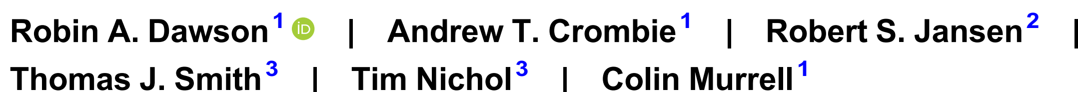
Received: 3 November 2022 | Accepted: 21 December 2022

DOI: 10.1111/1462-2920.16325

MINI REVIEW

ENVIRONMENTAL MICROBIOLOGY Applied Microbiology International

Peering down the sink: A review of isoprene metabolism by bacteria


Robin A. Dawson[1] | Andrew T. Crombie[1] | Robert S. Jansen[2] | Thomas J. Smith[3] | Tim Nichol[3] | Colin Murrell[1]

[1]School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, UK

[2]Department of Microbiology, Radboud University, Nijmegen, The Netherlands

[3]Biomolecular Sciences Research Centre, Sheffield Hallam University, Sheffield, UK

Correspondence
Robin A. Dawson, School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 5 7TJ UK.
Email: R.Dawson@uea.ac.uk

Funding information
H2020 European Research Council, Grant/Award Number: 694578-IsoMet; Leverhulme Trust, Grant/Award Number: ECF-2016-626; Natural Environment Research Council, Grant/Award Number: NE/J009725/1

Abstract

Isoprene (2-methyl-1,3-butadiene) is emitted to the atmosphere each year in sufficient quantities to rival methane (>500 Tg C yr^{-1}), primarily due to emission by trees and other plants. Chemical reactions of isoprene with other atmospheric compounds, such as hydroxyl radicals and inorganic nitrogen species (NO_x), have implications for global warming and local air quality, respectively. For many years, it has been estimated that soil-dwelling bacteria consume a significant amount of isoprene (∼20 Tg C yr^{-1}), but the mechanisms underlying the biological sink for isoprene have been poorly understood. Studies have indicated or confirmed the ability of diverse bacterial genera to degrade isoprene, whether by the canonical *iso*-type isoprene degradation pathway or through other less well-characterized mechanisms. Here, we review current knowledge of isoprene metabolism and highlight key areas for further research. In particular, examples of isoprene-degraders that do not utilize the isoprene monooxygenase have been identified in recent years. This has fascinating implications both for the mechanism of isoprene uptake by bacteria, and also for the ecology of isoprene-degraders in the environments.

INTRODUCTION

Our understanding of the isoprene biogeochemical cycle (Figure 1), responsible for distributing carbon from the ∼500 Tg yr^{-1} isoprene emitted to the atmosphere, has been greatly improved in recent years thanks to efforts to understand the biological isoprene sink. Bacterial metabolism of isoprene is estimated to be responsible for the uptake by soils of approximately 20.4 Tg yr^{-1} isoprene (Cleveland & Yavitt, 1997), with additional uptake by bacteria in the phyllosphere of isoprene-emitting trees (Crombie et al., 2018; Singh et al., 2019) and in certain aquatic environments (Acuña Alvarez et al., 2009; Johnston et al., 2017), meaning that the total biological sink for isoprene may be considerably greater. A number of studies have been conducted in recent years to improve our understanding of this sink. Molecular ecology studies, reviewed by Carrión, Gibson, et al. (2020), Carrión, McGenity, and Murrell (2020), have begun to reveal the significant diversity of isoprene-degrading genera associated with isoprene-emitting trees. Bacterial metabolism of isoprene was first studied in detail in a member of the Actinobacteria, *Rhodococcus* sp. AD45, isolated from freshwater sediment due to its ability to degrade chlorinated ethenes (van Hylckama Vlieg et al., 1998). Metabolic versatility has been noted in various isoprene-degrading isolates (Crombie et al., 2015; Dawson et al., 2020; Gibson et al., 2020; Johnston et al., 2017; Uttarotai et al., 2022). In particular, *Alcaligenes* sp. Strain 13f and *Variovorax* sp. WS11 are capable of growing and consuming isoprene in the presence of organic acids or sugars (Dawson et al., 2022; Uttarotai et al., 2022), thus, indicating that isoprene-degraders in the environment are supported by multiple metabolic pathways.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2022 The Authors. *Environmental Microbiology* published by Applied Microbiology International and John Wiley & Sons Ltd.

14622920, 0, Downloaded from https://ami-journals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.16325 by Sheffield Hallam University, Wiley Online Library on [05/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

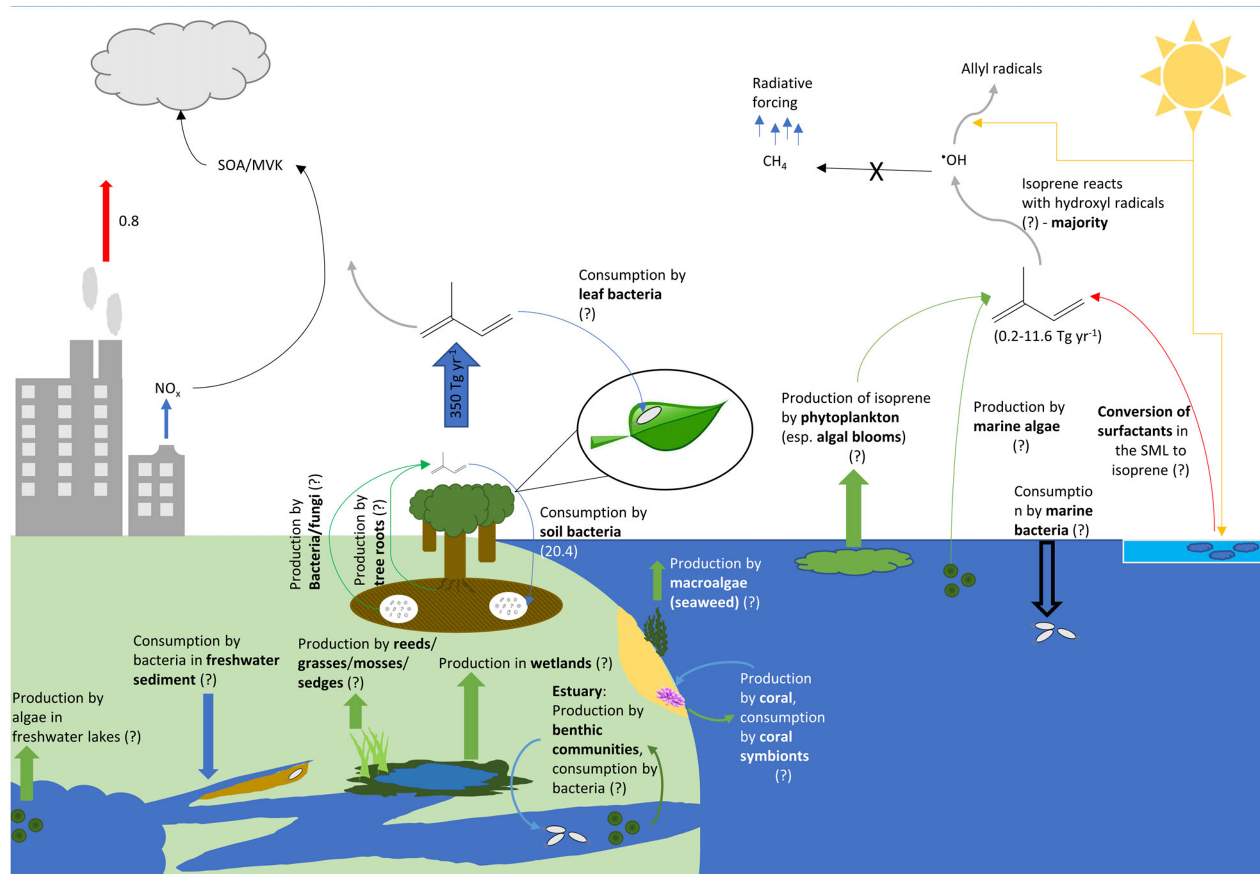


FIGURE 1 Sources (upward arrows) and sinks (downward arrows) of isoprene, listed with production/consumption estimates in Tg C yr^{-1}. Anthropogenic production is shown in red, biological production in green, anthropogenic sinks are shown in grey, and biological sinks in blue. Other processes are shown in black. MV, methyl vinyl ketone; •OH, hydroxyl radicals; SML, sea-surface microlayer; SOA, secondary organic aerosol.

Studying the bacteria which contribute to the global isoprene cycle is essential to understand the biological sink for isoprene. Our knowledge of *iso*-type isoprene-degraders (employing the conserved *iso* metabolic gene cluster (*isoGHIJABCDEF*) first identified in *Rhodococcus* sp. AD45) has improved significantly in recent years (Crombie et al., 2015; Dawson et al., 2020; van Hylckama Vlieg et al., 2000). Iso proteins have been purified from *Rhodococcus* sp. AD45 (Sims et al., 2022), permitting in-depth biochemical analyses. Evidence of a β-oxidative metabolic pathway for the assimilation of carbon from isoprene via propionyl-CoA, initially predicted by van Hylckama Vlieg et al. (2000), has also been reported (Dawson et al., 2022), leading to the prediction of the complete pathway by which isoprene-derived carbon is assimilated. In addition, Srivastva et al. (2015) isolated isoprene-degrading bacteria from soils in a rubber dump site. This study isolated the first example of an isoprene-degrading *Pseudomonas* sp. strain, which was subsequently grown in a continuous bioreactor. The current state of research into isoprene metabolism is reviewed here, and the critical areas for further study of the cycling of this abundant but neglected volatile organic compound (VOC) are highlighted. Although isoprene-degraders containing *isoA* can and have been identified, a growing volume of data (DNA-stable isotope probing (DNA-SIP) experiments (Gibson et al., 2021) and extant isoprene-degraders) suggest that an alternative pathway may exist and that in addition, co-metabolism of isoprene may be important. Therefore, the potential of non-*iso* isoprene-degraders (those bacteria not employing the *iso* metabolic gene cluster, Figure 2) to contribute to the global isoprene biogeochemical cycle is also discussed. This is an area of research that requires further molecular and physiological studies to elucidate alternative mechanisms of isoprene metabolism. Such knowledge would permit in-depth molecular ecology studies with the aim of revealing the extended diversity of isoprene-degrading bacteria.

ECOLOGY OF ISOPRENE-DEGRADING BACTERIA: AN UNTAPPED METABOLIC DIVERSITY

When studying the ecology of isoprene-degrading microorganisms, the diverse sources of isoprene must

14622920, 0, Downloaded from https://ami-journals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.16325 by Sheffield Hallam University, Wiley Online Library on [05/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

ENVIRONMENTAL MICROBIOLOGY Applied Microbiology International

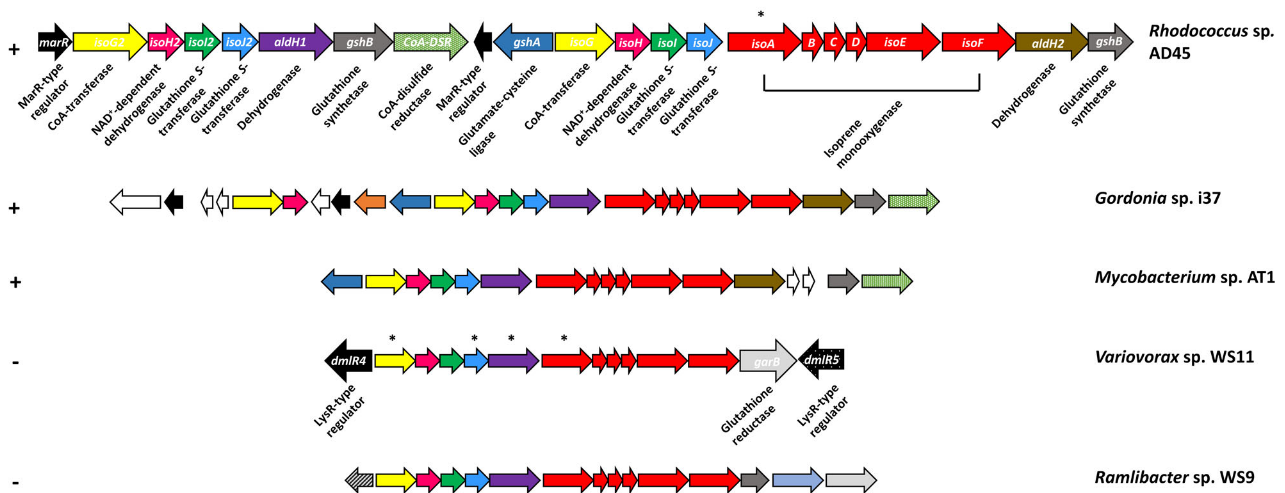


FIGURE 2 Conserved *iso* metabolic gene cluster required for isoprene metabolism by *iso* isoprene-degraders, adapted from Dawson et al. (2021). Asterisks (*) denote essential genes which have been characterized by targeted mutagenesis in *Rhodococcus* sp. AD45 (Crombie et al., 2015) and *Variovorax* sp. WS11 (Dawson et al., 2020, 2022).

be considered. The vast majority of isoprene is emitted by plants, ranging from crop plants to a variety of tree species (Ekberg et al., 2009; Sharkey et al., 2008), predominantly from the leaves but also to a lesser extent from the roots (van Doorn et al., 2020). Additional biogenic sources of isoprene are bacteria (Kuzma et al., 1995), fungi (Bäck et al., 2010), algae (Exton et al., 2013) and even humans (DeMaster & Nagasawa, 1978). As terrestrial ecosystems around trees are the most likely to be exposed to isoprene, the soil and phyllosphere of isoprene-emitting trees, both tropical and temperate, have been studied in detail, revealing a previously unappreciated diversity of both *iso*-type (Figure 2) and non-*iso* isoprene-degraders (Carrión, McGenity, & Murrell, 2020; El Khawand et al., 2016; Larke-Mejía et al., 2019; Singh et al., 2019). In fact, the presence of isoprene-emitting plant species may modulate the local isoprene-degrading community, as the rate of isoprene consumption by grassland mesocosms decreased in the presence of non-emitting plants (Spielmann et al., 2017). Inconsistencies in enrichment technique and in the choice of marker gene (16 S rRNA vs. *isoA*) used during molecular ecology surveys of isoprene-degrading communities may hinder our understanding of the most significant contributors to the isoprene sink. For example, bacteria and fungi have been enriched by isoprene in soils (Gray et al., 2015) and certain bacteria have been enriched in aquatic environments (Acuña Alvarez et al., 2009), determined by 16 S rRNA gene sequencing or sequencing of the internal transcribed spacer (ITS1) region of rRNA in fungi, but this does not guarantee active isoprene uptake due to the potential for cross-feeding between members of a microbial community (Mooshammer et al., 2021). DNA-SIP improves the specificity of identification efforts by labelling the active isoprene-degrading community with carbon from ^{13}C-isoprene, followed by separation of labelled and unlabelled DNA using isopycnic ultracentrifugation (Carrión, McGenity, & Murrell, 2020; Crombie et al., 2018; Nkongolo & Narendrula-Kotha, 2020), thereby revealing those organisms which specifically incorporated carbon from isoprene. However, this does not entirely eliminate the issue of cross-feeding, as increased incubation times can exacerbate the uptake of metabolites containing ^{13}C-labelled carbon by non-isoprene-degraders (Neufeld et al., 2007). Difficulties are also introduced by the choice of target genes for sequencing; functional gene probes have been developed specifically to target the *isoA* gene (encoding the conserved α-oxygenase component of isoprene monooxygenase [IsoMO]) (Carrión, McGenity, & Murrell, 2020), but the design of such probes requires a database of known *isoA* sequences. The diversity of *isoA* sequences subsequently identified by these primers is determined (or hindered) by the initial diversity of *isoA* sequences in the database. Such drawbacks may be avoided by employing high-throughput sequencing techniques to non-selectively identify the microbial community in an environmental sample.

Members of the Actinobacteria, typically *Rhodococcus* species, have dominated in many studies of the diversity of isoprene-degrading bacteria in enrichments from terrestrial soils and phyllosphere, and from coastal and salt marsh sediments (Carrión et al., 2018; Crombie et al., 2018; El Khawand et al., 2016), particularly in earlier studies in which isoprene-degraders

14622920, 0, Downloaded from https://ami-journals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.16325 by Sheffield Hallam University, Wiley Online Library on [05/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

were enriched at higher concentrations of isoprene (2000–5000 ppmv isoprene) (El Khawand et al., 2016; Johnston et al., 2017). More recently, improved enrichment methods have allowed the identification of a wider diversity of isoprene-degraders, with the use of lower concentrations of isoprene during enrichments (25–500 ppmv) (Carrión, Gibson, et al., 2020; Crombie et al., 2018; Larke-Mejía et al., 2019). The isolation of a greater diversity of isoprene-degraders then permitted molecular studies which, in turn, revealed a previously unappreciated diversity of isoprene-degraders in the environment. For example, members of the β-proteobacteria, particularly *Variovorax* spp., were abundant in willow soil (Larke-Mejía et al., 2019) and the phyllosphere of willow and poplar trees (Crombie et al., 2018; Gibson et al., 2021). Framework trees, pioneer species used to aid the regeneration of lost forests, and economic crops, such as oil palm and sugar cane were the sources of previously unknown isoprene-degrading genera; *Cellulosimicrobium*, *Friedmanniella*, *Isoptericola*, *Ochrobactrum* (Uttarotai et al., 2021). *Alcaligenes* sp. Strain 13f was isolated from one of these framework trees, prompting further evaluation of the variation in isoprene metabolic pathways (Uttarotai et al., 2022). *Alcaligenes* sp. Strain 13f lacked a complete *iso* metabolic gene cluster, suggesting that an entirely different mechanism of aerobic isoprene metabolism was used. Other putative non-*iso* isoprene-degraders, so-called because they were not confirmed to contain an *iso* metabolic gene cluster, have been isolated from the soils in a rubber dumping site and in a rice field (Srivastva et al., 2015, 2017), and in the soils and phyllosphere of tropical *Madhuca latifolia* and *Tectona grandis* trees (Singh et al., 2019). In all of these cases, the presence or absence of an *iso* gene cluster was not confirmed, although each tropical isoprene-degrader had an affinity for isoprene which was considerably lower than previously described IsoMO-catalyzed reactions. For example, the K_M of IsoMO from *Rhodococcus* sp. AD45 for isoprene is 0.8 μM, determined in whole cells (van Hylckama Vlieg et al., 1998), while the tropical isoprene-degraders had a K_M (K_M apparent) between 1.47 and 3.70 mM, more than 1000-times greater. Bacteria that express soluble diiron monooxygenases (SDIMO) other than the IsoMO must also be factored into the global isoprene sink due to co-oxidation of isoprene. Methanotrophs such as *Methylobacterium* sp. PV1 (which was reported to grow on methane, unlike most other members of this genus) (Srivastva et al., 2017), *Methylococcus capsulatus* (Bath) (Dawson et al., 2020) and *Methylocella silvestris* BL2 (Crombie, 2011) all co-oxidize isoprene, with the last two known to use the soluble methane monooxygenase (sMMO) to achieve this and the first using an unconfirmed mechanism of isoprene oxidation. The relative contributions of isoprene-degraders and co-oxidisers can be analysed in greater detail through the use of linear 1-alkynes, specific mechanism-based inhibitors of SDIMO (Yeager et al., 1999), with acetylene specifically inhibiting substrate oxidation by the sMMO (Dawson et al., 2020; Prior & Dalton, 1985) and octyne inhibiting isoprene oxidation by the IsoMO (Dawson et al., 2020). It would be revealing to explore microbial metabolism of isoprene in peatlands by using alkynes, as methanotrophs are highly abundant in peat-rich, moss-covered land (Larmola et al., 2010; Raghoebarsing et al., 2005). As a result, methanotrophs may compete with isoprene-degraders in such settings where mosses emit isoprene (Ekberg et al., 2009, 2011). Further study is also required to determine the relative contributions of other non-SDIMO oxygenases to isoprene oxidation, such as the copper-requiring ammonia monooxygenase (AMO) and particulate methane monooxygenase (pMMO). Although it is currently not known whether these oxygenases are capable of oxidizing isoprene, each is differentially inhibited by linear alkynes (Wright et al., 2020) and may need to be included in considerations of the relative contributions of microorganisms to isoprene degradation.

Aquatic sources of isoprene are yet to be studied in detail, although it is known that isoprene can be a major BVOC associated with wetlands due to its emission from mosses (e.g., *Sphagnum* spp.) and sedges (Ekberg et al., 2009, 2011; Hellén et al., 2020; Seco et al., 2020). Isoprene fluxes have been measured in lakes (Seco et al., 2020; Steinke et al., 2018), estuaries (Exton et al., 2012), reefs (Hrebien et al., 2020) and oceans (reviewed by Dawson et al., 2021), but we know practically nothing about the distribution, diversity and relative impact of isoprene-degraders in these environments. Members of the Actinobacteria made up a significant portion of the isoprene-degrading community in brackish waters and in estuarine water and sediment (Acuña Alvarez et al., 2009; Johnston et al., 2017), while *Stappia* spp. were a significant member of marine isoprene-degrading communities (Acuña Alvarez et al., 2009). Isoprene-degraders also inhabit isoprene-emitting corals (*Acropora* spp.) (Dawson et al., 2021), although the identity of these isoprene-degraders is unknown. Isoprene concentrations in the ocean generally very low, as low as 0.6 pmol L^{-1} in surface oceans (Ooki et al., 2015), but local concentrations of isoprene increase significantly during phytoplankton blooming events (Tripathi et al., 2020), a fact which may support concomitant increases in the abundance of isoprene-degrading bacteria. Oxidative breakdown of isoprene may not be the only biological driver of isoprene consumption in the ocean. Moore et al. (2020) reported that the marine heterotroph *Pelagibacter* sp. HTCC1062 (SAR11) was capable of consuming isoprene which was emitted from phototrophic diatoms. Unlike the conventional oxidative breakdown of isoprene facilitated by the *iso*-type

14622920, 0, Downloaded from https://ami-journals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.16325 by Sheffield Hallam University, Wiley Online Library on [05/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

degradation pathway, and potentially by non-*iso* pathways, isoprene was unable to support the growth of SAR11 in the absence of other heterotrophic carbon sources, although a small increase in ATP was observed in SAR11 when isoprene was supplied (Moore et al., 2020). Instead, SAR11 may consume freely available isoprene in the ocean to support isoprenoid biosynthesis (Moore et al., 2022), as this is a metabolically costly process (Zhao et al., 2013). Given that SAR11 is extremely abundant in the ocean (estimated global population 2.4 $\times 10^{26}$ cells) (Morris et al., 2002), the maximum potential contribution to global isoprene uptake was estimated at 11.9 Tg C yr^{-1} (Moore et al., 2022), resembling the previously predicted consumption by oceans of 11.6 Tg C yr^{-1} (Luo & Yu, 2010). The mechanism of metabolism of isoprene by SAR11 is yet to be elucidated, but this represents a fascinating, and potentially environmentally very relevant, aspect of the global isoprene biogeochemical cycle for further study.

Despite the diversity in the sites of isolation of isoprene-degrading bacteria (Acuña Alvarez et al., 2009; Carrión et al., 2018; Ewers et al., 1990; Johnston et al., 2017; Larke-Mejía et al., 2019; van Hylckama Vlieg et al., 1998) aerobic conditions were always used in isolation efforts. Kronen et al. (2019), however, demonstrated the ability of homoacetogens in activated sludge microcosms (dominated by *Acetobacterium* and *Geobacterium* species) to reduce isoprene to methylbutenes under anaerobic conditions. The significance of anaerobic isoprene uptake in the environment is unknown; isoprene biogenesis in plants is an aerobic process which is largely dependent on photosynthesis (Broadgate et al., 2004; Sharkey & Yeh, 2001; Zuo, 2019), suggesting that isoprene reduction by homoacetogens (Kronen et al., 2019) in anoxic regions where isoprene is not predicted to be abundant, may be a coincidental conversion by an established metabolic pathway rather than a dedicated isoprene metabolic pathway. This could be tested further by challenging these homoacetogenic microcosms with other alkenes and dialkenes, as they may reduce more than just isoprene. Soil-dwelling and aquatic bacteria also produce isoprene by the methylerythritol phosphate (MEP) pathway (Wagner et al., 2000; Yu et al., 2022), permitting isoprene formation in the absence of sunlight, potentially supplying isoprene to soil-dwelling anaerobes closer to the oxic-anoxic interface. *Rhodobacter sphaeroides*, a purple non-sulfur bacterium, also produced isoprene via the MEP pathway, with the authors noting an antimicrobial effect of isoprene on Gram-positive and Gram-negative bacteria (Yu et al., 2022). This raises further questions as to the role of isoprene emission in bacteria, and also to the benefits of isoprene consumption by bacteria. Further studies may focus on identifying additional examples of anaerobic isoprene uptake. It is interesting to note that no representative *Acetobacterium* strains were capable of isoprene reduction in pure culture (Kronen et al., 2019), a fact that complicates in-depth molecular studies which would serve to determine the basis for anaerobic isoprene metabolism. Further studies are required to determine the genetic and biochemical basis for anaerobic isoprene metabolism, and also to reveal the phylogenetic diversity of isoprene-reducing microorganisms.

Genetics of isoprene degradation

The genetic basis for isoprene metabolism has only been studied in detail in the *iso*-type isoprene-degraders (Crombie et al., 2015; Dawson et al., 2020; van Hylckama Vlieg et al., 2000). Crombie et al. (2015) reported increased transcription of a cluster of 22 megaplasmid-encoded genes (Figure 2) when *Rhodococcus* sp. AD45 was incubated with isoprene or epoxyisoprene, the first metabolic intermediate in the *iso* metabolic pathway. This *iso* gene cluster contains a core set of 11 genes which were subsequently identified in the genomes of extant isoprene degraders and as partially complete gene clusters in metagenome-assembled genomes (MAGs) from isoprene-enriched environmental samples (Carrión, McGenity, & Murrell, 2020; Crombie et al., 2018; Dawson et al., 2020; Johnston et al., 2017; Larke-Mejía et al., 2020). These 11 core genes comprise *isoABCDEF* (encoding IsoMO), *isoG* (putative CoA-transferase), *isoI* (glutathione *S*-transferase—GST), *isoH* (NAD^+-dependent dehydrogenase), *isoJ* (GST), and *aldH* (putative aldehyde dehydrogenase). The combinations of *iso* metabolic genes vary between phylogenetically distinct isoprene-degraders, since one or more of the *isoGHIJ* gene clusters are found in Gram-positive isoprene-degraders compared to a single copy of the *isoGHIJ* cluster in Gram-negative isoprene-degraders (Figure 2). Many *iso* metabolic gene clusters contain genes that indicate a role for thiol recycling in isoprene metabolism, such as *garB* (encoding glutathione reductase) in *Variovorax* sp. WS11 and *Ramlibacter* sp. WS9 (Dawson et al., 2020), or a putative CoA-disulfide reductase in *Rhodococcus* sp. AD45 and *Gordonia* sp. i37 (Crombie et al., 2015; Johnston et al., 2017). The organization of the *iso* gene cluster is largely conserved, but the common genetic origin of the cluster and the extent to which it has been transmitted via lateral gene transfer have not been investigated in detail. The potential for horizontal gene transfer was indicated by the fact that the *iso* metabolic gene clusters from *Rhodococcus* sp. AD45 and *Variovorax* sp. WS11 are both encoded on megaplasmids, and the *iso* cluster from strain AD45 is found on a region of the plasmid which is rich in insertion sequences (Crombie et al., 2015).

Gene knockout studies show that *isoA* is essential for *iso*-type isoprene metabolism (Crombie et al., 2015;

14622920, 0, Downloaded from https://ami-journals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.16325 by Sheffield Hallam University, Wiley Online Library on [05/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Dawson et al., 2020), thus, validating the use of *isoA* as a target for functional gene probing (Carrión, McGenity, & Murrell, 2020). During phylogenetic analysis, IsoA amino acid sequences do not form a monophyletic clade (Dawson et al., 2020), likely due to the considerable conservation among SDIMO α-oxygenase components (Leahy et al., 2003). Further molecular genetics studies have been conducted with *Variovorax* sp. WS11, leading to the confirmation that *isoG*, *isoJ*, and *aldH* are essential for isoprene metabolism (Dawson et al., 2022). However, the putative glutathione-disulfide reductase *garB* was non-essential, since a *Variovorax* sp. WS11 mutant lacking *garB* (Δ*garB*) was able to grow on isoprene. Further analysis of the individual genes in the *iso* metabolic gene cluster would provide valuable insights into the *iso*-type isoprene metabolic pathway. It would also be interesting to investigate the physiological and evolutionary significance of the duplication and deletion events that are evident in the *iso* gene clusters of certain Gram-positive isoprene-degraders (Carrión, McGenity, & Murrell, 2020).

Molecular genetics techniques, ideally informed by proteomics, are required to determine the genetic basis for isoprene degradation in the non-*iso* *Alcaligenes* sp. Strain 13f (Uttarotai et al., 2022), as this is the only aerobic isoprene-degrader that has been definitively shown not to possess an *iso* gene cluster. Proteomics is likely to be valuable for the prediction of the isoprene metabolic pathway used by *Alcaligenes* sp. Strain 13f, as it is not certain if carbon from isoprene may be assimilated via the same β-oxidative metabolic pathway proposed by van Hylckama Vlieg et al. (2000), or whether an entirely distinct isoprene metabolic pathway is employed. A number of enzymes are capable of catalzsing the oxidation of isoprene, including the cytochrome P450-dependent monooxygenases (Watson et al., 2001) and sMMO (Crombie, 2011; Dawson et al., 2020), although each of these processes causes the accumulation of toxic epoxide intermediates. *Alcaligenes* sp. Strain 13F is a Gram-negative bacterium and thus would be expected to produce glutathione (Johnson et al., 2009), but no glutathione biosynthesis genes were evident on the genome of Strain 13F (accession no. PRJNA734706). This raises the question of how strain 13F would detoxify epoxyisoprene following isoprene oxidation, particularly as it lacked an *isoI* (GST) gene (Uttarotai et al., 2022). Genetic studies are also required to determine the basis for reductive isoprene metabolism reported by Kronen et al. (2019), as it is currently unknown whether the homoacetogenic community responsible for methylbutene formation was capable of doing so via a dedicated metabolic process, or whether a pre-existing metabolic pathway opportunistically reduced isoprene. As was discussed earlier, testing the isoprene-reducing community for the ability to reduce other alkenes may provide further information. A cluster of five genes was found on the genome of *Acinetobacter wieringae* ISORED-2 which were significantly more expressed in isoprene-reducing cultures. This cluster included genes for an FAD-dependent oxidoreductase (suggested as a putative isoprene reductase), Hyp proteins involved in incorporating nickel into [NiFe]-hydrogenases, and a 4Fe-4 S-superfamily protein (Kronen, 2019). A functional role for the encoded proteins has yet to be confirmed.

The genetics of isoprene degradation is a relatively understudied area, a fact that must be addressed if knowledge of the microbial sink for isoprene is going to be advanced. Molecular ecology studies of *iso*-type isoprene-degraders were made possible by *isoA*-specific molecular gene probes, as this essential gene is retained in all extant *iso*-type isoprene-degraders (reviewed by Carrión, Gibson, et al., 2020; Carrión, McGenity, & Murrell, 2020). Finding similarly essential genes for non-*iso* metabolic pathways would be a key step in revealing the diversity and distribution of isoprene-degraders in the environment.

Metabolism of isoprene by bacteria

Detailed knowledge of the metabolism of isoprene is essential to understand the biological sink for isoprene in nature. The only metabolic pathway for isoprene degradation which has been characterized in detail is the *iso*-type pathway (Figure 3), the latter stages of which closely resemble the β-oxidation of isoleucine to propionyl-CoA and acetyl-CoA (Conrad et al., 1974). For many years, only the first three enzyme-catalyzed steps of the *iso*-type pathway were known (van Hylckama Vlieg et al., 1998, 1999, 2000). Isoprene is oxidized to epoxyisoprene by the IsoMO, a reaction which requires NADH and O_2. SDIMO enzymes have previously been studied for their ability to catalyze enantiospecific transformations (Cheung et al., 2013; Takagi et al., 1990), producing commercially valuable chiral epoxides. *Rhodococcus* sp. AD45 was reported to produce (*R*)-epoxyisoprene with a 95% enantiomeric excess (van Hylckama Vlieg et al., 1999), although the details of this reaction were not published. The alkene monooxygenase of *Rhodococcus rhodochrous* B-276 was similarly enantioselective, producing (*R*)-epoxypropane from propylene with 83% enantiomeric excess (Gallagher et al., 1997; Miura & Dalton, 1995). Chiral epoxyisoprene formation was tested in whole-cells using isoprene-grown *Rhodococcus* sp. AD45 and *Variovorax* sp. WS11 (Figure S1), both well-characterized model isoprene-degraders, with 1 mM epoxyhexane included in the reaction to inhibit IsoI activity (van Hylckama Vlieg et al., 1998), thereby causing epoxyisoprene to accumulate. The IsoMO from *Rhodococcus* sp. AD45 and *Variovorax* sp. WS11 produced (*R*)-epoxyisoprene with an enantiomeric excess

14622920, 0, Downloaded from https://ami-journals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.16325 by Sheffield Hallam University, Wiley Online Library on [05/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

14622920, 0, Downloaded from https://ami-journals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.16325 by Sheffield Hallam University, Wiley Online Library on [05/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

of 93.2 ± 0.4% and 90.6 ± 1.4% (Figure S1 F, G), respectively, consistent with the previous report (van Hylckama Vlieg et al., 1998). Further work is required to determine whether IsoMO is able to produce commercially valuable epoxides in sufficient quantities, and with sufficient enantiomeric specificity, to be viable as a biological catalyst in the industry.

Epoxyisoprene is conjugated with glutathione (GSH) by IsoI, forming 1-hydroxy-2-glutathionyl-2-methyl-3-butene (HGMB), which is then oxidized in two steps by IsoH to form 2-glutathionyl-2-methyl-3-butanoic acid (GMBA). These enzymatic steps have been confirmed using purified IsoI and IsoH from *Rhodococcus* sp. AD45 (van Hylckama Vlieg et al., 1998, 1999). The following steps were proposed by Dawson et al. (2022) using a combination of proteomics and transcriptomics (Figure 3). IsoG, a putative CoA-transferase, catalyzes the conversion of GMBA into a CoA-thioester (GMBA-CoA), although it is unknown which CoA-donor is required for this reaction. MBE-CoA (2-methyl-3-hydroxy-butenyl-CoA) is then formed by the removal of oxidized glutathione by IsoJ, a GST which has no activity towards epoxyisoprene or GMBA (van Hylckama Vlieg et al., 2000), while the disulfide (GSSG) may be recycled to reduced glutathione by the non-essential, but highly expressed, glutathione-disulfide reductase GarB (Dawson et al., 2022). Gram-positive isoprene-degraders typically encode a putative CoA-disulfide reductase instead of *garB* (Figure 2), with the translated gene product potentially fulfilling the same role. An isoprene-induced enoyl-CoA hydratase catalyzes the transfer of the alkene bond of MBE-CoA to the 2–3 position, indicated by isomerase activity conferred by the enzyme's conserved Glu164 residue (Dawson et al., 2022; Onwukwe et al., 2015), followed by the formation of a hydroxyl group in 2-methyl-3-hydroxybutyryl-CoA (MHB-CoA). The enzyme responsible for oxidizing MHB-CoA to 2-methylacetoacetyl-CoA (MAA-CoA) is unknown, although this step was inferred due to similarities with the β-oxidative degradation of isoleucine to the same metabolic intermediate (Conrad et al., 1974). Acetyl-CoA acetyltransferase is then predicted to catalyze the reaction of an additional molecule of coenzyme A with MAA-CoA to yield acetyl-CoA (assimilated via the tricarboxylic acid (TCA)-cycle) and propionyl-CoA (which is likely to be assimilated via the methylmalonyl-CoA and methylcitrate pathways) (Dawson et al., 2022; Textor et al., 1997). Examination of the genome sequences of isoprene-degrading bacteria consistently indicated the ability to assimilate propionyl-CoA via one or both of the above pathways (Dawson et al., 2022), although growth of *Rhodococcus* sp. AD45 on isoprene did not cause an increase in transcription of the methylmalonyl-CoA pathway or methylcitrate cycle-related genes (Crombie et al., 2015).

Variovorax sp. WS11 was subjected to metabolomics analysis to confirm that HGMB and GMBA, the isoprene-derived metabolites initially identified in *Rhodococcus* sp. AD45 (van Hylckama Vlieg et al., 1998, 1999), were produced by the cell during growth on isoprene. Interestingly, HGMB, although considerably more abundant in cells grown in the presence of isoprene, was also present in cells grown on pyruvate (Figure 4A, B). It is unclear how HGMB would be

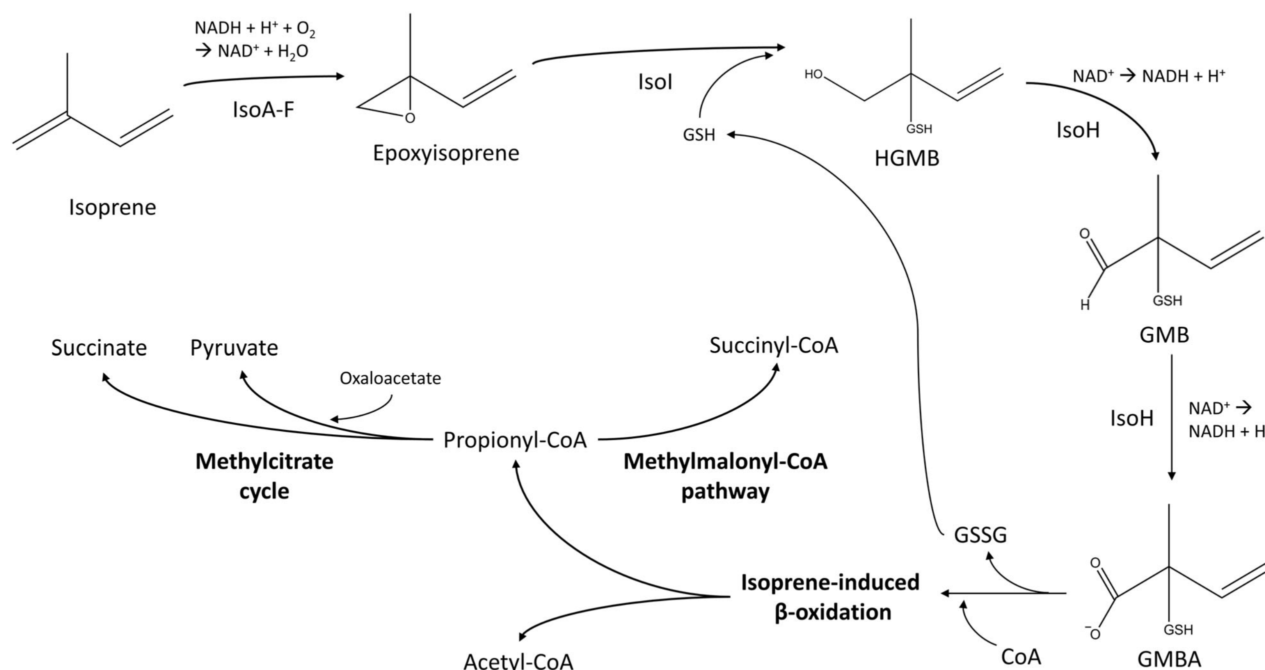


FIGURE 3 Simplified scheme of the *iso*-type isoprene metabolic pathway, adapted from Dawson et al. (2022).

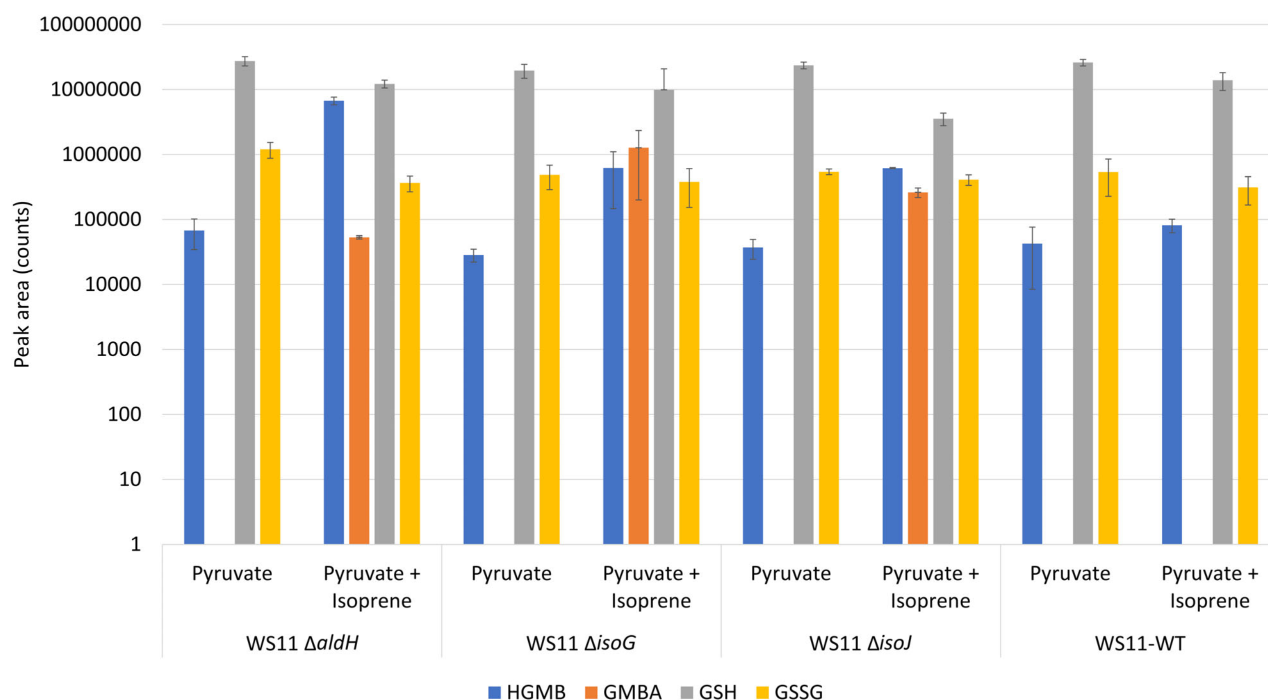


FIGURE 4 The presence of key isoprene metabolic pathway intermediates (HGMB, GMBA) and thiols (GSH, GSSG) were measured in wild-type *Variovorax* sp. WS11 and with targeted mutants (Δ*isoG*, Δ*isoJ*, Δ*aldH*) using metabolomics (see supplementary methods). LC–MS-derived peak areas were determined using Profinder software (Agilent). HGMB, GMBA, GSH, and GSSG were measured in cell extracts from *Variovorax* sp. WS11 strains grown on 10 mM pyruvate only, or 10 mM pyruvate +1% (v/v) isoprene. HGMB and GMBA were putatively identified based on their accurate mass and MS2 spectra (see supplementary methods).

produced without the isoprene precursor, indicating the need for a greater understanding of the intermediate metabolites from the *iso*-type metabolic pathway. GMBA, on the other hand, was not detected in wild-type *Variovorax* sp. WS11 grown with or without isoprene, (Figures 4A,B, Figures S2 and S3), likely due to the fact that GMBA was rapidly metabolized by the isoprene metabolic pathway (Dawson et al., 2022). This hypothesis was tested by analysing strains inactivated in one or other of the genes required for later steps of the pathway. Compared to isoprene-free conditions, when isoprene was present the levels of HGMB increased substantially in the *Variovorax* sp. WS11 strains Δ*isoG*, Δ*isoJ*, and Δ*aldH* (Figure 4), mutants lacking a specific essential *iso* metabolic gene but still able to oxidize isoprene in a truncated isoprene metabolic pathway (Dawson et al., 2022). Interestingly, the greatest accumulation of HGMB was observed in *Variovorax* sp. WS11 Δ*aldH*, whereas accumulation of GMBA was less than in the other mutant strains. Currently, *aldH* is the only *iso* metabolic gene which lacks a predicted function in the isoprene pathway (Dawson et al., 2022). These data suggest that AldH is involved in the isoprene metabolic pathway subsequent to the production of HGMB. A role for AldH was previously suggested in the functionally similar styrene metabolic pathway (Lienkamp et al., 2021), in which StyH (catalyzing the equivalent reactions as IsoH) and AldH1 (equivalent to AldH in isoprene metabolism) may both catalyze the second NAD^+-reducing oxidation step, equivalent to the conversion of GMB to GMBA in isoprene metabolism (Figure 3). The metabolomics data support the role of AldH in the conversion of HGMB to GMBA in *Variovorax* sp. WS11. The continued accumulation of GMBA in *Variovorax* sp. WS11 Δ*aldH* when incubated with isoprene was likely to be due to the activity of IsoH, which is known to oxidize HGMB through to GMBA via the GMB intermediate (van Hylckama Vlieg et al., 1999). GMBA accumulated to a greater degree in the *isoG* mutant than in the *isoJ* mutant (Figure 4), consistent with the prediction that IsoG catalyzes the conversion of GMBA to a GMBA-CoA conjugate, while IsoJ is predicted to remove oxidized glutathione from GMBA-CoA, meaning that GMBA-CoA would be the key accumulated intermediate in WS11 Δ*isoJ* during incubation with isoprene. Levels of reduced glutathione were variable in all strains of *Variovorax* sp. WS11 supplemented with isoprene compared to cells grown on pyruvate alone. The amount of GSH in the cells decreased in the presence of isoprene, likely due to the consumption of GSH during the conversion of epoxyisoprene to HGMB (Figure 3). This is consistent with the observation that glutathione is involved in the detoxification of epoxyisoprene (van Hylckama Vlieg et al., 1998). Overall, the metabolomics data provide support for the predicted

14622920, 0, Downloaded from https://ami-journals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.16325 by Sheffield Hallam University, Wiley Online Library on [05/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

isoprene metabolic pathway (Dawson et al., 2022; van Hylckama Vlieg et al., 2000), but the CoA-conjugated intermediates of the isoprene metabolic pathway are yet to be confirmed. This confirms that isoprene metabolism is an area of research that is ripe for further study using metabolomics combined with other 'omics techniques.'

Singh et al. (2019) isolated isoprene-degraders from tropical trees (*Madhuca latifolia* and *Tectona grandis*), but did not perform molecular characterization of the mechanism for isoprene metabolism. However, Fourier transform infrared spectroscopy (FTIR) analysis indicated that isoprene was broken down to form products that contained amine (possible from glutathione) carboxylic acid and hydroxyl groups, potentially corresponding to known isoprene pathway intermediates (Figure 3). HGMB could account for the hydroxyl and amine peaks, while GMBA could account for the carboxyl group. However, as detailed earlier, the tropical isoprene-degrading isolates had a much lower affinity for isoprene than the IsoMO-expressing *Rhodococcus* sp. AD45 (van Hylckama Vlieg et al., 1998) (up to 3.70 mM, compared to 0.8 μM). Other SDIMO enzymes also typically exhibit similarly high affinities for their respective substrates (Green & Dalton, 1986), suggesting that the tropical isoprene-degraders isolated by Singh et al. (2019) use an alternative mechanism of isoprene uptake. Despite the apparently lower affinity for isoprene of these tropical isoprene-degraders, *iso*-type isoprene-degraders withstand 2% v/v isoprene (van Hylckama Vlieg et al., 1998), while tropical isolates could not grow above 500 ppmv (Singh et al., 2019), equivalent to approximately 6.5 μM isoprene according to the Henry's law constant for isoprene of 0.013 mol/m^3Pa (Sander, 2015). FTIR analysis also indicated the presence of –OH and –NH-containing compounds during the co-oxidation of isoprene by *Methylobacterium* sp. PV1 (Srivastva et al., 2017), a methanotroph which oxidized low concentrations of isoprene while growing on 20% (v/v) methane. It is unclear whether these signals were specific to isoprene-derived metabolic intermediates, as it is unknown which organic compounds would be formed by the breakdown of epoxyisoprene in the cell without conversion into HGMB. Further study is required to determine whether *Methylobacterium* sp. PV1 expresses the sMMO to oxidize isoprene, or indeed if strain PV1 expresses a pMMO which is capable of oxidizing isoprene, which would be unusual since no extant *Methylobacterium* species have been shown to contain MMO. Many isoprene-degraders have been isolated from rubber-contaminated soils and tropical soils (Singh et al., 2019; Srivastva et al., 2015, 2017). Now, further study is required to definitively state whether these novel isoprene-degraders employ a canonical or alternative isoprene metabolic pathway. Many questions still remain to be answered when considering the microbial sink for isoprene.

Future perspectives

The biochemistry isoprene metabolism

The biochemistry of isoprene metabolism is an emerging area of research. Recently, Sims et al. (2022) described the expression and purification of the isoprene monooxygenase from *Rhodococcus* sp. AD45 in homologous and heterologous hosts, paving the way for detailed biochemical characterizations and further study of the ability of this enzyme to produce valuable chiral epoxides. Current study of IsoMO from *Rhodococcus* sp. AD45 has relied on the use of *Rhodococcus* sp. AD45-id as a host strain for homologous protein expression (Crombie et al., 2018; Sims et al., 2022), increasing the difficulty of such studies when compared to using more well-characterized bacteria such as *Escherichia coli* as heterologous hosts. Expressing IsoMO from the Gram-negative *Variovorax* sp. WS11 may be simpler in standard protein expression hosts. IsoMO from *Variovorax* sp. WS11 oxidizes a wide range of alkenes (Dawson et al., 2020), presumably to the corresponding epoxides although this remains to be experimentally verified. The biochemistry of non-*iso* isoprene metabolism remains to be elucidated. A candidate isoprene reductase was proposed in *Acetobacterium wieringae* ISORED-2 (Kronen, 2019), which now requires characterization to confirm its role in anaerobic isoprene metabolism. Currently, no candidate enzymes have been suggested which could facilitate the proposed aerobic non-*iso* metabolic pathways (Table 1). The identification of the enzymes which drive these processes will inform future molecular ecology studies, and metagenomes previously derived from isoprene-enriched environmental samples (see Carrión, McGenity, & Murrell, 2020) can be re-examined with the aim of determining the relative contributions of *iso*-type and alternative isoprene-degraders.

The ecology of isoprene degraders

Many bacteria which express SDIMOs may contribute to isoprene oxidation without being able to assimilate carbon from isoprene, meaning that enrichments and DNA-SIP are not sufficient to gain a full understanding of the microbial sink for isoprene. For example, *Methylococcus capsulatus* (Bath) and *Methylocella silvestris* BL2 both co-oxidize isoprene to epoxyisoprene using the soluble methane monooxygenase (Crombie, 2011; Dawson et al., 2020), and methane-grown *Methylobacterium* sp. PV1 also co-oxidizes isoprene to a currently un-confirmed product (Srivastva et al., 2017). Dawson et al. (2020) proposed that octyne and acetylene could be applied to environmental samples to differentiate between the contributions of isoprene-degraders and co-oxidisers to the microbial isoprene sink,

14622920, 0, Downloaded from https://ami-journals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.16325 by Sheffield Hallam University, Wiley Online Library on [05/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 1 Putative isoprene metabolic processes which do not, or are not known to utilize the *iso*-type isoprene metabolic pathway

Mechanism/features of isoprene degradation	Candidate organisms	Reference
Isoprenoid-generating	*Pelagibacter* sp. HTCC1062 (SAR11)	(Moore et al., 2022)
Unknown	*Alcaligenes* sp. Strain 13f	(Uttarotai et al., 2022)
Unknown, low affinity for isoprene	*Pseudomonas* sp. strains BHU FT1 and LFM1, *Arthrobacter* sp. strains BHU FT2 and FM3, *Bacillus* sp. strain BHU FM1, *Sphingobacterium* sp. strain BHU LFT1, *Pantoea* sp. strain BHU LFM3, *Sphingobium* sp. strain BHU LFT2	(Singh et al., 2019)
Unknown, likely co-oxidation sMMO/pMMO	*Methylobacterium* sp. PV1	(Srivastva et al., 2017)
Anaerobic reduction, methylbutene-forming Energy-conserving	Homoacetogenic enrichment (dominated by *Acetobacterium* spp.)	(Kronen et al., 2019)

respectively. A key consideration during inhibitor-based studies is the fact that increasingly more isoprene-degraders are being identified which do not seem to possess an isoprene monooxygenase (Kronen et al., 2019; Singh et al., 2019; Uttarotai et al., 2022). Genetic and biochemical analysis of non-*iso* isoprene-degraders will be key to gaining a better understanding of the biological component of the global isoprene sink.

Non-*iso* isoprene degraders

Research currently indicates the existence of multiple groups of isoprene-degraders, other than the *iso*-type isoprene-degraders. Gray et al. (2015) reported that members of the Zygomycota and Trichomaceae were enriched when soil harvested from beneath a pine tree (*Pinus contorta*) was incubated with isoprene. The amino acid sequences of IsoA from *Variovorax* sp. WS11 (JAAGOW000000000), *Ramlibacter* sp. WS9 (NZ RKMB00000000), *Rhodococcus* sp. AD45 (AJ249207), *Nocardioides* sp. WS12 (MK176348), and *Sphingopyxis* sp. OPL5 (MK176354) failed to return a single result when queried by tBLASTn (Altschul et al., 1990) against the nucleotide collection (nr/nt) specific to fungi (taxid: 4751). Without the use of DNA-SIP, it cannot be ruled out that the fungi identified by Gray et al. (2015) were enriched due to cross-feeding of metabolites supplied by isoprene-degrading bacteria, a frequent issue during enrichment studies (Mooshammer et al., 2021). Currently, no archaeal isoprene-degraders have been identified. The abundant marine heterotroph *Pelagibacter* sp. HTCC1062 (SAR11) does not possess an *iso* gene cluster but still consumed exogenously supplied isoprene to generate isoprenoids (Moore et al., 2022). The contribution of such an abundant heterotrophic bacterium to aquatic isoprene cycling may become an important consideration in future modelling efforts. Once a mechanism for isoprene consumption and isoprenoid production by SAR11 has been identified, it will be fascinating to search for the same or similar biosynthetic pathways elsewhere in nature.

The putative *iso*-type isoprene metabolic pathway

Dawson et al. (2022) recently proposed the first complete isoprene metabolic pathway using data from proteomics and transcriptomics experiments in *Variovorax* sp. WS11 (Figure 3). These data indicated that carbon from isoprene was specifically assimilated as acetyl-CoA and propionyl-CoA, with the latter being incorporated via the methylcitrate cycle and methylmalonyl-CoA pathway. Further analysis of the competing, or possibly colluding, methylcitrate cycle and methylmalonyl-CoA pathway could be done by targeted deletion studies. Transcription of genes encoding the methylcitrate cycle or methylmalonyl-CoA pathway did not increase during growth of *Rhodococcus* sp. AD45 on isoprene (Crombie et al., 2018), although no information was available regarding the abundance of the translated protein products within the cell during growth on isoprene. This further indicates the importance of proteomics for further studies of isoprene-degrading bacteria. The *iso*-type metabolic pathway proposed by Dawson et al. (2022), while likely in the context of the available proteomics and transcriptomics data, requires further experimental validation. Metabolomics analysis confirmed that HGMB and GMBA are produced by *Variovorax* sp. WS11 specifically when isoprene is present, but many predicted isoprene metabolic intermediates are unconfirmed.

Regulation of isoprene metabolism

Isoprene metabolism is an inducible trait in several isoprene-degrading bacteria (Crombie et al., 2015; Dawson et al., 2020; Johnston et al., 2017). Epoxyisoprene is likely to be the primary inducer of isoprene

14622920, 0, Downloaded from https://ami-journals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.16325 by Sheffield Hallam University, Wiley Online Library on [05/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

metabolism in *iso*-type bacteria (Crombie et al., 2015; Dawson et al., 2020), but the putative transcriptional regulators found on Gram-positive and Gram-negative *iso* gene clusters differs, with one or more MarR-type regulators found in the former and one or more LysR-type regulators in the latter. The transcription of these regulatory genes was affected by the presence of isoprene/epoxyisoprene in both *Rhodococcus* sp. AD45 and *Variovorax* sp. WS11 (Crombie et al., 2015; Dawson et al., 2022), but the mechanism of these regulators has not been confirmed. Protein-DNA association studies, such as chromatin immunoprecipitation with sequencing (ChIP-seq), would be valuable for identifying the regions of DNA-binding for distinct transcriptional regulators.

To conclude, there have been significant advances in the field of isoprene metabolism in recent years. *iso*-type isoprene-degraders have been well-characterized at the genomic level, promoting rigorous molecular ecology studies which have begun to demonstrate the abundance, diversity and distribution of isoprene-degraders in the environment. However, recent evidence of alternative methods of isoprene consumption require further attention if we are to fully understand the contribution of bacteria, or indeed fungi, to the global isoprene cycle. The relative contributions of bacteria which utilize alternative SDIMOs, such as the sMMO, must also be considered. Studies of isoprene degradation versus co-oxidation will be conducted in the future through the use of the linear alkynes acetylene and octyne, as these specifically inhibit substrate oxidation by the sMMO and IsoMO, respectively. Although we have known for many years that soil microbes constitute a sink for isoprene, our knowledge of the underlying mechanisms which permit this activity was relatively poor for many years. Recent years have seen significant improvements in our understanding of the *iso*-type metabolic pathway, but reports of additional types of isoprene-degrading bacteria clearly indicate the need for a greater diversity of physiological, biochemical and molecular studies to characterize these non-*iso* isoprene-degraders.

AUTHOR CONTRIBUTIONS

Robin A. Dawson: Conceptualization (equal); data curation (lead); formal analysis (lead); investigation (lead); methodology (equal); visualization (equal); writing – original draft (lead); writing – review and editing (lead). **Andrew T. Crombie:** Conceptualization (equal); data curation (supporting); formal analysis (supporting); funding acquisition (supporting); investigation (supporting); methodology (equal); supervision (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Robert S. Jansen:** Conceptualization (supporting); data curation (equal); formal analysis (equal); investigation (supporting); methodology (equal); visualization (supporting); writing – review and editing (supporting). **Thomas J. Smith:** Conceptualization (supporting); formal analysis (supporting); investigation (supporting); methodology (supporting); writing – review and editing (supporting). **Tim Nichol:** Data curation (supporting); formal analysis (supporting); investigation (supporting); methodology (supporting). **Colin Murrell:** Conceptualization (equal); formal analysis (supporting); funding acquisition (lead); investigation (supporting); methodology (equal); project administration (equal); supervision (lead); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting).

ACKNOWLEDGEMENTS

This research was funded through a European Research Council Advanced grant to JCM (694578—IsoMet) and the Earth and Life Systems Alliance (ELSA) at the University of East Anglia. A Natural Environment Research Council grant (NE/J009725/1) was awarded to JCM, and a Leverhulme Trust Early Career Fellowship (ECF-2016-626) was awarded to ATC.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Robin A. Dawson https://orcid.org/0000-0002-7635-2251

REFERENCES

Acuña Alvarez, L., Exton, D.A., Timmis, K.N., Suggett, D.J. & McGenity, T.J. (2009) Characterization of marine isoprene-degrading communities. *Environmental Microbiology*, 11, 3280–3291.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403–410.

Bäck, J., Aaltonen, H., Hellén, H., Kajos, M.K., Patokoski, J., Taipale, R. et al. (2010) Variable emissions of microbial volatile organic compounds (MVOCs) from root-associated fungi isolated from scots pine. *Atmospheric Environment*, 44, 3651–3659.

Broadgate, W.J., Malin, G., Küpper, F.C., Thompson, A. & Liss, P.S. (2004) Isoprene and other non-methane hydrocarbons from seaweeds: a source of reactive hydrocarbons to the atmosphere. *Marine Chemistry*, 88, 61–73.

Carrión, O., Gibson, L., Elias, D.M.O., McNamara, N.P., Van Alen, T. A., Op Den Camp, H.J.M. et al. (2020) Diversity of isoprene-degrading bacteria in phyllosphere and soil communities from a high isoprene-emitting environment: a Malaysian oil palm plantation. *Microbiome*, 8, 81. Available from: https://doi.org/10.1186/s40168-020-00860-7

Carrión, O., Larke-Mejía, N.L., Gibson, L., Farhan Ul Haque, M., Ramiro-garcía, J., McGenity, T.J. et al. (2018) Gene probing reveals the widespread distribution, diversity and abundance of isoprene-degrading bacteria in the environment. *Microbiome*, 6, 219. Available from: https://doi.org/10.1186/s40168-018-0607-0

14622920, 0, Downloaded from https://ami-journals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.16325 by Sheffield Hallam University, Wiley Online Library on [05/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Carrión, O., McGenity, T.J. & Murrell, J.C. (2020) Molecular ecology of isoprene-degrading bacteria. *Microorganisms*, 8(7), 967. Available from: https://doi.org/10.3390/microorganisms8070967

Cheung, S., McCarl, V., Holmes, A.J., Coleman, N.V. & Rutledge, P. J. (2013) Substrate range and enantioselectivity of epoxidation reactions mediated by the ethene-oxidising *Mycobacterium* strain NBB4. *Applied Microbiology and Biotechnology*, 97, 1131–1140.

Cleveland, C. & Yavitt, J. (1997) Consumption of atmospheric isoprene in soil. *Geophysical Research Letters*, 24, 2379–2382.

Conrad, R.S., Massey, L.K. & Sokatch, J.R. (1974) D and L isoleucine metabolism and regulation of their pathways in *Pseudomonas putida*. *Journal of Bacteriology*, 118, 103–111.

Crombie, A.T. (2011) *Metabolism of methane and propane and the role of the glyoxylate bypass enzymes in Methylocella silvestris BL2*. PhD Thesis. UK: University Warwick.

Crombie, A.T., El Khawand, M., Rhodius, V.A., Fengler, K.A., Miller, M.C., Whited, G.M. et al. (2015) Regulation of plasmid-encoded isoprene metabolism in *Rhodococcus*, a representative of an important link in the global isoprene cycle. *Environmental Microbiology*, 17, 3314–3329.

Crombie, A.T., Larke-Mejia, N.L., Emery, H., Dawson, R.A., Pratscher, J. & Murphy, G.P. (2018) Poplar phyllosphere harbors disparate isoprene-degrading bacteria. *Proceedings of the National Academy of Sciences*, 115, 13081–13086.

Dawson, R.A., Crombie, A.T., Pichon, P., Mcgenity, T.J. & Murrell, J. C. (2021) The microbiology of isoprene cycling in aquatic ecosystems. *Aquatic Microbial Ecology*, 87, 79–98.

Dawson, R.A., Larke-Mejía, N.L., Crombie, A.T., Farhan Ul Haque, M. & Murrell, J.C. (2020) Isoprene oxidation by the gram-negative model bacterium *Variovorax* sp. WS11. *Microorganisms*, 8(3), 349. Available from: https://doi.org/10.3390/microorganisms8030349

Dawson, R.A., Rix, G.D., Crombie, A.T. & Murrell, J.C. (2022) Omics-guided prediction of the pathway for metabolism of isoprene by Variovorax sp. WS11. *Environmental Microbiology*, 24, 5151–5164.

DeMaster, E.G. & Nagasawa, H.T. (1978) Isoprene, an endogenous constituent of human alveolar air with a diurnal pattern of excretion. *Life Sciences*, 22, 91–97.

Ekberg, A., Arneth, A., Hakola, H., Hayward, S. & Holst, T. (2009) Isoprene emission from wetland sedges. *Biogeosciences*, 6, 601–613.

Ekberg, A., Arneth, A. & Holst, T. (2011) Isoprene emission from *sphagnum* species occupying different growth positions above the water table. *Boreal Environment Research*, 16, 47–59.

El Khawand, M., Crombie, A.T., Johnston, A., Vavlline, D.V., McAuliffe, J.C., Latone, J.A. et al. (2016) Isolation of isoprene degrading bacteria from soils, development of isoA gene probes and identification of the active isoprene-degrading soil community using DNA-stable isotope probing. *Environmental Microbiology*, 18, 2743–2753.

Ewers, J., Freier-Schröder, D. & Knackmuss, H. (1990) Selection of trichloroethene (TCE) degrading bacteria that resist inactivation by TCE. *Archives of Microbiology*, 154, 410–413.

Exton, D.A., Suggett, D.J., McGenity, T.J. & Steinke, M. (2013) Chlorophyll-normalized isoprene production in laboratory cultures of marine microalgae and implications for global models. *Limnology and Oceanography*, 58, 1301–1311.

Exton, D.A., Suggett, D.J., Steinke, M. & McGenity, T.J. (2012) Spatial and temporal variability of biogenic isoprene emissions from a temperate estuary. *Global Biogeochem Cycles*, 26, 1–13.

Gallagher, S.C., Cammack, R. & Dalton, H. (1997) Alkene monooxygenase from *Nocardia corallina* B-276 is a member of the class of dinuclear iron proteins capable of stereospecific epoxygenation reactions. *European Journal of Biochemistry*, 247, 635–641.

Gibson, L., Crombie, A.T., McNamara, N.P. & Murrell, J.C. (2021) Isoprene-degrading bacteria associated with the phyllosphere of *Salix fragilis*, a high isoprene-emitting willow of the northern hemisphere. *Environmental Microbiomes*, 16, 1–13.

Gibson, L., Larke-Mejía, N.L. & Murrell, J.C. (2020) Complete genome of isoprene degrading *Nocardioides* sp. WS12. *Microorganisms*, 8(6), 889. Available from: https://doi.org/10.3390/microorganisms8060889

Gray, C.M., Helmig, D. & Fierer, N. (2015) Bacteria and fungi associated with isoprene consumption in soil. *Elementa*, 3, 000053. Available from: https://doi.org/10.12952/journal.elementa.000053

Green, J. & Dalton, H. (1986) Steady-state kinetic analysis of soluble methane mono-oxygenase from *Methylococcus capsulatus* (Bath). *The Biochemical Journal*, 236, 155–162.

Hellén, H., Schallhart, S., Praplan, A.P., Tykkä, T., Aurela, M., Lohila, A. et al. (2020) Sesquiterpenes dominate monoterpenes in northern wetland emissions. *Atmospheric Chemistry and Physics*, 20, 7021–7034.

Hrebien, V., Deschaseaux, E., Eickhoff, W., Swan, H.B. & Eyre, B.D. (2020) Quantification of isoprene in coastal ecosystems by gas chromatography–mass spectrometry using cumulative headspace injections. *Limnology and Oceanography: Methods*, 18, 374–382.

Johnson, T., Newton, G.L., Fahey, R.C. & Rawat, M. (2009) Unusual production of glutathione in Actinobacteria. *Archives of Microbiology*, 191, 89–93.

Johnston, A., Crombie, A.T., El Khawand, M., Sims, L., Whited, G.M., McGenity, T.J. et al. (2017) Identification and characterisation of isoprene-degrading bacteria in an estuarine environment. *Environmental Microbiology*, 19, 3526–3537.

Kronen, M. (2019) *Anaerobic microbial metabolism of isoprene*. PhD Thesis. Australia: University of New South Wales.

Kronen, M., Lee, M., Jones, Z.L. & Manefield, M.J. (2019) Reductive metabolism of the important atmospheric gas isoprene by homoacetogens. *The ISME Journal*, 13, 1168–1182.

Kuzma, J., Nemecek-Marshall, M., Pollock, W.H. & Fall, R. (1995) Bacteria produce the volatile hydrocarbon isoprene. *Current Microbiology*, 30, 97–103.

Larke-Mejía, N.L., Carrión, O., Crombie, A.T., McGenity, T.J. & Murrell, J.C. (2020) *Sphingopyxis* sp. strain OPL5, an isoprene-degrading bacterium from the sphingomonadaceae family isolated from oil palm leaves. *Microorganisms*, 8(10), 1557. Available from: https://doi.org/10.3390/microorganisms8101557

Larke-Mejía, N.L., Crombie, A.T., Pratscher, J., McGenity, T.J. & Murrell, J.C. (2019) Novel isoprene-degrading proteobacteria from soil and leaves identified by cultivation and metagenomics analysis of stable isotope probing experiments. *Frontiers in Microbiology*, 10, 2700. Available from: https://doi.org/10.3389/fmicb.2019.02700

Larmola, T., Tuittila, E.-S., Tiirola, M., Nykänen, H., Martikainen, P.J., Yrjälä, K. et al. (2010) The role of *Sphagnum* mosses in the methane cycling of a boreal mire. *Ecology*, 91, 2356–2365.

Leahy, J.G., Batchelor, P.J. & Morcomb, S.M. (2003) Evolution of the soluble diiron monooxygenases. *FEMS Microbiology Reviews*, 27, 449–479.

Lienkamp, A.C., Burnik, J., Heine, T., Hofmann, E. & Tischler, D. (2021) Characterization of the glutathione S-transferases involved in styrene degradation in *Gordonia rubripertincta* CWB2. *Microbiology Spectrum*, 9, e00474–e00421.

Luo, G. & Yu, F. (2010) A numerical evaluation of global oceanic emissions of α-pinene and isoprene. *Atmospheric Chemistry and Physics*, 10, 2007–2015. Available from: https://doi.org/10.5194/acp-10-2007-2010

Miura, A. & Dalton, H. (1995) Purification and characterization of the alkene monooxygenase from *Nocardia corallina* B-276. *Bioscience, Biotechnology, and Biochemistry*, 59, 853–859.

Moore, E.R., Davie-Martin, C.L., Giovannoni, S.J. & Halsey, K.H. (2020) *Pelagibacter* metabolism of diatom-derived volatile

14622920, 0, Downloaded from https://ami-journals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.16325 by Sheffield Hallam University, Wiley Online Library on [05/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

organic compounds imposes an energetic tax on photosynthetic carbon fixation. *Environmental Microbiology*, 22, 1720–1733.
Moore, E.R., Weaver, A.J., Davis, E.W., Giovannoni, S.J. & Halsey, K.H. (2022) Metabolism of key atmospheric volatile organic compounds by the marine heterotrophic bacterium *Pelagibacter* HTCC1062 (SAR11). *Environmental Microbiology*, 24, 212–222.
Mooshammer, M., Kitzinger, K., Schintlmeister, A., Ahmerkamp, S., Nielsen, J.L., Nielsen, P.H. et al. (2021) Flow-through stable isotope probing (flow-SIP) minimizes cross-feeding in complex microbial communities. *The ISME Journal*, 15, 348–353. Available from: https://doi.org/10.1038/s41396-020-00761-5
Morris, R.M., Rappé, M.S., Connon, S.A., Vergin, K.L., Siebold, W.A., Carlson, C.A. et al. (2002) SAR11 clade dominates ocean surface bacterioplankton communities. *Nature*, 420, 806–810.
Neufeld, J.D., Dumont, M.G., Vohra, J. & Murrell, J.C. (2007) Methodological considerations for the use of stable isotope probing in microbial ecology. *Microbial Ecology*, 53, 435–442.
Nkongolo, K.K. & Narendrula-Kotha, R. (2020) Advances in monitoring soil microbial community dynamic and function. *Journal of Applied Genetics*, 61, 249–263.
Onwukwe, G.U., Kursula, P., Koski, M.K., Schmitz, W. & Wierenga, R.K. (2015) Human Δ3,Δ2-enoyl-CoA isomerase, type 2: A structural enzymology study on the catalytic role of its ACBP domain and helix-10. *The FEBS Journal*, 282, 746–768.
Ooki, A., Nomura, D., Nishino, S., Kikuchi, T. & Yokouchi, Y. (2015) A global-scale map of isoprene and volatile organic iodine in surface seawater of the Arctic, Northwest Pacific, Indian, and southern oceans. *Journal of Geophysical Research: Oceans*, 120, 4108–4128.
Prior, S.D. & Dalton, H. (1985) Acetylene as a suicide substrate and active site probe for methane monooxygenase from *Methylococcus capsulatus* (Bath). *FEMS Microbiology Letters*, 29, 105–109.
Raghoebarsing, A.A., Smolders, A.J.P., Schmid, M.C., Rijpstra, W.I.C., Wolters-Arts, M., Derksen, J. et al. (2005) Methanotrophic symbionts provide carbon for photosynthesis in peat bogs. *Nature*, 436, 1153–1156.
Sander, R. (2015) Compilation of Henry's law constants (version 4.0) for water as solvent. *Atmospheric Chemistry and Physics*, 15, 4399–4981.
Seco, R., Holst, T., Matzen, M.S., Westergaard-Nielsen, A., Li, T., Simin, T. et al. (2020) Volatile organic compound fluxes in a subarctic peatland and lake. *Atmos Chem Phys Discuss*, 20, 1–32.
Sharkey, T.D., Wiberley, A.E. & Donohue, A.R. (2008) Isoprene emission from plants: why and how. *Annals of Botany*, 101, 5–18.
Sharkey, T.D. & Yeh, S. (2001) Isoprene emission from plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 52, 407–436.
Sims, L.P., Lockwood, C.W.J., Crombie, A.T., Bradley, J.M., Le Brun, N.E. & Murrell, J.C. (2022) Purification and characterization of the isoprene monooxygenase from *Rhodococcus* sp. strain AD45. *Applied and Environmental Microbiology*, 88, e0002922. Available from: https://doi.org/10.1128/aem.00029-22
Singh, A., Srivastava, N. & Dubey, S.K. (2019) Molecular characterization and kinetics of isoprene degrading bacteria. *Bioresource Technology*, 278, 51–56.
Spielmann, F.M., Langebner, S., Ghirardo, A., Hansel, A., Schnitzler, J.P. & Wohlfahrt, G. (2017) Isoprene and α-pinene deposition to grassland mesocosms. *Plant and Soil*, 410, 313–322.
Srivastva, N., Shukla, A.K., Singh, R.S., Upadhyay, S.N. & Dubey, S.K. (2015) Characterization of bacterial isolates from rubber dump site and their use in biodegradation of isoprene in batch and continuous bioreactors. *Bioresource Technology*, 188, 84–91.
Srivastva, N., Vishwakarma, P., Bhardwaj, Y., Singh, A., Manjunath, K. & Dubey, S.K. (2017) Kinetic and molecular analyses reveal isoprene degradation potential of *Methylobacterium* sp. *Bioresource Technology*, 242, 87–91.
Steinke, M., Hodapp, B., Subhan, R., Bell, T.G. & Martin-Creuzburg, D. (2018) Flux of the biogenic volatiles isoprene and dimethyl sulfide from an oligotrophic lake. *Scientific Reports*, 8, 630. Available from: https://doi.org/10.1038/s41598-017-18923-5
Takagi, M., Uemura, N. & Furuhashi, K. (1990) Microbial transformation processes of aliphatic hydrocarbons. *Annals of the New York Academy of Sciences*, 613, 697–701.
Textor, S., Wendisch, V.F., De Graaf, A.A., Müller, U., Linder, M.I., Linder, D. et al. (1997) Propionate oxidation in *Escherichia coli*: evidence for operation of a methylcitrate cycle in bacteria. *Archives of Microbiology*, 168, 428–436.
Tripathi, N., Sahu, L.K., Singh, A., Yadav, R. & Karati, K.K. (2020) High levels of isoprene in the marine boundary layer of the Arabian Sea during spring inter-monsoon: role of phytoplankton blooms. *ACS Earth and Space Chemistry*, 4, 583–590.
Uttarotai, T., McKew, B.A., Benyahia, F., Murrell, J.C., Mhuantong, W., Wangkarn, S. et al. (2021) Isoprene-degrading bacteria from soils associated with tropical economic crops and framework forest trees. *Microorganisms*, 9(5), 1024. Available from: https://doi.org/10.3390/microorganisms9051024
Uttarotai, T., Sutheeworapong, S., Crombie, A.T., Murrell, J.C., Mhuantong, W., Noirungsee, N. et al. (2022) Genome characterisation of an isoprene-degrading *Alcaligenes* sp. isolated from a tropical restored Forest. *Biology (Basel)*, 11, 519.
van Doorn, M.M., Merl-Pham, J., Ghirardo, A., Fink, S., Polle, A., Schnitzler, J.-P. et al. (2020) Root isoprene formation alters lateral root development. *Plant, Cell & Environment*, 43, 2207–2223.
van Hylckama Vlieg, J.E.T., Kingma, J., Kruizinga, W. & Janssen, D.B. (1999) Purification of a glutathione *S*-transferase and a glutathione conjugate-specific dehydrogenase involved in isoprene metabolism in *Rhodococcus* sp. strain AD45. *Journal of Bacteriology*, 181, 2094–2101.
van Hylckama Vlieg, J.E.T., Kingma, J., van den Wijngaard, A.J. & Janssen, D.B.D. (1998) A glutathione *S*-transferase with activity towards cis-1,2-dichloroepoxyethane is involved in isoprene utilization by *Rhodococcus* sp. strain AD45. *Applied and Environmental Microbiology*, 64, 2800–2805.
van Hylckama Vlieg, J.E.T., Leemhuis, H., Jeffrey, H., Spelberg, L. & Janssen, D.B. (2000) Characterization of the gene cluster involved in isoprene metabolism in *Rhodococcus* sp. strain AD45. *Journal of Bacteriology*, 182, 1956–1963.
Wagner, W.P., Helmig, D. & Fall, R. (2000) Isoprene biosynthesis in *Bacillus subtilis* via the methylerythritol phosphate pathway. *Journal of Natural Products*, 63, 37–40.
Watson, W.P., Cottrell, L., Zhang, D. & Golding, B.T. (2001) Metabolism and molecular toxicology of isoprene. *Chemico-Biological Interactions*, 135–136, 223–238.
Wright, C.L., Schatteman, A., Crombie, A.T., Murrell, J.C. & Lehtovirta-Morley, L.E. (2020) Inhibition of ammonia monooxygenase from ammonia-oxidizing archaea by linear and aromatic alkynes. *Applied and Environmental Microbiology*, 86, e02388–e02319.
Yeager, C.M., Bottomley, P.J., Arp, D.J. & Hyman, M.R. (1999) Inactivation of toluene 2-monooxygenase in *Burkholderia cepacia* G4 by alkynes. *Applied and Environmental Microbiology*, 65, 632–639.
Yu, J., Moon, S.-K., Kim, Y.-H. & Min, J. (2022) Isoprene production by *Rhodobacter sphaeroides* and its antimicrobial activity. *Research in Microbiology*, 173, 103938.
Zhao, L., Chang, W.W., Xiao, Y., Liu, H.H. & Liu, P. (2013) Methylerythritol phosphate pathway of isoprenoid biosynthesis. *Annual Review of Biochemistry*, 82, 497–530.

14622920, 0, Downloaded from https://ami-journals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.16325 by Sheffield Hallam University, Wiley Online Library on [05/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Zuo, Z. (2019) Why algae release volatile organic compounds—the emission and roles. *Frontiers in Microbiology*, 10, 491. Available from: https://doi.org/10.3389/fmicb.2019.00491

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Dawson, R.A., Crombie, A.T., Jansen, R.S., Smith, T.J., Nichol, T. & Murrell, C. (2023) Peering down the sink: A review of isoprene metabolism by bacteria. *Environmental Microbiology*, 1–14. Available from: https://doi.org/10.1111/1462-2920.16325

14622920, 0, Downloaded from https://ami-journals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.16325 by Sheffield Hallam University, Wiley Online Library on [05/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License