Microbial Cycling of Methanethiol

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Abstract

Methanethiol (MT) is an organic sulfur compound with a strong and disagreeable odour. It has biogeochemical relevance as an important compound in the global sulfur cycle, where it is produced as a reactive intermediate in a number of different pathways for synthesis and degradation of other globally significant sulfur compounds such as dimethylsulfoniopropionate, dimethylsulfide and methionine. With its low odour threshold and unpleasant smell, MT can be a significant cause of malodour originating from animal husbandry, composting, landfill operations, and wastewater treatment and is also associated with faeces, flatus and oral malodour (halitosis). A diverse range of microorganisms drives the production and degradation of MT, including its aerobic and anaerobic metabolism. MT producing and degrading organisms are known to be present in terrestrial, freshwater and marine environments but may also be important in association with plant and animal (including human) hosts. This chapter considers the role of MT as an intermediate of the global sulfur cycle and discusses current knowledge of microbial pathways of MT production and degradation.

Introduction

Methanethiol (MT), also known as methylmercaptan, is a one-carbon organic sulfur compound with the formula CH_3SH. With a boiling point of 6°C it is a colourless gas at room temperature characterized by a pungent and disagreeable odour that has been likened to rotten cabbage. As a product of decaying biomass, it can be found widely in the environment, for instance as a compound emitted from rotting fruit and vegetables but it is also found in association with humans and animals where it is a component of the smell of faeces, flatus and can be associated with bad breath (halitosis) (NCBI Pubchem database, n.d.). The human sense of smell has a low threshold for detection of MT at 1–2 ppb (Devos *et al*., 1990), which is exploited in its use as an additive to natural gas distribution systems in order to facilitate the detection of leaks. A leak of an unspecified quantity of MT from a chemical factory in northern France in 2013 illustrated the potential of MT as a significant malodour. A bad smell was reported in parts of northern France and as far away as across the Channel in parts of Southern England, due to the chemical having been dispersed by the wind (Reuters, 2013).

Environmental concentrations, production and degradation of MT

Environmental concentrations of MT

Only relatively few studies have measured MT concentrations in seawater, anoxic environments and industrial settings. A detailed study of production of volatile sulfur compounds in anoxic freshwater sediments in a peat bog by Lomans *et al*. (1997) showed that MT was one of the dominant volatile sulfur compounds in anoxic sediments. MT concentrations ranged from 3–76 nM and its production pathway was biological as shown by heat

killed controls. Accumulation of MT in sediment slurries to which bromoethanesulfonate had been added, suggested that methanogens were mainly responsible for MT removal in these freshwater sediments. Surface freshwater MT concentrations in the peat bog were 1–8 nM and in a similar range to those of H_2S, DMS and CS_2, demonstrating that MT contributes to emission of S from such freshwater environments (Lomans *et al.*, 1997).

In marine environments, MT concentrations have been reported to be in the range of 0.02–2 nM. In a study primarily reporting carbonyl sulfide (COS) emissions from the Aegean Sea, MT concentrations detected at time zero in incubation experiments with natural seawater samples were in a range of 50–500 pM and it was suggested that MT was subject to photodegradation and could potentially be a precursor for COS photoproduction (Ulshöfer *et al.*, 1996). Measurements of MT were also reported for a sample transect of the Atlantic by Kettle and colleagues, who reported average MT concentrations in surface seawater of 420 pM, with localized higher concentrations up to ≈1700 pM in the North African upwelling area, around ≈1500 pM in a coastal region close to Montevideo and up to ≈1000 pM on a transect between Montevideo and the Falkland Islands (Kettle *et al.*, 2001). In addition to above mentioned photodegradation, the reaction of MT with sulfate, dissolved organic matter (DOM) and trace metals to form sulfate–DOM–metal complexes has also been suggested as an abiotic degradation pathway (Kiene *et al.*, 2000).

Biological Production of MT

Biological MT production is well known as an intermediate in metabolism of dimethylsulfoniopropionate (DMSP) (Kiene and Taylor, 1988; Kiene *et al.*, 2000; Reisch *et al.*, 2011a,b) and DMS (De Bont *et al.*, 1981; Suylen and Kuenen, 1986; Pol *et al.*, 1994; Borodina *et al.*, 2000; Schäfer, 2007) which itself can be produced through microbial degradation of DMSP (Curson *et al.*, 2011b). In marine, estuarine and salt marsh environments, DMSP, an abundant metabolite released by phytoplankton and macroalgae, acts as a key precursor of dimethylsulfide (DMS). Degradation of dissolved DMSP releases DMS by the activity of DMSP-lyases (Johnston *et al.*, 2008), which are found in many aerobic and anaerobic organisms (Todd *et al.*, 2009; Todd *et al.*, 2011; Curson *et al.*, 2011a,b, 2012; Sun *et al.*, 2016). Once produced, DMS is degraded to MT by a DMS monooxygenase, which was reported in some strains of *Hyphomicrobium* and *Arthrobacter* (De Bont *et al.*, 1981; Borodina *et al.*, 2000; Boden *et al.*, 2011).

MT production also occurs as an intermediate of microbial DMSP degradation via the demethylation pathway. In a study of the sulfur metabolism of several isolates from the abundant marine roseobacter clade, Gonzales and colleagues noted the ability of several strains to produce MT from DMSP and other precursors including DMS, dimethyl sulfoxide (DMSO), 3-methylmercaptopropionate (MMPA) and α-ketomethiol-butyrate (González *et al.*, 1999). Subsequent characterization of the metabolic pathway producing MT from DMSP showed that, in this pathway, DMSP is initially demethylated to MMPA by DMSP demethylase encoded by *dmdA,* which was first discovered in the marine roseobacter *Ruegeria pomeroyi* DSS-3 (Howard *et al.*, 2006). Subsequently, Reisch *et al.* (2011) demonstrated that after the demethylation step, MMPA is catabolized first to methylthioacryloyl-CoA (MTA-CoA) and then to MT via the demethiolation pathway (Reisch *et al.*, 2011b). The genes designated as *dmdB, dmdC* and *dmdD* were shown to encode the enzymes catalysing the transformation from MMPA to MT in *R. pomeroyi*. The presence of these genes (with the exception of *dmdD*) in the genome of ubiquitous marine bacterium *Pelagibacter ubique, Pelagibacter* HTCC1062 and *Ruegeria lacuscaerulensis* as well as in marine metagenomes reiterate the significance of this pathway in the marine environment (Reisch *et al.*, 2011b; Sun *et al.*, 2016). Sun and colleagues also demonstrated that eight *Pelagibacterales* genomes contain homologues of the *dmdABC* genes, but not *dmdD*, but *Pelagibacter* is still able to produce MT from DMSP. This suggests that the MT formation from MMPA is widespread in marine ecosystems, however a novel enzyme may be catalysing the last step of MT production from DMSP in some marine bacteria.

It was also noted that several aerobic bacteria from the genera *Corynebacterium, Rhizobium, Flavobacterium, Erwinia, Aeromonas, Pseudomonas* and *Yersinia* isolated from soil, sediment and marine algae cultures have the capacity to methylate hydrogen sulfide to produce MT. The activity of a thiol

S-methyltransferases was demonstrated in distinct fractions of crude cell free extracts of *Pseudomonas fluorescens* PF4 subjected to gel-filtration and ion-exchange chromatography; S-adenosylmethionine was identified as a methyl donor (Drotar *et al.*, 1987). The authors speculated that further methylation of the product MT to DMS would be a possibility and might be carried out by the same enzyme, but they did not observe DMS production in their activity assays, noting that this would depend on the K_m of the second methyl transfer reaction (Drotar *et al.*, 1987).

Anaerobic mechanisms

In anaerobic soils and sediments, MT is primarily produced via the degradation of sulfur containing amino acids and methylation of sulfide (Lomans *et al.*, 2002). Degradation of methionine was shown to lead to formation of MT in anoxic lake sediments (Zinder and Brock, 1978). Similarly, in anoxic salt marsh sediments, addition of methionine and S-methyl cysteine led to production of MT, less MT production was noted from DMSP (Kiene and Capone, 1988). The methyl-thiol group of methionine is cleaved (demethiolation) via the methionine-gamma-lyase enzyme (MegL), which has been purified and characterised from various bacteria such as *Pseudomonas*, *Brevibacter* and *Trichomonas* species (Bentley and Chasteen, 2004). Another route to anaerobic MT production is by the activity of thiol S-methyltransferases, which transfer methyl groups from S-adenosylmethionine to sulfide resulting in MT formation or methylate MT to form DMS (Bentley and Chasteen, 2004).

Methoxylated aromatic compounds are another precursor from which MT is produced in soil and sediments. Bak and colleagues (1992) isolated two anaerobic homoacetogenic species from the genus *Pelobacter* that produce MT during growth on trimethoxybenzoate or syringate by transferring the methyl group of the aromatic ring to hydrogen sulfide via thiol S-methyltransferase (Bak *et al.*, 1992). Several anaerobic isolates have been described including members of the genera *Holophaga*, *Sporobacter*, *Sporobacterium* and *Parasporobacterium* that can methylate sulfide to MT during the degradation of aromatic methoxylated compounds (Kreft and Schink, 1993; Grech-Mora *et al.*, 1996; Mechichi *et al.*, 1999; Lomans *et al.*, 2001). This process is suggested to take place at aerobic/anaerobic interfaces of organic rich freshwater sediments as methoxylated aromatic compounds are produced during the degradation of lignin, an abundant biopolymer (Lomans *et al.*, 2002).

Microbial degradation of MT

The main sink for MT in the environment is its degradation by microorganisms. Owing to the toxicity and foul odour of MT, only few studies have actually attempted to enrich and grow microorganisms on MT as a sole source of carbon and energy source. Therefore, most of what is known about microbial MT degradation is based on isolates in which MT is degraded as an intermediate of the metabolism of other organic sulfur compounds.

Aerobic mechanisms

Aerobic degradation of MT was shown in *Hyphomicrobium* sp. S, which was obtained from soil using DMSO as the enrichment substrate (De Bont *et al.*, 1981). Following this, a wide range of aerobic bacteria that degrade MT have been isolated from several environments including soil, peat biofilter, lake and marine sediments, seawater and marine algal cultures. These methylotrophic and sulfur-oxidizing species were affiliated with the genera *Hyphomicrobium*, *Thiobacillus, Rhodococcus* and *Methylophaga* (De Bont *et al.*, 1981; Suylen *et al.*, 1987; Cho *et al.*, 1991; Gould and Kanagawa, 1992; Visscher and Taylor, 1993a,b; Pol *et al.*, 1994; Borodina *et al.*, 2000; Schäfer, 2007; Boden *et al.*, 2010). Recently, *Methylotenera mobilis* JLW8 was shown to degrade MT as the sole carbon and energy source (Carrión *et al.*, 2017).

Aerobic bacteria degrade MT by methanethiol oxidase (MTO). This enzyme has been purified from *Hyphomicrobium* and *Thiobacillus* species and shown to degrade MT to formaldehyde, hydrogen sulfide and hydrogen peroxide (Suylen *et al.*, 1987; Gould and Kanagawa, 1992). Recently, the MTO enzyme in *Hyphomicrobium* sp. VS has been characterised in more detail, showing that it requires Cu for activity and suggesting presence of a tryptophan-tryptophylquinone (TTQ) co-factor. The identification of the encoding gene revealed that MTO it is a homologue of the so-called selenium-binding protein family whose function had previously been poorly constrained (Eyice *et al.*,

2018). However, identification and characterisation of the human form of selenium-binding protein SELENBP1 by Pol and colleagues demonstrated that the human homologue is also a methanethiol oxidase and that a genetic defect in this gene is the underlying cause of extra-oral, or blood-borne, halitosis (Pol *et al.*, 2018).

Genes encoding MTO are present in genomes of a wide range of microorganisms known to degrade DMS (e.g. *Hyphomicrobium* and *Thiobacillus* spp.), DMSP (*Ruegeria pomeroyi* and other roseobacter clade bacteria), and indeed a number of methanotrophic and methylotrophic bacteria. Detection of *mtoX* in metagenomes as well as the application of specific PCR primers for *mtoX* demonstrated that *mtoX* and thus MT-degrading bacteria are present in a wide range of marine and terrestrial environments. Application of the stable isotope probing method with ^{13}C-labelled DMS has also been used indirectly to identify active MT-degraders in soil and lake sediment samples (Eyice *et al.*, 2018). MT oxidases with different molecular weights to that found in *Hyphomicrobium* sp. VS have been reported in *Rhodococcus* and *Thiobacillus* strains, suggesting that other methanethiol oxidases may yet have to be characterized in detail at the biochemical and genetic level (Kim *et al.*, 2000; Lee *et al.*, 2002).

An alternative sink for MT removal in aerobic environments is MT-dependent DMS production. A recent study demonstrated that MT can be methylated to DMS in aerobic bacteria through the activity of a membrane bound methyltransferase encoded by the gene *mddA* (Carrion *et al.*, 2015). The *mddA* gene was found widely distributed in phylogenetically diverse bacteria and several isolates tested showed that the presence of the *mddA* gene correlated with the ability to form DMS from MT. Based on metagenomic datasets, it was estimated that the *mddA* gene may be present in 5–76% of soil bacteria (Carrión *et al.*, 2015). In a subsequent study, Carrion and colleagues showed that although only a small proportion of MT (≈0.1%) added to a grassland soil was converted to DMS via this pathway, the soil microbial community contained a phylogenetically diverse group of bacteria, mainly *Pseudomonas, Acinetobacter, Gemmobacter, Phyllobacterium, Rhizobium, Ensifer* and *Sinorhizobium* that encoded *mddA*. (Carrión *et al.*, 2017). It is notable that the gene encoding the MddA enzyme can be found abundantly in metagenomes, particularly in soils. The relatively low conversion of MT to DMS observed in a grassland soil in the study could be due to competing pathways of MT and DMS removal and the environmental significance of this pathway requires additional analysis (Carrión *et al.*, 2017).

Anaerobic mechanisms

DMS and MT degradation by microorganisms has been studied in several ecosystems, yet, our knowledge on microbial populations that degrade MT in anoxic environments is very limited.

MT is primarily used by methane-producing archaea (methanogens) and sulfate-reducing bacteria (SRB) in anoxic marine, freshwater and terrestrial ecosystems (Fig. 9.1; Zinder and Brock, 1978; Lomans *et al.*, 1999b). The first study that showed that MT is degraded to methane and carbon dioxide was carried out on samples from Lake Mendota (Wisconsin, USA) using radiolabelled ^{14}C-methyl-methionine (Zinder and Brock, 1978). However, pure methanogenic species that

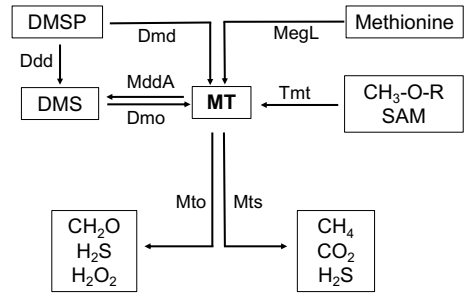

Figure 9.1 Simplified MT cycle and the key synthesis/degradation enzymes/pathways identified. Main MT sources include degradation of DMSP by the demethylation/demethiolation pathway (Dmd), degradation of methionine by methionine-gamma-lyase (MegL) or methyl transfer to sulfide by thiol methyltransferases (Tmt) from methoxylated aromatic compounds (CH_3-O-R) or S-adenosylmethionine (SAM). Cleavage of DMSP by DMSP-lyases (Ddd) produces DMS which can be oxidized to MT by DMS monooxygenase (Dmo). Sinks include methanethiol oxidase (Mto) which degrades MT to formaldehyde (CH_2O), hydrogen sulfide (H_2S) and hydrogen peroxide (H_2O_2), methylation of MT to DMS by methyltransferase (Mdd), and degradation in methanogens to methane (CH_4), carbon dioxide (CO_2) and hydrogen sulfide (H_2S) via activity of Mts methyltransferases.

could utilize MT as the carbon and energy source were not isolated from the samples. Later, Kiene *et al.* (1986) demonstrated that methane is produced in sediment samples from a variety of habitats including freshwater, alkaline and hypersaline lakes as well as estuarine salt marshes. They also obtained, from an estuarine salt marsh sediment, the first methanogenic strain, which was capable of metabolizing MT as the sole source of carbon and energy, yet did not identify this strain (Kiene *et al.*, 1986). Following this, Ni and Boone (1991) identified the first pure methanogenic strain (*Methanolobus siciliae*) from oilfield water samples using DMS as the substrate (Ni and Boone, 1991). The cultures were subsequently shown to use MT as the catabolic substrate (Ni and Boone, 1993; Table 9.1). Another methanogenic strain, *Methanosarcina* sp. MTP4, was isolated from marine sediment using MT as the sole carbon and energy source (Finster *et al.*, 1992; Table 9.1). The biochemical and genetic basis of methanethiol-dependent methanogenesis of *Methanosarcina* spp. has recently been identified. Three fused methyltransferase-corrinoid enzymes (MtsD, MtsF and MtsH) were shown by mutational analysis to be involved in formation of DMS as a metabolic intermediate during carboxidotrophic growth of *Methanosarcina acetivorans*. Further characterization indicated that these were also required for methylotrophic growth of *M. acetivorans* on DMS (Oelgeschläger and Rother, 2009). Work by Fu and Metcalf (2015) further showed that MtsF, which was highly up-regulated during growth on MT, and MtsH were capable of transferring the methyl group from MT to coenzyme M, the latter also seemed to accept DMS as a substrate (Fu and Metcalf, 2015). Other methanogens that can grow on methylated sulfur compounds have been described which appear to be obligately methylotrophic. *Methanomethylovorans hollandica* DMS1 is a MT-degrading methanogen that has been isolated from the sediment of a eutrophic freshwater pond in Nijmegen, The Netherlands, using a chemostat, which enabled high DMS degradation rates by removing inhibitory metabolites (i.e. hydrogen sulfide) (Lomans *et al.*, 1999a). A closely related strain, *Methanomethylovorans uponensis*, was obtained from a wetland sediment and shared the ability to grow using methylated sulfur compounds MT and DMS (Cha *et al.*, 2013). Methanogens related to *Methanomethylovorans hollandica* as well as *Methanolobus* were also present in a lab-scale bioreactor able degrade 6mM MT in the inflowing

Table 9.1 Anaerobic archaea and bacteria that can utilize MT as a carbon and energy source

Isolate	Isolation source	Reference
Archaea		
Methanolobus siciliae HI350	Oil field water	Ni and Boone (1991)
Methanolobus bombayensis B-1[a]	Marine sediment	Kadam *et al*. (1994)
Methanolobus taylorii GS-16	Estuarine sediment	Oremland and Boone (1994)
Methanolobus sp. strain SODA	Bioreactor treating MT at pH 10	van Leerdam *et al*. (2008a)
Methanolobus sp. strain WR1	Bioreactor treating MT at pH ≥ 8	van Leerdam *et al*. (2008b)
Methanosarcina sp. MTP4	Marine sediment	Finster *et al*. (1992)
Methanosarcina semesiae MD1	Mangrove sediment	Lyimo *et al*. (2000)
Methanomethylovorans hollandica DMS1	Lake sediment	Lomans *et al*. (1999a)
Methanohalophilus zhilinae WeN5T[a]	Alkaline lake sediment	Mathrani *et al*. (1988)
Methanohalophilus oregonense WAL1[a]	Alkaline saline aquifer	Liu *et al*. (1990)
Bacteria		
Desulfotomaculum sp. MTS5	Anaerobic fermentor	Tanimoto and Bak (1994)
Desulfotomaculum sp. SDN4	Anaerobic fermentor	Tanimoto and Bak (1994)
Desulfosarcina sp. SD1	Mangrove sediment	Lyimo *et al*. (2009)
Thiobacillus sp. ASN1	Salt marsh	Visscher and Taylor (1993b)

[a]These strains were isolated using DMS as the carbon and energy source but not tested directly for their ability to grow on MT.

medium which had been inoculated with sludge from a paper pulp mill wastewater treatment plant (de Bok *et al*., 2006). Further *Methanolobus* isolates (strains WR1 and SODA) were obtained by van Leerdam and colleagues from bioreactors treating MT under alkaline conditions at pH of 8 and above and pH10, respectively, and were shown to grow on MT as sole carbon source (van Leerdam *et al*., 2008a,b). Additionally, a number of methanogens from the genera *Methanosarcina*, *Methanohalophilus* and *Methanolobus*, which can transform DMS to methane were isolated from anoxic environments including alkaline lake and marine sediments (Table 9.1). Some of these were not tested for their growth on MT directly; however, it appears likely that because of MT being an intermediate of DMS metabolism in other methanogens, that these species can also catabolize MT.

In high-sulfate ecosystems such as marine and salt marsh sediments, SRB degrade MT to hydrogen sulfide and carbon dioxide using sulfate as the final electron acceptor (Kiene and Visscher, 1987; Kiene and Capone, 1988; Tanimoto and Bak, 1994). A relatively small number of SRB that grow on MT and sulfate have been isolated so far. These belong to the genera *Desulfotomaculum* and *Desulfosarcina*, which were obtained from laboratory scale methanogenic fermenters (Tanimoto and Bak, 1994) and mangrove sediments (Lyimo *et al*., 2009).

A number of studies have been carried out to understand the interaction between methanogens and SRB using 2-bromoethanesulfonic acid (BES) and molybdate as specific inhibitors of methanogenesis and sulfate reduction, respectively (Kiene *et al*., 1986; Lomans *et al*., 1997). MT was reported to be a non-competitive substrate for methanogens although competition between methanogens and SRB was observed for DMS at low substrate concentrations (< 10 μM DMS) (Kiene *et al*., 1986). This microbial interaction may have significant impact on the fate of MT in the environment, particularly in marine sediments which have high sulfate concentrations.

In addition to sulfate, nitrate is also used as an electron acceptor by MT-degrading microorganisms. One example for this metabolism is *Desulfotomaculum* sp. SDN4 that was isolated from a methanogenic thermophilic fermentor and which was able to use nitrate as terminal electron acceptor (Tanimoto and Bak, 1994). DMS-grown cells of *Thiobacillus* ASN-1, which was isolated from a *Spartina*-dominated salt marsh, were also shown to metabolize MT with nitrate and nitrite as electron acceptor (Taylor and Visscher, 1993b).

In a study that used cultivation-independent methods to identify methanogen and SRB populations degrading MT (and DMS) in the environment, Lomans and colleagues found methanogens closely related to *Methanomethylovorans hollandica* to be the dominant MT-degraders in freshwater sediments (Lomans *et al*, 2001). This suggests that *M. hollandica* might be a major player in the MT cycle in freshwater habitats. To our knowledge, there are as yet no studies focusing on the characterization of anaerobic MT-degrading populations using post-genomic approaches, which limits our understanding of the identity and distribution of MT-degrading microorganisms in anoxic environments.

Conclusions

The biogeochemical cycling of methylated sulfur compounds is brought about by a wide range of interconnected and interacting metabolic pathways and microorganisms. The intense study of the metabolism of DMSP and DMS during recent years has brought the role of MT as an intermediate of their microbial degradation into focus. MT is relevant as a malodourous compound in a range of industries but also in a medical context, therefore a more detailed understanding of the biochemistry, genetics and ecology of MT degrading microorganisms has considerable benefit to aid in the exploitation of the properties of microorganisms for removal of MT, and even to help understand the role of organic sulfur metabolism in plants, animals and humans. The identification of several key genes that encode enzymes of MT metabolism facilitates a more holistic analysis of the role of diverse bacteria and archaea using metagenomics and related approaches in the future and will lead to a better understanding of MT cycling in the environment. At the same time, advances in uncovering the role of MT metabolism in human disease may only represent the beginning of a better understanding how sulfur metabolism affects human health, providing an important scope to explore both host and microbiome associated pathways of MT production and degradation in more detail.

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