Genetics and Ecology of Isoprene Degradation

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Abstract

Approximately 550 million tonnes of the monoterpene, isoprene, are emitted to the atmosphere annually, principally from terrestrial plants. In contrast to methane, which is emitted in similar quantities, little is known about the biodegradation of isoprene. However, 30 years ago, bacteria capable of living on isoprene as a sole source of carbon and energy were described, although they were not investigated in detail. Recently there has been renewed interest in the potential of bacteria living in soils, marine sediments, and on the leaves of plants to degrade isoprene. Isolates capable of isoprene metabolism use a multicomponent soluble monooxygenase, which contains a diiron center at the active site, to oxidize isoprene to the epoxide, and all isolates described to date depend on glutathione for subsequent metabolic steps. The diversity of isoprene degraders

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has been investigated in terrestrial and marine environments using DNA-stable isotope probing (DNA-SIP), together with the use of gene probes targeting the monooxygenase active-site subunit. Gaps in our knowledge and future research directions are described.

1 Introduction

Isoprene (2-methyl-1,3-butadiene) is one of the most highly produced biogenic volatile organic compounds (BVOCs) emitted to the atmosphere, accounting for approximately 550 Tg C y^{-1}, or 1/3 of total BVOCs (Guenther et al. 2006, 2012). This is similar in magnitude to the release of methane, with all other BVOCs comprising the remaining third. Isoprene is volatile, with a boiling point of 34 °C and can be considered as a trace gas; its high reactivity in the atmosphere has a major influence on Earth's climate. The effects of isoprene on climate are complex and not fully understood. It reacts with hydroxyl radicals, reducing the oxidative capacity of the atmosphere and resulting in a slower turnover of methane, a potent greenhouse gas, leading to global warming. Isoprene also reacts with oxides of nitrogen in the atmosphere, resulting in increased ozone levels, with effects on air quality and health (Sanderson et al. 2003; Pacifico et al. 2009). The oxidation products can result in secondary organic aerosols which promote increased cloud formation and global cooling (Carlton et al. 2009). Therefore under different circumstances, isoprene can act as both a global warming and a global cooling gas.

Isoprene is a key building block for isoprenoids, which consist of two or more isoprene units and are produced by all free-living organisms. This large family of molecules includes, for example, carotenoids, sterols, chlorophyll, quinones, archaeal lipids, and hopanoids. The precursor molecules for isoprenoids are dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP), which are synthesized in animals, fungi, archaea, some bacteria, and the cytosol of plants using the mevalonate (MVA) pathway or by the methylerythritol phosphate (MEP) pathway in chloroplasts and most bacteria (Rohmer 1999). The vast majority of isoprene (approximately 90%) is produced globally by terrestrial plants by the action of isoprene synthase on DMAPP in chloroplasts (Sharkey et al. 2008). Interestingly, not all plants produce isoprene, with both high and low producers, even among closely related species – for example, all American oaks emit isoprene whereas many European oaks do not (Loreto et al. 1998). High isoprene-emitting trees typically divert 2% of fixed carbon to isoprene production, and in some cases considerably more (Sharkey et al. 1996), so it is striking that the reasons for this outlay of carbon and energy are not fully understood. There is good evidence that isoprene can alleviate heat and oxidative stress (Sharkey et al. 2008; Zeinali et al. 2016) and other proposed roles for isoprene biosynthesis include plant signaling, prevention from herbivory and as a way to dissipate excess energy from photosynthesis (Magel et al. 2006; Loivamäki et al. 2008). Certain emerging crop plants, for example palm oil, are high-isoprene emitters, and there is an increasing interest in the effects on air quality of the development of isoprene-emitting agroforestry (Hewitt et al. 2009).

The remaining 10% of isoprene produced in the biosphere is attributed to bacteria, fungi, algae, and animals in both terrestrial and aquatic environments (Gelmont et al. 1981; Fares et al. 2008; Bäck et al. 2010; Exton et al. 2015). For example, *Bacillus subtilis* has been shown to produce isoprene, maybe as a consequence of stress in this bacterium (Kuzma et al. 1995). In the marine environment, macro- and microalgae are the major producers, responsible for a poorly constrained flux of 0.1–10 Tg C y^{-1} (Palmer and Shaw 2005; Luo and Yu 2010; Shaw et al. 2010; Srikanta Dani et al. 2017). As seen with plants, isoprene emissions by marine microalgae increase in response to higher temperature and light intensity and so may protect these organisms during periods of stress (Exton et al. 2013). Isoprene is also produced industrially (approximately 0.8 Tg y^{-1}) and used primarily for polyisoprene elastomer (synthetic rubber) production (Morais et al. 2015). Since little is known about bacterial isoprene synthases, strategies have been developed to express isoprene synthase genes from plants in heterologous systems, including *Escherichia coli*, *Saccharomyces*, and *Synechocystis* (Marienhagen and Bott 2013; Lv et al. 2014).

In comparison to the global methane cycle, information regarding the biogeochemical cycling of isoprene is rather sparse and there are many unknowns, particularly estimates of both production and consumption in the biosphere and the internal recycling of isoprene in soils, on the surface of plants, and in the marine environment. Microbial production and/or consumption of isoprene have previously been reviewed by Fall and Copley (2000), Shennan (2006), and McGenity et al. (2017). The global isoprene cycle is shown in Fig. 1. Here we review the mechanisms by which microbes degrade isoprene, and their diversity in the environment.

2 Microbial Consumption of Isoprene

Soils have been recognized as a sink for isoprene for over 20 years. In the terrestrial environment, temperate, tropical, and boreal soils were shown to rapidly consume isoprene at concentrations of 500 ppbv in laboratory-based experiments. Field chamber experiments in temperate forests also revealed that soils could deplete isoprene added to chambers at ~400 ppbv down to below the detection limits of about 5 ppbv within 1 h (Cleveland and Yavitt 1997, 1998). Temperate agriforest and model tropical rainforest mesocosm experiments revealed rapid in situ consumption of isoprene (Pegoraro et al. 2005, 2006). More recently, continuous flow experiments with temperate forest soils revealed that these systems consume isoprene over a range of concentrations (2–200 ppbv), with substantial rates of isoprene removal even at low (20 ppbv) concentrations (Gray et al. 2015). The first demonstration of microbial consumption of isoprene in the marine environment was by Acuña Alvarez et al. (2009), who observed isoprene-degrading bacteria in estuarine, coastal, and open marine waters. They also demonstrated that isoprene produced by marine microalgae cultures could be consumed by isoprene-degrading bacteria, an important observation proving that marine microbes could benefit directly from isoprene, produced by microalgae and without using artificially high laboratory

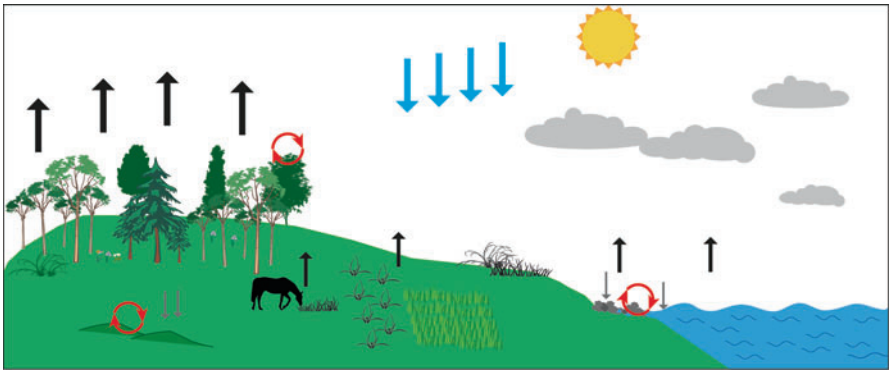

Fig. 1 Isoprene is emitted to the atmosphere by trees, plants, algae, animals, fungi, and bacteria in the terrestrial and marine environment (black arrows), where it is rapidly photochemically oxidized (blue arrows). Isoprene may also be taken up from the atmosphere by microbes in soils and aquatic ecosystems and sediments (grey arrows). Consumption of isoprene by microbes at the point of release is shown as red circular arrows

concentrations. This study also resulted in the isolation of a number of isoprene-degrading bacteria from these marine environments (see later).

3 Bacterial Degradation of Isoprene

The first reports of isolation of bacteria growing on isoprene as sole carbon and energy source were by van Ginkel et al. (1987a, b), Ewers et al. (1990), and Cleveland and Yavitt (1997). Soil enrichments with isoprene under aerobic conditions yielded bacteria assigned to the actinobacterial genera *Rhodococcus, Nocardia*, and *Arthrobacter* and to the proteobacterial genus *Alcaligenes*. More recently aerobic isoprene-degrading *Pseudomonas*, *Klebsiella*, and *Alcaligenes* strains have been reported (Srivastva et al. 2015), but, as with earlier studies, these have not been characterized in any detail. Our recent work has provided collections of aerobic isoprene-degrading bacteria for further study with a number of *Rhodococcus* species being isolated from soils and the leaves of isoprene-producing trees such as Poplar and Willow (El Khawand et al. 2016; Murphy 2017). Marine sediments have also yielded a variety of Gram-positive and Gram-negative aerobic isoprene degraders (Acuña Alvarez et al. 2009; Johnston et al. 2017) (Fig. 2). To our knowledge, no anaerobic bacteria, archaea, or fungi growing on isoprene have yet been reported.

The most well-characterized isoprene-degrading bacterium described so far is *Rhodococcus* sp. AD45, isolated from freshwater sediment by Janssen and colleagues (van Hylckama Vlieg et al. 1998). In this aerobe, the initial oxidation of isoprene to 1,2-epoxyisoprene is carried out by the enzyme isoprene monooxygenase (IsoMO), a multicomponent soluble diiron monooxygenase (SDIMO) belonging to the same large family of enzymes as soluble methane monooxygenase, toluene monooxygenase, and alkene monooxygenase (Leahy et al. 2003; Holmes

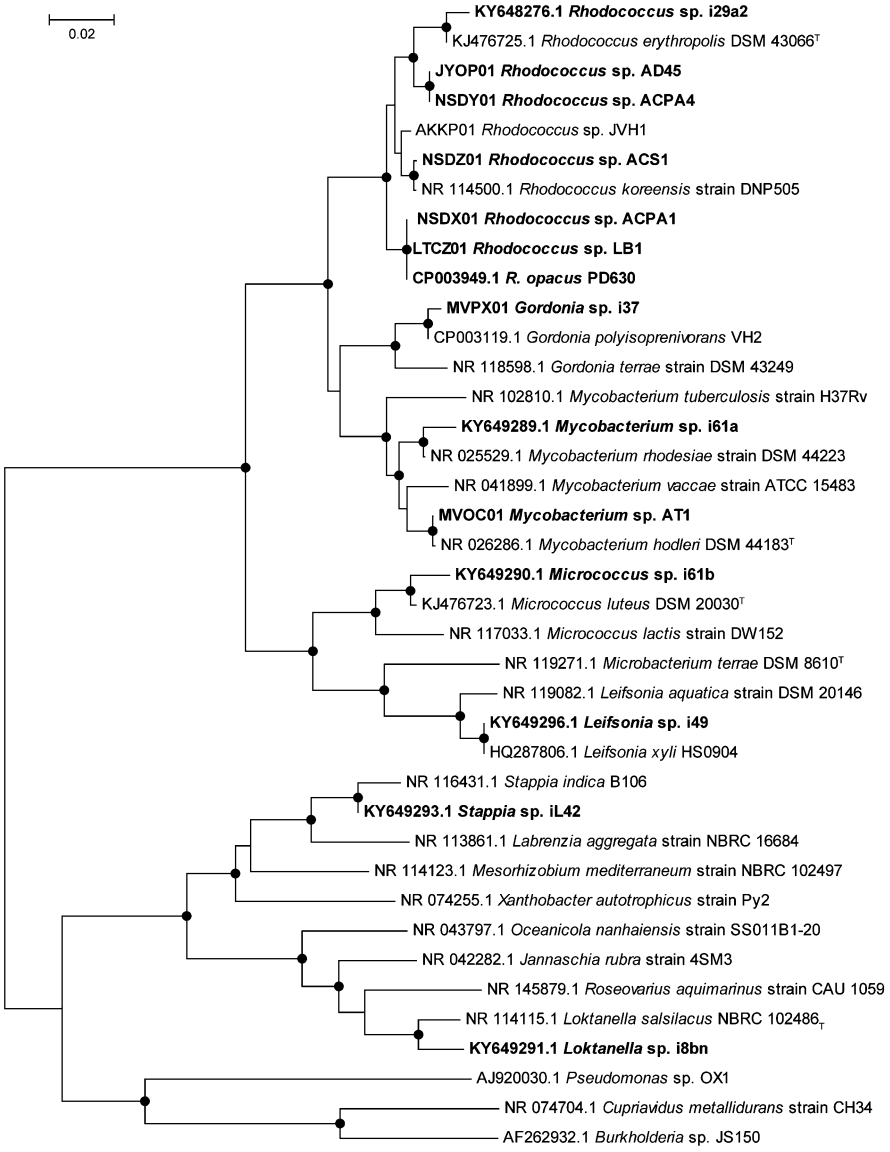

Fig. 2 The 16S rRNA gene-based phylogeny of isoprene degraders (shown in bold) together with other representative strains. The tree was drawn using the Neighbor Joining method in Mega6 (Tamura et al. 2013) with pairwise deletion, resulting in 1594 nucleotide positions in the analysis. Bootstrap values (1000 replications) greater than 75% are shown by black circles at the nodes. The scalebar shows base substitutions per site

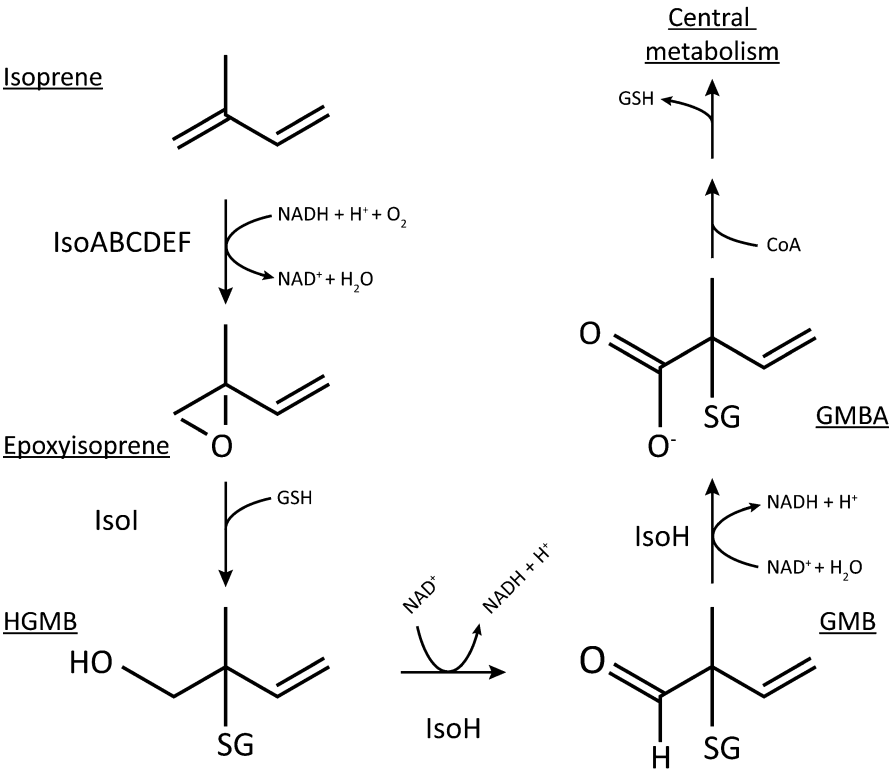

Fig. 3 The isoprene degradation pathway. HGMB, 1-hydroxy-2-glutathionyl-2-methyl-3-butene; GMB, 2-glutathionyl-2-methyl-3-butenal; GMBA, 2-methyl-2-glutathionyl butenoic acid; GS, glutathione.

and Coleman 2008). The reactive epoxide produced in the first step of isoprene metabolism is conjugated with glutathione by a glutathione-*S*-transferase to form 1-hydroxy-2-glutathionyl-2-methyl-3-butene (HGMB). HGMB is further metabolized by a dehydrogenase to glutathionyl-2-methyl-3-butenoate (GMBA) (van Hylckama Vlieg et al. 1998, 1999). The subsequent fate of GMBA remains to be elucidated but it can be assumed that the subsequent removal of the glutathione moiety and β-oxidation of the intermediates of isoprene metabolism allows *Rhodococcus* sp. AD45 to grow on isoprene as a sole carbon and energy source (Fig. 3).

van Hycklama Vleig et al. (2000) showed that the isoprene monooxygenase is encoded by the genes *isoABCDEF*. Subsequent sequencing of the 6.8 Mbp genome of *Rhodococcus* sp. AD45 (Crombie et al. 2015) revealed that all of the genes necessary for the metabolism of isoprene are carried on a 300 kbp megaplasmid in this *Rhodococcus* strain (Fig. 4). This clustering of isoprene metabolic genes seems to be a common feature in all isoprene degraders studied to date, although in some other isoprene-degrading *Rhodococcus* strains (e.g., *Rhodococcus opacus* PD630)

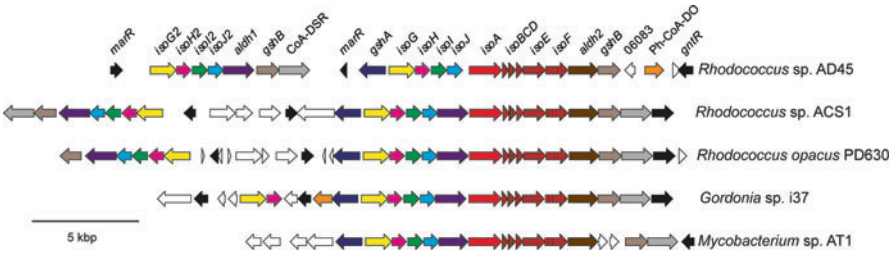

Fig. 4 The isoprene metabolic gene cluster from representative terrestrial and marine strains. The isoprene monooxygenase genes are shown in red, and homologous genes are in corresponding colors

these isoprene-related genes appear to be chromosomally located rather than on a plasmid (Chen et al. 2014; Crombie et al. 2015). Genes *isoA*, *isoB*, and *isoE* encode the diiron ($\alpha_2\beta_2\gamma_2$) oxygenase component; *isoF* encodes a flavoprotein NADH reductase; *isoC* encodes a Rieske-type ferredoxin; and *isoD* encodes a coupling protein, which together form the multicomponent isoprene monooxygenase. The four genes *isoGHIJ*, preceding the IsoMO genes, encode a putative coenzyme A transferase, a dehydrogenase, and two glutathione transferases (van Hylckama Vlieg et al. 1998, 1999, 2000). An additional copy of this *isoGHIJ* cluster is located on the *Rhodococcus* sp. AD45 megaplasmid (Crombie et al. 2015), and these and other isoprene metabolic genes are duplicated in many isoprene degraders (Fig. 4). Upstream of *isoG*, *gshA* encodes glutamate cysteine ligase, which catalyzes the first step of glutathione biosynthesis. Two copies of *gshB*, encoding glutathione synthetase, are also present. The cluster also contains genes predicted to encode two aldehyde dehydrogenases (*aldh1* and *ald2*), both of which are present in the genomes of all other sequenced isoprene degraders. In *Rhodococcus opacus* PD630, the former of these, *aldh1*, was shown to have glyceraldehyde-3-phosphate and $NADP^+$-dependent activity (MacEachran and Sinskey 2013), possibly suggestive of a requirement for NADPH in isoprene metabolism, although, to our knowledge, alternative substrates of this enzyme were not investigated in detail. Genes encoding a putative coenzyme A disulfide reductase and three putative transcriptional regulators (*marR*1, *marR*2, and *gntR*) are also present. These *iso* genes are essential for isoprene metabolism since removal of the megaplasmid of *Rhodococcus* sp. AD45 by "curing" resulted in loss of the ability to grow on isoprene, as did deletion of *isoA* by mutagenesis (unpublished observation; Crombie et al. 2015).

Actinobacteria such as *Rhodococcus* are metabolically versatile and it appears that isoprene metabolism in *Rhodococcus* sp. AD45 and other isoprene degraders tested to date (*Gordonia* and *Mycobacterium*) is an inducible trait (Crombie et al. 2015; Johnston et al. 2017). Carbon sources such as glucose and succinate repress isoprene metabolism but isoprene monooxygenase polypeptides are readily observed in cell extracts of isoprene-grown *Rhodococcus* sp. AD45. The regulation of expression of isoprene metabolic genes in this strain has been studied in some detail. A replicated time course experiment was conducted in which succinate-grown

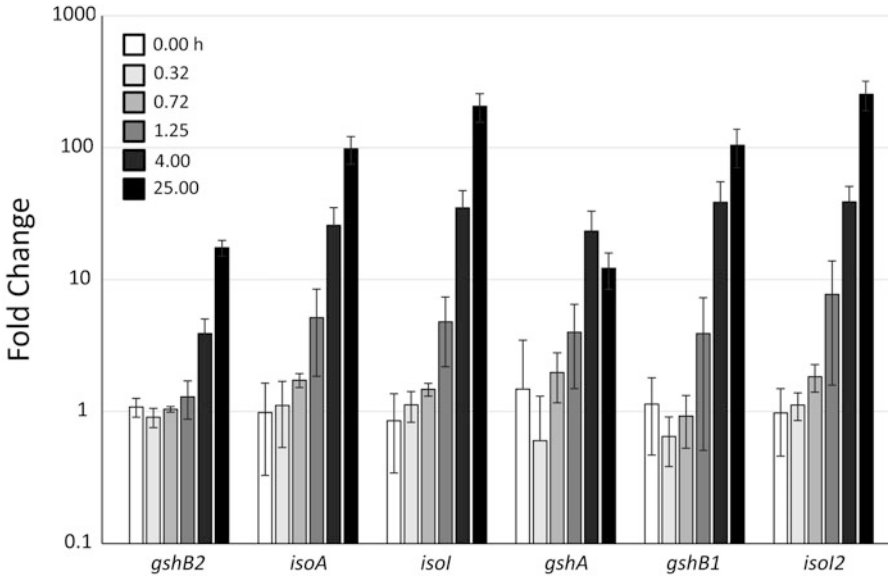

Fig. 5 Induction of representative isoprene-degradation genes in *Rhodococcus* sp. AD45 after incubation with isoprene, at six timepoints from zero to 25 h, quantified by RNAseq. The bar chart shows the fold change at each time point relative to controls incubated without carbon substrate (Data from Crombie et al. 2015)

cells were subcultured into medium containing isoprene, epoxyisoprene, glucose, succinate, or no substrate (Crombie et al. 2015). Isoprene- and epoxyisoprene-induced expression of *iso* genes were examined by sequencing the transcriptome and comparison with cells grown on glucose and succinate (at 0, 19, 43, 75, 240 min and 25 h). Both isoprene and epoxyisoprene induced high levels of expression of *isoABCDEF, isoGHJI*, and all other putative isoprene metabolism genes present in the 22-gene cluster on the megaplasmid of *Rhodococcus* sp. AD45 (Fig. 5). Under isoprene-induced conditions, these genes represented over 25% of all transcripts observed. These results were subsequently confirmed by targeted RT-PCR. There was no significant over-expression of genes on the chromosome in response to isoprene or epoxyisoprene, suggesting that all isoprene metabolic genes reside on this plasmid (Crombie et al. 2015). The dynamics of gene transcription in response to isoprene or epoxyisoprene indicated that the inducer was not isoprene itself, but rather epoxyisoprene or a subsequent metabolic product of isoprene oxidation, subsequently confirmed by transcriptional analysis of a mutant with an inactivated IsoMO in which isoprene (which could not be metabolized) did not induce *iso* gene transcription. These data provide further targets for mutagenesis and expression studies in order to elucidate the full pathway of isoprene metabolism in *Rhodococcus* sp. AD45. The use of reporter strains to analyze transcriptional regulation and analysis of putative regulators encoded by *marR* and *gntR* may also be a fruitful approach.

4 Ecology of Isoprene Degraders

Analysis of the genomes of a number of other isoprene-degrading *Rhodococcus, Gordonia, Mycobacterium*, and *Variovorax* strains has revealed that the clustering of *isoABCDEF* along with *isoGHIJ* and genes involved in glutathione biosynthesis seems to be a common feature in isoprene degradation (Fig. 4) (Crombie et al. 2015; El Khawand et al. 2016; Johnston et al. 2017). The genes encoding isoprene monooxygenase are often misannotated as toluene or alkene monooxygenases, other members of the SDIMO family, but the close proximity of *isoGHIJ* to *isoABCDEF* is a good indication of isoprene metabolism in newly isolated strains. The availability of the *isoABCDEF* sequence has also provided tools for cultivation-independent studies aimed at assessing the distribution, diversity, and activity of isoprene degraders in the environment. A fruitful approach in molecular ecology studies of methane-oxidizing bacteria has been to use methane monooxygenase marker genes ("functional genes") encoding key components of methane monooxygenases (*pmoA* or *mmoX*) to examine different environments (Dumont and Murrell 2005b; McDonald et al. 2008). We have used a similar approach with isoprene monooxygenase. The homologue of *mmoX* (which encodes the large subunit of the oxygenase component of the soluble methane monooxygenase) in IsoMO is *isoA*, which encodes the putative active site component. This polypeptide appears to be highly conserved in all isoprene degraders studied to date. Phylogenetic analysis of IsoA of isoMO from known isoprene degraders and comparison with the corresponding components of SDIMO enzymes such as toluene monooxygenase and alkene monooxygenases (Fig. 6) has confirmed that *isoA* is a suitable marker gene for cultivation-independent studies and that derived IsoA homologs can be readily distinguished from those of non-isoprene-degraders containing SDIMOs other than IsoMO. Alignment of IsoA from bona fide isoprene degraders has allowed the design of *isoA* PCR primer sets targeting these bacteria. These primers did not amplify SDIMO genes from non-isoprene degraders but gave *isoA* gene products with DNA from a range of isoprene-degrading isolates and enrichment cultures originating from various soils, sediments, and leaf samples (El Khawand et al. 2016). Alignments of the IsoA sequences retrieved from environmental samples, with those of characterized isoprene degraders, showed that the IsoA sequences were relatively highly conserved (>86% identity) and could be broadly separated into two groups, those from marine environments and those from terrestrial environments, predominantly actinobacterial isoprene degraders (El Khawand et al. 2016). However, the subsequent isolation and analysis of more isoprene degraders suggests that there is variation in their *isoA* sequences which will, as has been the case with methane monooxygenase functional gene PCR primers, necessitate redesign of *isoA* PCR primer sets. This functional gene probing approach has extended knowledge of the diversity of isoprene-degraders in both terrestrial and marine environments (El Khawand et al. 2016; Johnston et al. 2017).

In order to determine which isoprene degraders are active in the environment, other cultivation-independent techniques such as DNA Stable Isotope Probing (DNA-SIP) (Dumont and Murrell 2005a) need to be used. DNA-SIP has been used to identify active isoprene degraders in both the terrestrial and marine

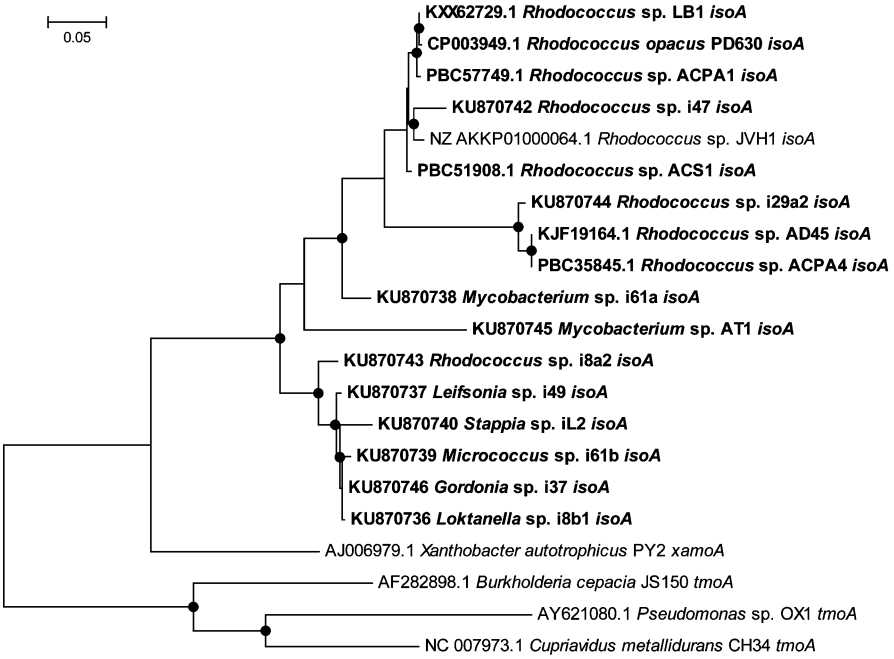

Fig. 6 The relationship between the *isoA* gene sequences of isoprene degraders (shown in bold) and other homologous sequences. The tree was drawn in Mega6 (Tamura et al. 2013) using the Maximum Likelihood method. Sites with less than 95% coverage were deleted, resulting in 1005 nucleotide positions in the analysis. Bootstrap values (1000 replications) greater than 75% are shown by black circles at the nodes. The scalebar shows base substitutions per site. Based on the presence of adjacent *isoGHIJ* genes, we can predict that *Rhodococcus* sp. JVH1 can grow on isoprene, although this has not, to our knowledge, been tested

environment. For example, surface soil samples from around Willow trees (*Salix fragilis*) were supplied with ^{13}C–labelled isoprene in microcosms and isoprene uptake was monitored by gas chromatography. After sufficient "heavy" isoprene was incorporated into biomass, ^{13}C–labelled DNA was isolated by buoyant density gradient centrifugation and used as template in PCR reactions with primers targeting 16S rRNA genes. The analysis revealed a considerable enrichment of several species of *Rhodococcus* in these microcosms, indicating that under the incubation conditions used, *Rhodococcus* represented the majority of active isoprene degraders in these soils (El Khawand et al. 2016). Interestingly, 16S rRNA gene sequences from the Betaproteobacteria *Comamonas* and *Variovorax* were also enriched in ^{13}C–labelled DNA. In addition to soils, DNA-SIP has also been used to identify active isoprene degraders on leaves of White Poplar (*Populus alba*). Microbes washed from the leaves of this tree were incubated in microcosms with ^{13}C–labelled isoprene. After sufficient ^{13}C–labelling, "heavy" DNA was isolated and used in shotgun metagenomics experiments. This enabled the reconstruction of a considerable proportion of the draft genome of an isoprene-degrading *Variovorax* species, including the *iso*

metabolic gene clusters *isoABCDEF* and *isoGHIJ* (Crombie et al., manuscript in preparation). This information has subsequently been used in the targeted isolation of isoprene-degrading *Variovorax* species (Mejia-Florez et al., unpublished).

The functionality of these *Variovorax iso* genes retrieved directly from the environment using DNA-SIP was confirmed by expression studies. The putative IsoMO genes *isoABCDE* from the reconstructed *Variovorax* genome were expressed in a heterologous expression system: a non-isoprene-degrading variant of *Rhodococcus* sp. AD45 without the 300 kbp megaplasmid carrying the *iso* genes (Crombie et al., manuscript in preparation). When expressed, the *Variovorax isoABCDEF* genes conferred the ability of the *Rhodococcus* strain to oxidize isoprene, thus proving that it is a bona fide isoprene monooxygenase. Metatranscriptome data obtained from the same isoprene incubation experiments used for DNA-SIP also confirmed that these *Variovorax* genes were expressed under the enrichment conditions. Targeted isolations have now yielded isoprene-degrading *Variovorax* strains from leaves, thus providing further *isoA* genes to refine PCR primer sets and a new model Gram-negative isoprene degrader to complement the Gram-positive strains available (Crombie et al. manuscript in preparation).

The diversity of isoprene degraders in the marine environment has also been investigated using cultivation-independent methods. Surface estuarine sediments from the Colne Estuary (UK), incubated with ^{13}C–labelled isoprene, yielded ^{13}C–DNA which when analyzed revealed the development of isoprene-degrading communities dominated by Actinobacteria including *Gordonia, Mycobacterium, Microbacterium*, and *Rhodococcus* (Johnston et al. 2017). Enrichments of similar environmental samples from the Colne Estuary yielded isolates *Gordonia* sp. i37 and *Mycobacterium* sp. AT1, which grew on isoprene as a sole source of carbon and energy. Analysis of their genomes revealed the same gene arrangements of *isoABCDEF* and *isoGHIJ* seen in *Rhodococcus* sp. AD45 (Fig. 4). As with *R.* sp. AD45, isoprene oxidation was inducible in the presence of isoprene (Johnston et al. 2017). A second SDIMO was identified in the genomes of both of these isoprene-degraders which had significant sequence identity to the propane monooxygenase from *Gordonia* TY-5 (Kotani et al. 2003). This second SDIMO system enabled these bacteria to grow on propane, which, interestingly, appears to be a common feature of many isoprene-degrading bacteria (although not *Rhodococcus* sp. AD45) (Acuña Alvarez et al. 2009; Johnston et al. 2017). These marine isolates, together with other isoprene degraders isolated and characterized from the Colne Estuary and other marine environments (Acuña Alvarez et al. 2009; Johnston et al. 2017), are yielding valuable genome sequence information to refine *isoA* PCR primers and confirming the presence of both *isoABCDEF* and *isoGHIJ* gene clusters in bona fide isoprene degraders.

5 Conclusions and Research Needs

All isoprene-degrading bacteria studied so far possess an isoprene monooxygenase of the SDIMO family, which is required for the initial oxidation of isoprene. This enzyme is induced by isoprene or a further oxidation product of isoprene metabolism. It will be

interesting to explore the structure and substrate range of this new class of enzymes in relation to others in the SDIMO family; for example, soluble methane monooxygenase found in some methanotrophs and alkene monooxygenases of propene degraders. These enzymes can also (co-)oxidize isoprene but the bacteria lack the additional metabolic machinery to allow growth on isoprene (Johnston et al. 2017). A second unifying feature of extant isoprene-degraders is the use of glutathione to detoxify epoxyisoprene, the first oxidation product of isoprene. This is in contrast to other alkene degraders, which often use coenzyme M as cofactor (Krishnakumar et al. 2008), and glutathione biosynthesis genes have so far always been found in close proximity to *iso* genes in isoprene degraders. The subsequent steps in isoprene metabolism require further study but the identification of *iso* genes in *Rhodococcus* sp. AD45 and the availability of a mutagenesis and expression system in this "workhorse" organism will now allow us to characterize the mechanisms by which bacteria regulate isoprene metabolism and subsequently incorporate carbon from isoprene into biomass. In some cases (*Rhodococcus* sp. AD45), all necessary *iso* genes reside on a megaplasmid, hinting at the possibility that they are transferred between bacteria by horizontal gene transfer. This notion is supported by the lack of congruence between the phylogeny derived from *isoA* and 16S rRNA gene sequences (Figs. 1 and 6).

The studies summarized here clearly indicate that isoprene-degrading bacteria are widespread in the environment and that soils possess the capability to deplete isoprene at environmental concentrations. Cultivation-independent techniques, such as DNA-, RNA- or protein-SIP, or single cell technologies such as Raman microspectroscopy, will help reveal new isoprene degraders (Murrell and Whiteley 2011; Wang et al. 2016). Challenges for the future include conducting these sequence-independent experiments at conditions that mimic those in the environment, which will identify isoprene-degrading microbes which may exploit specific micro niches. Given that intercellular isoprene concentrations inside leaves may be up to three orders of magnitude higher than atmospheric (Fini et al. 2017), reaching the low ppmv range, the possibility of isoprene-degrading endophytes should be explored. It will also be necessary to increase the diversity of isoprene-degrading strains in cultivation. Characterization of isolates may reveal other pathways for metabolism of isoprene by bacteria and indeed may identify other isoprene-degrading microbes such as Archaea and fungi. To our knowledge there is so far no evidence for anaerobic degradation of isoprene but this possibility should also be borne in mind. To determine the impact that biological isoprene uptake has on global fluxes it will be necessary to quantify microbial activity in the environment. Approaches being pursued in our laboratory range from construction of biosensor strains to express reporter genes under conditions where isoprene-related genes are expressed, through to purification and characterization of isoprene metabolic enzymes, including IsoMO, to determine the affinity and kinetics of isoprene degradation in different isolates.

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