Rasmussen, R., Arnts, R. R., and Guenther, A. (2000). A review and synthesis of monoterpene speciation from forests in the United States. Atmos. Environ. 34, 1761–1781. doi: 10.1016/S0304-4068(99)00364-7

Ghirardo, A., Gutknecht, J., Zimmer, L., Bruggemann, N., and Schnitzer, J. P. (2011). Biogenic volatile organic compound and respiratory CO2 emissions after 13C-labeling: online tracing of C translocation dynamics in poplar plants. PLoS ONE 6:e17393. doi: 10.1371/journal.pone.0017393

Griffiths, E. T., Harries, P. C., Jeffcoat, R., and Trudgill, P. W. (1987). Purification and properties of alpha-pinene oxide lyase from Nocardia sp. strain P18.3. J. Bacteriol. 169, 4980–4983.

Guenter, A., Hewitt, C. N., Erickson, D., Fall, R., Geron, C., Graedel, T., et al. (1995). A global model of natural volatile organic compound emissions. J. Geophys. Res. Atmos. 100, 8873–8892. doi: 10.1029/94JD02950

Harayama, S., Reich, M., Nagi, K. L., and Ormsen, L. N. (1989). Physically associated enzymes produce and metabolize 2-hydroxy-2,4-diеноate, a chemically unstable intermediate formed in catechol metabolism via meta-cleavage in Pseudomonas putida. J. Bacteriol. 171, 6251–6258.

Harder, J., and Foss, S. (1999). Anaerobic formation of the aromatic hydrocarbon p-cymene from monoterpens by methanogenic enrichment cultures. Geomicrobiol. J. 16, 295–305. doi: 10.1080/146552249095001290

Harder, J., Heyen, U., Probian, C., and Foss, S. (2000). Anaerobic utilization of essential oils by denitrifying bacteria. Biodegradation 11, 55–63. doi: 10.1023/A:1026552724696

Hawkes, D. B., Adams, G. W., Burlingame, A. L., De Montellano, P. R. O., and De Voss, J. J. (2002). Cytochrome P450omer (CYP176a), isolation, expression, and characterization. J. Biol. Chem. 277, 2725–2732. doi: 10.1074/jbc.M203382200

Heyen, U., and Harder, J. (2000). Geranic acid formation, an initial reaction of anaerobic monoterpene metabolism in denitrifying Alcaligenes defrag- ans. Appl. Environ. Microbiol. 66, 3004–3009. doi: 10.1128/AEM.66.7.3004-3009.2000

Höschle, B., Gnau, V., and Jendrossek, D. (2005). Methylcrotonyl-CoA and geranyl-CoA carboxylases are involved in leucine/isolavate utilization (Liu) and acyclic terpene utilization (Atu), and are encoded by lutiluu1 and atuClaotf, in Pseudomonas aeruginosa. Microbiology 151(pt 11), 3649–3656. doi: 10.1099/micro.0.28260-0

Höschle, B., and Jendrossek, D. (2005). Utilization of geraniol is dependent on molybdenum in Pseudomonas aeruginosa: evidence for different metabolic routes for oxidation of geraniol and citronellol. Microbiology 151, 2277–2283. doi: 10.1099/micro.0.27957-0

Hu, Q. H., Xie, Z. Q., Wang, X. M., Kang, H., He, Q. F., and Zhang, P. F. (2013). Secondary organic aerosols over oceans via oxidation of isoprene and monoterpens from Arctic to Antarctic. Sci. Rep. 3:2280. doi: 10.1038/srep02280

Iwaki, H., Grosse, S., Bergeron, H., Leisch, H., Morley, K., Hasegawa, Y., et al. (2013). Camphor pathway reduct: functional recombiant expression of 2,3- and 3,6-diketocamphane monooxygenases of Pseudomonas putida ATCC 17453 with their cognate flavin reductase catalyzing Baeyer-Villiger reactions. Appl. Environ. Microbiol. 79, 3282–3293. doi: 10.1128/AEM.03958-12

Iurescia, S., Marconi, A. M., Tofani, D., Gambacorta, A., Paterno, A., Devirgiliis, C., et al. (1999). Identification and sequencing of beta-myrcene cataholism genes from Pseudomonas sp. strain M1. Appl. Environ. Microbiol. 65, 2871–2876.

Jendrossek, D. (2006). Identification of genes and proteins necessary for utilization of geraniol is dependent on molybdenum in Pseudomonas aeruginosa NCIMB 10007 by identification of the two missing genes, and their cognate flavin reductase catalyzing Baeyer-Villiger reactions. Appl. Environ. Microbiol. 72, 4819–4828. doi: 10.1128/AEM.00835-06

Kadow, M., Loschinski, K., Sass, S., Schmidt, M., and Bornscheuer, U. T. (2013). Concentrations of secondary componds in Scots pine needles at different stages of decomposition. Soil Biol. Biochem. 43, 37–42. doi: 10.1016/j.soilbio.2010.01.0147-X

Kesslermeier, J., and Staudt, M. (1999). Biogenic volatile organic compounds (VOC): an overview on emission, physiology and ecology. J. Atmos. Chem. 33, 23–88. doi: 10.1023/A:1006127516791
Liu, W. G., and Rosazza, J. P. N. (1993). A soluble Bacillus cereus.

Li, H. J., Lan, W. J., Cai, C. H., Zhou, Y. P., and Lin, Y. C. (2006). Biotransformation of limonene by microorganisms.

Li, H. J., and Lan, W. J. (2011). Biotransformation of limonene by microorganisms.

Lueddeke, F., and Harder, J. (2011). Enantiospecific (+)-linalool formation from beta-myrcene by linalool dehydrogenase-isomerase. Z. Naturforsch. C. 66, 409–412. doi: 10.5560/ZNC.2011.66c409

Lueddeke, F., Wueffing, A., Timke, M., Germer, F., Weber, J., Dikfidoan, A., et al. (2012). Geraniol and geranial dehydrogenases induced in anaerobic monoterpene degradation by Castellaniella defragrans. Appl. Environ. Microbiol. 78, 2128–2136. doi: 10.1128/AEM.07226-11

Luo, G., and Yu, F. (2010). A numerical evaluation of global oceanic emissions of α-pinene and isoprene. Atmos. Chem. Phys. 10, 2007–2015. doi: 10.5194/acp-10-2007-2010

Mahnoud, S. S., and Croteau, R. B. (2002). Strategies for transgenic manipulation of monoterpene biosynthesis in plants. Trends Plant Sci. 7, 366–373. doi: 10.1016/S1360-1385(02)02303-8

Mars, A. E., Gorissen, J. P. L., Van Den Beld, I., and Eggink, G. (2001). Microbial metabolism of monoterpenes - recent developments. Appl. Microbiol. Biotechnol. 56, 101–107. doi: 10.1007/s002530100625

Martínez, J. L., Sánchez, M. B., Martínez-Solano, L., Hernandez, A., Garmedina, L., Fajardo, A., et al. (2009). Functional role of bacterial multidrug efflux pumps in microbial natural ecosystems. FEMS Microbiol. Rev. 33, 430–449. doi: 10.1111/j.1574-6976.2008.00157.x

Ougham, H. J., Taylor, D. G., and Trudgill, P. W. (1983). Camphor revisited - vide further evidence of conservation of meta-cleavage pathway gene sequences. Tetrahedron Lett. 34, 946–950. doi: 10.1016/S0040-4039(05)80389-3

Poulos, T. L., Finzel, B. C., Gunalsus, I. C., Wagner, G. C., and Kraut, J. (1985). The 2.6-A crystal structure of Pseudomonas putida cytochrome-P-450. J. Biol. Chem. 260, 6122–6130.

Prakash, O., Kumari, K., and Lal, R. (2007). Pseudomonas delhiensis sp. nov., from a fly ash dumping site of a thermal power plant. Int. J. Syst. Evol. Microbiol. 57, 527–531. doi: 10.1099/ijs.0.64456-0

Rasmussen, J. A. M., Henderson, K. A., Strafmon, M. J., Dumsday, G. J., Coulton, J., and Zachariou, M. (2005). Two new biocatalysts for improved biological oxidation of 1,8-cineole. Aust. J. Chem. 58, 912–916. doi: 10.1071/CH05204

Savithry, N., Gage, D., Fu, W. J., and Oriel, P. (1998). Degradation of pinene by Bacillus pallidus BR425. Biodegradation 9, 337–341. doi: 10.1023/A:1008304603734

Scheewe, H., Mirata, M. A., Holtmann, D., and Schrader, J. (2011). Biotransformation of monoterpenes with bacterial monoxygenases. Proces Biochem. 46, 1885–1899. doi: 10.1016/j.procbio.2011.06.010

Seubert, W. (1960). Degradation of isoprenoid compounds by microorganisms. i. isolation and characterization of an isoprenoid-degrading bacterium, Pseudomonas citronellolis n. sp. J. Bacteriol. 79, 426–434.

Seubert, W., and Fass, E. (1964). Untersuchungen über den bakteriellen Abbau von Isoprenoiden. 5. Der Mechanismus des Isoprenenbaus. Biochem. Z. 331, 35–44.

Seubert, W., Fass, E., and Remberger, U. (1963). Untersuchungen über den bakteriellen Abbau von Isoprenoiden. 3. Reinigung und Eigenschaften der Geranylcarboxylase. Biochem. Z. 338, 263–275.

Seubert, W., and Remberger, U. (1963). Untersuchungen über den bakteriellen Abbau von Isoprenoiden. 2. Die Rolle der Kohlsäure. Biochem. Z. 338, 245–264.

Sharkey, T. D., Wibleber, A. E., and Donouhie, A. R. (2008). Isoprene emission from plants: why and how. Ann. Bot. 101, 5–18. doi: 10.1093/aob/mcm240

Shaw, T. D., and Yeh, S. S. (2001). Isoprene emission from plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52, 407–436. doi: 10.1146/annurev.arplant.52.1.407

Shaw, S. L., Gantt, B., and Meskhidze, N. (2010). Production and emissions of marine isoprene and monoterpenes: a review. Adv. Meteorol. 2010:408696. doi: 10.1155/2010/408696

Speelman, G., Bijsma, A., and Eggink, G. (1998). Limonene bioconversion to high concentrations of a stable and stable product, perillic acid, by a solvent-resistant Pseudomonas putida strain. Appl. Microbiol. Biotechnol. 50, 538–544. doi: 10.1007/s002530051331

Stotzky, G., and Schenck, S. (1976). Volatile organic compounds and microorganisms. Crit. Rev. Microbiol. 4, 333–382. doi: 10.3109/10408417609102303

Tanaka, K., Him, J. S., Saito, K., Takahashi, H. G., Watanabe, M., Yokohata, T., et al. (2012). How have both cultivation and warming influenced annual global isoprene and monoterpane emissions since the preindustrial era? Atmos. Chem. Phys. 12, 9753–9781. doi: 10.5194/acp-12-9753-2012

Taylor, D. G., and Trudgill, P. W. (1986). Camphor revisited: studies of 2,5-diketocamphane 1,2-monooxygenase from Pseudomonas putida ATCC 17453. J. Bacteriol. 165, 489–497.

Thompson, M. L., Marriott, R., Dowle, A., and Grogan, G. (2010). Biotransformation of beta-myrcene to geranial by a strain of Rhodococcus erythropolis isolated by selective enrichment from hot plants. Appl. Microbiol. Biotechnol. 85, 721–730. doi: 10.1007/s00253-009-2182-6

Tong, W.-Y. (2013). "Biotransformation of terpenoids and steroids," in Natural Products, eds K. G. Ramawat and J.-M. Mérillon (Berlin; Heidelberg: Springer), 2733–2739. doi: 10.1007/978-3-642-21144-6_122

Trudgill, P. W. (1990). Metabolism of monoterpenes - recent developments. Biodegradation 1, 93–105. doi: 10.1007/BF00058829

Trudgill, P. W. (1994). Microbial Metabolism And Transformation Of Selected Monoterpenes. Dordrecht; Norwell: Kluwer Academic Publishers.

Ullah, A. J. H., Murray, R. I., Bhattacharyya, P. K., Wagner, G. C., and Gunalsus, I. C. (1990). Protein components of a cytochrome P-450 linalool 8-methyl hydroxylase. J. Biol. Chem. 265, 1345–1351.

van der Werf, M., De Bont, J. A. M., and Leak, D. J. (1997). "Opportunities in microbial biotransformation of monoterpenes," in Advances In Biochemical Engineering/Biotechnology: Biotechnology Of Aroma Compounds, ed R. G. Berger (Berlin; New York: NY: Springer-Verlag), 147–177.

van der Werf, M. J., and Boot, A. M. (2000). Metabolism of carveol and dihydrocarveol in Rhodococcus erythropolis DCL14. Microbiology 146, 1129–1141.

van der Werf, M. J., Orru, R. V. A., Overkamp, K. M., Swarts, H. J., Osprian, I., Steinreiber, A., et al. (1999a). Substrate specificity and stereospecificity

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of limonene-1,2-epoxide hydrolase from *Rhodococcus erythropolis* DCL14; an enzyme showing sequential and enantioconvergent substrate conversion. *Appl. Microbiol. Biotechnol.* 52, 380–385.

van der Werf, M. J., Swarts, H. J., and De Bont, J. A. M. (1999b). *Rhodococcus erythropolis* DCL14 contains a novel degradation pathway for limonene. *Appl. Environ. Microbiol.* 65, 2092–2102.

Wing, Y., Lim, L., Diguistini, S., Robertson, G., Bohlmann, J., and Breuil, C. (2013). A specialized ABC efflux transporter GcABC-G1 confers monoterpene resistance to *Grosmannia clavigera*, a bark beetle-associated fungal pathogen of pine trees. *New Phytol.* 197, 886–898. doi: 10.1111/nph.12063

Williams, D. R., Trudgill, P. W., and Taylor, D. G. (1989). Metabolism of 1,8-cineole by a *Rhodococcus* species: ring cleavage reactions. *J. Gen. Microbiol.* 135, 1957–1967.

Wilt, F. M., Miller, G. C., Everett, R. L., and Hackett, M. (1993). Monoterpene concentrations in fresh, senescent, and decaying foliage of single-leaf pinyon (*Pinus monophylla* Torr And Frem; Pinaceae) from the Western Great-Basin. *J. Chem. Ecol.* 19, 185–194. doi: 10.1007/BF00993688

Wright, S. J., Caunt, P., Carter, D., and Baker, P. B. (1986). Microbial oxidation of α-pinene by *Serratia marcescens*. *Appl. Microbiol. Biotechnol.* 23, 224–227.

Yang, J. E., Park, Y. J., and Chang, H. C. (2007). Cloning of four genes involved in limonene hydroxylation from *Enterobacter cowanii* 6L. *J. Microbiol. Biotechnol.* 17, 1169–1176.

Yassaa, N., Peeken, I., Zollner, E., Bluhm, K., Arnold, S., Spracklen, D., et al. (2008). Evidence for marine production of monoterpenes. *Environ. Chem.* 5, 391–401. doi: 10.1071/EN08047

Yoo, S. K., and Day, D. F. (2002). Bacterial metabolism of alpha- and beta-pinene and related monoterpenes by *Pseudomonas* sp. strain PIN. *Process Biochem.* 37, 739–745. doi: 10.1016/S0032-9592(01)00262-X

Yu, F. N. A., and Utsumi, R. (2009). Diversity, regulation, and genetic manipulation of plant mono- and sesquiterpenoid biosynthesis. *Cell. Mol. Life Sci.* 66, 3043–3052. doi: 10.1007/s00018-009-0066-7

Ziemann, P. J., and Atkinson, R. (2012). Kinetics, products, and mechanisms of secondary organic aerosol formation. *Chem. Soc. Rev.* 41, 6582–6605. doi: 10.1039/c2cs35122f

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