A novel pathway producing dimethylsulphide in bacteria is widespread in soil environments

O. Carrió\n1, A.R.J. Curson2, D. Kumaresan3, Y. Fu4, A.S. Lang4, E. Mercadé1 & J.D. Todd2

The volatile compound dimethylsulphide (DMS) is important in climate regulation, the sulphur cycle and signalling to higher organisms. Microbial catabolism of the marine osmolyte dimethylsulphonopropionate (DMSP) is thought to be the major biological process generating DMS. Here we report the discovery and characterization of the first gene for DMSP-independent DMS production in any bacterium. This gene, \textit{mddA}, encodes a methyltransferase that methylates methanethiol and generates DMS. MddA functions in many taxonomically diverse bacteria including sediment-dwelling pseudomonads, nitrogen-fixing bradyrhizobia and cyanobacteria, and mycobacteria including the pathogen \textit{Mycobacterium tuberculosis}. The \textit{mddA} gene is present in metagenomes from varied environments, being particularly abundant in soil environments, where it is predicted to occur in up to 76% of bacteria. This novel pathway may significantly contribute to global DMS emissions, especially in terrestrial environments and could represent a shift from the notion that DMSP is the only significant precursor of DMS.
globally, microbes, and particularly bacteria in the oceans and their margins, drive the production of dimethylsulphide (DMS), generating $\sim 3 \times 10^{11}$ g of this gas per annum through biotransformations of organosulphur molecules. Much of the DMS produced is either catabolized by bacteria or photochemically oxidized. However, $\sim 10%$ escapes into the atmosphere making it the most abundant biogenically derived form of sulphur transferred from the sea to the air. DMS oxidation products can act as cloud condensation nuclei, aiding cloud formation and affecting atmospheric chemistry. This in turn can influence climate regulation, although its significance has recently been questioned. DMS is also a signalling molecule used by some seabirds, crustaceans and marine mammals as a foraging cue.

Marine DMS is produced predominantly as a result of microbial catabolism of the algal osmolyte dimethylsulphoniopropionate (DMSP) through the action of DMSP lyase enzymes. However, there are microbial DMS-independent pathways generating DMS that are not limited to marine environments, such as the enzymatic degradation of S-methylmethionine, dimethyl sulfoxide (DMSO) or methoxyaromatic compounds. Also, many varied anoxic environments produce DMS at levels (for example, 1–44 nM) that can be in excess of those described for the upper marine water column, possibly through microbial methylation of methanethiol (MeSH) via sulphide and/or methionine (Met) catabolism. These environments include freshwater lake sediments, saltmarsh sediments, cyanobacterial mats and (Met) degraded sulphur transfer from the sea to the air. DMS production pathway.

**Results**

**DMS production by *Pseudomonas deceptionensis M1*.** We noted that *Pseudomonas deceptionensis M1*, a new species isolated from Antarctic marine sediments, produced DMS from minimal media containing magnesium sulphate as the only sulphur source (Table 1). *P. deceptionensis M1* does not synthesize DMS, nor does it catabolize DMS with its genome lacking any known genes for DMS catabolism. Therefore, *P. deceptionensis M1* has a novel DMS-independent DMS production pathway.

In *P. deceptionensis M1*, Met and MeSH are precursors for DMS since both these molecules significantly enhanced (four- to sevenfold) DMS production, with MeSH also being produced from Met (Table 1). The *P. deceptionensis M1* genome contains a homologous of the megl gene, whose Met gamma lyase product (EC4.4.1.11) would be predicted to cleave Met to MeSH. A *P. deceptionensis M1* megl mutant strain (J565) was constructed and found to no longer make MeSH or DMS from either minimal medium or medium supplemented with Met (Table 1). However, the megl mutant strain did make DMS when exogenous MeSH was added, supporting the pathway proposed in Fig. 1, namely that the direct precursor for DMS was MeSH, this reaction being mediated via an enzyme activity that we termed Mdd, for MeSH-dependent DMS.

**Identification of the mddA gene.** To identify the mddA gene(s) responsible for the conversion of MeSH to DMS, a genomic library of *P. deceptionensis M1* was constructed in the wide-host-range cosmid pLAFR3. This library was screened in the heterologous host *Rhizobium leguminosarum* and a clone, pBIO2219, was isolated that conferred the ability to produce DMS from MeSH at levels similar to that of *P. deceptionensis M1* itself (Table 1). Using transposon mutagenesis, a Tn5lac insertion in pBIO2219 was identified that abolished the Mdd $^{+}$ phenotype, and was mapped to a gene termed mddA. When cloned alone and expressed under the control of the T7 promoter in *Escherichia coli*, mddA conferred MeSH-dependent DMS production (Table 1). Furthermore, an insertional mutation in mddA of *P. deceptionensis M1* (strain J566) completely abolished DMS production, without affecting the ability to produce MeSH from Met (Table 1).

**Table 1 | The DMS and MeSH produced by key microbial strains used in this study.**

| Strain | Medium additive | pmol DMS min$^{-1}$ per mg protein | pmol MeSH min$^{-1}$ per mg protein |
|--------|----------------|-----------------------------------|-----------------------------------|
| *Pseudomonas deceptionensis M1* wild type | None | 7.65 ± 0.56 | ND |
| | DMSP | 6.99 ± 1.42 | ND |
| | Methionine | 55.22 ± 5.00 | 1,433.47 ± 3,26.01 |
| | MeSH | 34.42 ± 5.13 | ND |
| *P. deceptionensis M1* megl mutant (J565) | None | ND | ND |
| | Methionine | ND | ND |
| *P. deceptionensis M1* mddA mutant (J566) | None | ND | 4,926.16 ± 685.68 |
| | Methionine | ND | ND |
| *Rhizobium leguminosarum* J391 pBIO2219 (P. deceptionensis M1 mddA region cloned) | MeSH | 57.72 ± 2.65 | ND |
| *Rhizobium leguminosarum* J391 pBIO2220 (pBIO2219 mutated in mddA) | MeSH | ND | ND |
| *Escherichia coli* BL21 pBIO2223 (mddA of P. deceptionensis M1 cloned) | MeSH | 414.07 ± 88.06 | ND |
| *Bradyrhizobium diazoefficiens* USDA 110 | None | 385.00 ± 38.17 | ND |
| | MeSH | 596.79 ± 32.82 | ND |
| *Cyanobacteria* sp. ATCC 51142 | None | 5.69 ± 0.23 | ND |
| | MeSH | 7.53 ± 0.07 | ND |

DMS, dimethylsulphide; DMSP, dimethylsulphoniopropionate; MeSH, methanethiol; ND, not detected.

Strains were grown in the appropriate minimal media (see Methods), with and without the DMS precursors DMSP, Met and MeSH. The values for DMS and MeSH production are the averages of three biological replicates with the s.d. being shown.
DMS and MeSH production from H$_2$S. Given that it has been shown previously that MeSH is produced from H$_2$S, both *P. deceptionensis* M1$^1$ and *E. coli* expressing *mddA* were tested for MeSH and DMS production in the presence of H$_2$S, but neither produced increased levels of these gases when compared to controls without the addition of H$_2$S. This suggests that H$_2$S is not a significant contributor to DMS production through the MddA pathway in *P. deceptionensis* M1$^1$.

MddA is a membrane methyltransferase. The polypeptide sequence of MddA has no significant sequence identity to any characterized enzyme, but has limited similarity to S-isoprenylcysteine methyltransferases (COG2020, Fig. 2), supporting the proposed pathway (Fig. 1), whereby MddA would be a methyltransferase converting MeSH to DMS. Isoprenylcysteine methyltransferases, for example, Ste14p of *Saccharomyces cerevisiae*, which is localized in endoplasmic reticulum membranes, catalyse the S-adenosyl-L-Met (Ado-Met)-dependent carboxyl methylation of proteins$^{16}$. MddA is also predicted to be a membrane protein containing four to six membrane-spanning helices (Supplementary Table 1). With Ste14p, _in vitro_ Mdd activity in cell extracts of both *P. deceptionensis* M1$^1$ and *E. coli* expressing MddA (16,200 ± 2,130 and 1,750 ± 291 pmol DMS min$^{-1}$ per mg protein, respectively) required Ado-Met as methyl donor for MeSH to be converted to DMS. Also, subcellular fractionation of *P. deceptionensis* M1$^1$ showed that Mdd activity was only seen in the membrane fraction (6,170 ± 1,370 pmol DMS min$^{-1}$ per mg protein).

MddA functions in taxonomically diverse bacteria. The MddA polypeptide is highly conserved in specific *Pseudomonas* species (Figs 2 and 3, and Supplementary Table 2). In every case tested, the presence of *mddA* in pseudomonads correlated with the strain producing DMS. Both *Pseudomonas fragi* DSM 3456 and *Pseudomonas* sp. GM41 showed DMS production rates similar to *P. deceptionensis* M1$^1$, which were also enhanced by MeSH (Supplementary Table 3), and cloned *mddA* from *Pseudomonas* sp. GM41 conferred Mdd activity to *E. coli* (Supplementary Table 3). The *mddA* gene in pseudomonads is located between genes that have either not been characterized in any organism or which have no known connection to sulphur metabolism (Supplementary Fig. 1). Of the pseudomonads lacking *mddA*, for example, *Pseudomonas putida* and *Pseudomonas psychrophila* those tested showed no detectable levels of DMS production (Supplementary Table 3).

**Figure 1** | A schematic representation of DMSP-dependent and MeSH-dependent DMS production pathways. Blue arrows signify pathways occurring in *Pseudomonas deceptionensis* M1$^1$, shown as a blue box. The red arrow signifies the DMSP cleavage pathway, present in some bacteria, that is catalysed by DMSP lyase enzymes (Ddd) and generates DMS. The amino acid Methionine (Met) is converted to MeSH by the Met gamma methyltransferase converting MeSH to DMS by the Met gamma methyltransferase (MegL) in *P. deceptionensis* M1$^1$. The MddA enzyme methylates MeSH to DMS using Ado-Met as the methyl donor.

**Figure 2** | ClustalW alignment of representative bacterial MddA polypeptides and the *Saccharomyces cerevisiae* Ste14p polypeptide. Residues that are identical or have similar properties are highlighted in red, orange or yellow if they are conserved in all nine, at least seven or at least five polypeptides, respectively. Residues with similar properties that are conserved in all eight bacterial MddA sequences are marked with asterisks below. The position of the TnSlacZ insertions in the *Pseudomonas deceptionensis* M1$^1$ mddA mutant JS66 is marked with an ‘X’ below. Species names with the MddA polypeptide accession codes shown in brackets are: *Cyanothecaceae* sp. ATCC 51142 (YP_001803274), *Mycobacterium tuberculosis* H37Rv (NP_217755), *Bradyrhizobium diazoefficiens* USDA 110 (Blr1218; NP_767858.1), *Bradyrhizobium diazoefficiens* USDA 110 (Blr1218; NP_772381.1), *Pseudomonas* sp. GM41 (WP_008148420), *Pseudomonas deceptionensis* M1$^1$ (AJE75769), *Maricaulis maris* M510 (YP_757811.1), *Sulfurovum* sp. NBC37-1 (YP_001358232). Species names marked with a hash indicate those MddA sequences whose activity has been ratified in *Escherichia coli*. The *Saccharomyces cerevisiae* Ste14p accession is AAA156801.

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Importantly, MddA-like proteins are also present in a wide range of other bacterial taxa, including widely studied and important groups, and many which had not previously been suspected to make DMS. Proteins sharing \( \sim 50\% \) amino acid sequence identity to *P. deceptivora* M1\(^1\) MddA are present in multiple species of the actinobacteria *Gordonia*, *Rhodococcus* and *Mycobacterium*, including the pathogens *M. tuberculosis* and *M. avium*, Rhizobiales members including *N2*-fixing *Mesorhizobium* and *Bradyrhizobium* (two distinct forms, \( \sim 50\% \) identical to each other, exist in many, for example, *B. diaevoeficiens* USDA 110 Blr1218 and Blr5741) and *N2*-fixing cyanobacteria, including *Cyanathothece*, *Pseudanabaena* and *Nodosilinaea* species (Figs 2 and 3, and Supplementary Table 2). MddA-like proteins also exist, although less frequently, in some Planctomycetes, Flavobacteria, Spirochaetes, Verrucomicrobia and Alpha-, Beta-, Gamma- and Epsilonproteobacteria (Figs 2 and 3, and Supplementary Table 2).

It is known that some mycobacteria that contain *mddA* produce DMS, including *M. bovis BCG*\(^8\), but no soil-dwelling *Bradyrhizobium* or cyanobacteria, have previously been reported to produce DMS. We found that *B. diaevoeficiens* USDA 110 and *Cyanathothece* sp. ATCC 51142 DMS production was enhanced by addition of MeSH (Table 1). Our finding that some cyanobacteria are Mdd\(^{+}\) may explain why DMS production observed in a freshwater lake coincided with cyanobacterial blooms\(^3,13\). The genes encoding the MddA polypeptides from *B. diaevoeficiens* USDA 110 (blr1218 and blr5741, Rhizobiales), *M. tuberculosis* H37Rv (Actinomycetales) and *Cyanathothece* sp. ATCC 51142 (Cyanobacteria), which form distinct clades of the MddA phylogenetic tree (Fig. 3), were cloned and expressed in *E. coli*. In all cases, these genes gave significant Mdd activity, including both copies present in *B. diaevoeficiens* USDA 110 (Supplementary Table 3), perhaps explaining why this widely studied strain produces \( \sim 50\)-fold more DMS than other tested bacteria. We conclude that the *mddA* gene is widespread in taxonomically diverse bacteria, and that its occurrence in a bacterium is indicative of the capacity to generate DMS from MeSH.

*mddA* is particularly abundant in soil environments. The environmental abundance of the *mddA* gene was estimated by probing metagenomic data sets from a range of environments (Supplementary Table 4). MddA protein-encoding genes (E \(< e\^{-50}\) ) were represented in most metagenomes, but strikingly were much more abundant in terrestrial environments, especially soil metagenomes, where *mddA* was predicted to be present in 5–76% of bacteria (Fig. 4, Supplementary Table 5). Interestingly, *mddA* was much less abundant in marine metagenomes where it only occurred in \( \leq 0.5\% \) of bacteria, in contrast to some *ddd* DMSP lyase genes, whose products produce DMS from DMSP and are much more abundant in marine metagenomes\(^8\) (Fig. 4, Supplementary Table 5). Therefore, unlike previously identified DMS-producing enzymes, MddA is likely more important in the production of DMS from terrestrial rather than marine environments.

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**Figure 3 | Molecular phylogenetic analysis of MddA proteins.** Bacterial strains, their taxonomy and the accession codes of bacterial MddA proteins that have \( >40\% \) amino acid identity to *Pseudomonas deceptivora* M1\(^1\) MddA are shown. Sequences with \( >57\% \) identity to each other that are in bacteria of the same genus are shown with triangles; the size of the triangle reflects the number of sequences. Those cases where the cloned *mddA* genes were shown experimentally to confer MeSH-dependent DMS production to *Escherichia coli* are written in purple. The two MddA proteins of *Bradyrhizobium diaevoeficiens* USDA 110 (Blr1218 and Blr5741) are shown. *MddA* sequences that were aligned in Fig. 2 are in blue boxes. The neighbour-joining tree shown was obtained using the Jones–Taylor–Thornton (JTT) model of amino acid substitution. Bootstrap values \( \geq 50\% \) (based on 1,000 replicates) are shown at branch points. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The scale bar indicates 0.1 substitutions per site.
Potential roles of the MddA pathway. 

Many heterotrophic bacteria, including *P. putida* DS1 (ref. 22), oxidize DMS to DMSO, with some coupling this reaction to adenosine triphosphate synthesis and increased biomass production. 

*P. deceptionensis* M1\(^T\) can also use DMS as a sulphur source, but can use Met and MeSH. Therefore, to study why bacteria containing mddA might make DMS, the transcription of the mddA gene was examined using mddA-lac transcriptional fusions in *P. deceptionensis* M1\(^T\) and available microarray data from *B. diaeossacei* USDA 110 and *M. tuberculosis* H37Rv. In *P. deceptionensis* M1\(^T\), mddA was constitutively expressed (giving ~70 Miller units of β-galactosidase activity) irrespective of the potential inducer molecule or environmental conditions tested (see Methods). From experiments reported for *B. diaeossacei* USDA 110 and *M. tuberculosis* H37Rv, there were no conditions that significantly affected mddA gene expression, with the exception that in *M. tuberculosis* H37Rv the transcription of mddA was 4-fold induced by addition of the anti-tuberculosis drug thioridazine, which contains a methylated sulphur group.

In *Sulfitobacter* sp. EE-36, the DMSP lyase gene dddL is also constitutively expressed and it is proposed that DMS is generated as a signalling molecule. This is a possible explanation for the existence of the Mdd pathway in *P. deceptionensis* M1\(^T\) and other Mdd\(^+\) bacteria, since it is well documented that DMS is a chemoattractant and in some environments may play a major role in structuring bacterial communities.

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containing no sulphur or MgSO4. DMSP, Met, DMSO, DMS or MeSH as the sole sulphur source at 100 μM concentrations. Cultures were incubated at 28°C for 5 days and the growth was estimated by measuring the OD600 of the cultures with a spectrophotometer UV-1800 (Shimadzu).

**General in vivo and in vitro genetic manipulations.** Plasmids were transferred either by conjugation from E. coli to *R. leguminosarum* J391 or to *P. deceptionensis* M1 transconjugants using the method of genome transfer described in the Ion Xpress Plus gDNA Fragmentation Library Preparation manual (Life Technologies Inc.). A 400-bp library was prepared by size separation of 450–500-bp fragments from a 2% agarose gel. The Ion sphere particles were templated using the Ion OneTouch 2 instrument with the Ion PGM Template OT2 400 Kit, and then enriched using the Ion OneTouch ES. Sequencing was performed on an Ion PGM Sequencer with an Illumina 318 chip v2 and the Ion PGM Sequencing 400 Kit. The general features of the resultant genome sequence were determined using Galaxy (<http://usegalaxy.org/>). Ends were trimmed to remove all bases with quality scores less than 30 and all ambiguous bases from each end were trimmed. The remaining sequences were assembled using Lasergene (DNASTAR Inc.) and the default de novo assembly settings. The resulting contig sequences were analysed with Rapid Annotation using Subsystems Technology (RAST)35, Artemis v14.0.0 (http://www.sanger.ac.uk/Software/Artemis) and BioEdit36 v7.1.3, and were searched for genes of interest by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences of the cosmid insert from pBIO2219 and the megl gene have been submitted to the GenBank database (accession numbers KM030271 and KM030270, respectively).

**Sequencing of *P. deceptionensis* M1T genomic DNA.** Generation of a draft genomic sequence of *P. deceptionensis* M1T is in progress. High molecular weight *P. deceptionensis* M1T genomic DNA was extracted using a Qiagen genomic tip 100/G and Qiagen buffer set (Qiagen, Hilden, Germany). The genomic DNA was fragmented using Ion Shear reagents, followed by ligation with Ion Adapters, as described in the Ion Xpress Plus gDNA Fragmentation Library Preparation manual (Life Technologies Inc.). A 400-bp library was prepared by size separation of 450–500-bp fragments from a 2% agarose gel. The Ion sphere particles were templated using the Ion OneTouch 2 instrument with the Ion PGM Template OT2 400 Kit, and then enriched using the Ion OneTouch ES. Sequencing was performed on an Ion PGM Sequencer with an Illumina 318 chip v2 and the Ion PGM Sequencing 400 Kit. The general features of the resultant genome sequence were determined using Galaxy (<https://usegalaxy.org/>). Ends were trimmed to remove all bases with quality scores less than 30 and all ambiguous bases from each end were trimmed. The remaining sequences were assembled using Lasergene (DNASTAR Inc.) and the default de novo assembly settings. The resulting contig sequences were analysed with Rapid Annotation using Subsystems Technology (RAST)35, Artemis v14.0.0 (http://www.sanger.ac.uk/Software/Artemis) and BioEdit36 v7.1.3, and were searched for genes of interest by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences of the cosmid insert from pBIO2219 and the megl gene have been submitted to the GenBank database (accession numbers KM030271 and KM030270, respectively).

**Mutation of the megl gene in *P. deceptionensis* M1T.** For the targeted mutagenesis of the megl gene in *P. deceptionensis* M1T, a 0.5-kb internal PCR fragment of megl (Supplementary Table 9) was cloned into the suicide plasmid pBIO1879 (ref. 37) and transformed into *P. deceptionensis* M1T J564 selecting for recombination on LB medium containing spectinomycin (800 μg ml⁻¹) and kanamycin (20 μg ml⁻¹). Genomic insertion mutations in *P. deceptionensis* M1T J565 were confirmed by PCR.

**Construction of a *P. deceptionensis* M1T genomic library.** *P. deceptionensis* M1T genomic DNA was used to generate a genomic library in the cosmid pLAFR3 containing no sulphur or MgSO₄, DMSP, Met, DMSO, DMS or MeSH as the sole sulphur source at 100 μM concentrations. Cultures were incubated at 28°C for 5 days and the growth was estimated by measuring the OD₆₀₀ of the cultures with a spectrophotometer UV-1800 (Shimadzu).

**Analysis of volatile organic sulphur compounds and DMSP.** *P. deceptionensis* M1T wild type and J566 were grown on tryptone-soya agar (Oxoid) slants in 20 ml gas chromatography headspace vials (Chromlab) for 72 h at 15°C. Volatile organic sulphur compounds and DMSO produced were identified by solid-phase micro-extraction using a carboxen-polydimethylsiloxane fibre (CAR/PDMS, Supelco) coupled to gas chromatography—mass spectrometry (Trace GC Ultra + DSQII, Thermo Scientific) and a capillary column 1.8 μm DB-624 (60 m × 0.32 mm, Agilent Technologies).

**DMSP production and consumption assays.** *P. deceptionensis* M1T was grown in M9 media in the presence and absence of 1 mM DMSP at 28°C for 24 h. The culture was then pelleted and the supernatant was analysed for DMSP versus buffer control samples via the addition of 10 mM NaOH to dilutions in gas-tight 2 ml vials (12 × 32 mm, Altech Associates) and the subsequent liberation of DMSP was quantified by gas chromatography, as below.

**Assays of microbial DMSP and MeSH production.** To measure DMSP and MeSH production in pseudomonads, *R. leguminosarum*, *B. diazoefficiens* and *Cyanothece* sp. ATCC 51142, each strain was first grown overnight (1 week for *Cyanothece* sp. ATCC 51142) in its appropriate minimal medium and, where indicated, 5 mM DMSP, 0.5 mM MeSH, 5 mM Met or 1 mM H₂S. For *Cyanothece* sp. ATCC 51142, 300 μl of cultures were harvested and resuspended in the same volume of fresh medium. Met, 2 ml vials and incubated overnight at 26°C, as above, before being assayed for DMSP and MeSH production. All other bacterial cultures were adjusted to an OD₆₀₀ of 0.3 and the cells were diluted 10-fold into 300 μl of minimal medium supplemented with the additives as indicated above and incubated overnight at 28°C in 2 ml vials before assaying for DMSP, DMS and MeSH production in the headspace of vials which was gas chromatographed, using a flame photometric detector (Agilent 7890A GC fitted with a 7693 autosampler) and a HP-INNOWax 30 m × 0.320 mm column (Agilent Technologies J&W Scientific capillary column). The detection limits for DMSP and MeSH were 0.15 and 4 nmol, respectively. An eight-point calibration curve of DMSP and MeSH standards was used as in *Curson et al.*44 The protein content in the cells were estimated by a Bradford method (BioRad) and rates of MeSH and DMS production were expressed as pmol min⁻¹ mg protein⁻¹.

**Mddl activity assays in *P. deceptionensis* M1T cell lysates.** *P. deceptionensis* M1T was grown in LB media for 24 h at 28°C and 2 ml cell aliquots were pelleted, and resuspended in 500 μl of cold Tris 100 mM, MES 50 mM, acetic acid 50 mM (pH 7) buffer. Cell suspensions were sonicated (5 s × 10 s) using an ultrasonic processor VC50 sonicator (Sonics) and the cell debris was pelleted. Triplicate 50 μl aliquots of the cell lysates were incubated with 1 mM Ado-Met and 1 mM MeSH for 2 h before quantifying the DMS produced in the headspace and protein concentrations, as above.

**Cell fractionation and MddA activity assays.** *P. deceptionensis* M1T was grown in LB media for 24 h at 28°C. After that period, 2 ml aliquots were fractionated into the periplasmic, cytoplasmic and membrane fractions using a PeriPreps Periplastin Kit (Epicentre) as in Curson et al.*44 Triplicate 50 μl of each fraction were incubated with 1 mM Ado-Met and 1 mM MeSH for 2 h before quantifying the DMS produced in the headspace and protein concentrations, as above.

**Assays of MddA activity in *E. coli*.** *E. coli* BL21 strains containing cloned mddA genes were grown at 37°C in 5 ml of LB broth containing ampicillin (100 μg ml⁻¹) to an OD₆₀₀ of 0.8. The cells were diluted 10-fold into 300 μl of M9 media containing 100 μM isopropyl β-D-thiogalactopyranoside and 0.5 mM MeSH or 1 mM H₂S in 2 ml vials. Vials were incubated at 28°C for 18 h and then DMS, MeSH and protein concentrations were assayed as above.

**Cell lysate activity assays.** For cell lysate experiments, *E. coli* BL21 containing cloned mddA genes were grown to an OD₆₀₀ of 0.4 and then induced with 100 μM isopropyl β-D-thiogalactopyranoside and grown for 18 h at 28°C. Cells were pelleted and resuspended in cold Tris 100 mM, MES 50 mM, acetic acid 50 mM buffer (pH 7) and then sonicated (5 s × 10 s) with an ultrasonic processor VC50 sonicator (Sonics). The cleared supernatant was incubated with 1 mM Ado-Met and 1 mM MeSH in vials for 20 min before being analysed for DMS, MeSH and protein content, as above.

**Transcriptional assays of mddA in *P. deceptionensis* M1T.** The sequence upstream of the *P. deceptionensis* M1T mddA gene containing the predicted promoter region was cloned into the lacZ reporter plasmid pBIO1878 (ref. 46; Supplementary Tables 8 and 9). The resulting construct pBIO2232 was transformed into *P. deceptionensis* M1T and transformants were grown overnight under...
varying conditions. Transformants were grown in the presence and absence of the potential inducer molecules 1 mM DMSP, 1 mM Met, 1 mM DMSO, 0.5 mM DMS, 0.5 mM MesH, but also with 1 mM H2O2: 1000 mM paraquat. Transformants were also incubated at 14, 15 and 28 °C, in rich versus minimal media, sulphur-limited (M9 lacking MgSO4); under constant light (∼50 μE m−2 s−1) versus dark incubation; and under aerobic versus microaerobic conditions. Finally, the cultures were assayed for β-galactosidase activities following the protocol described by Wexler et al. 

P. deceptionis M17 wild type and J566 growth curves. Growth curves of P. deceptionis M17 wild type and J566 strains were plotted for differing conditions including temperature (0 and 28 °C), salinity (NaCl 1–6%) and the absence and presence of 2 mM Met or 0.5 mM MesH. Growth was estimated by measuring the OD600 of the cultures at different time points by spectrophotometer UV-1800 (Shimadzu).

Oxidative stress tests. For disk inhibition assays, P. deceptionis M17 wild type and J566 strains were grown in Mueller Hinton Broth (Oxoid) at 15 °C for 24 h and cultures adjusted to an OD600 of 0.5. A swab was soaked in the cultures and spread onto Mueller Hinton Agar (Oxoid) plates. Staphylococcus aureus discs (Whatman) containing 10 μl of 100 mM H2O2 or 25 mM paraquat (Sigma Aldrich) were placed on the top of the plates. After an incubation period of 48 h at 15 °C, zones of growth inhibition were recorded.

Minimal inhibitory concentration assays with P. deceptionis M17 wild type and J566 were performed by the broth microdilution method described by Wiegradt et al. with H2O2 ranging from 0.4 to 100 mM or Paraquat (Sigma Aldrich) ranging from 0.1 to 25 mM. OD600 of the wells was measured with a Synergy HT microplate reader (BioTek) after an incubation of 48 h at 15 °C.

Bioinformatics analysis. The protein sequence of P. deceptionis M17 MddA was analysed for membrane-spanning domains using the bioinformatics search engines detailed in Supplementary Table 1.

The MddA phylogenetic tree was constructed with the MEGA6 Package49 and analysed for conserved domains using GeneDoc51 v2.5.010. H37Rv (NP_217755.1), M1T (AJE75769), P. putida M1T (WP_008148240.1), Cytophaga sp. ATCC 51142 (YP_001358232.1), B. diazoeaicus USDA 110 B18212 (NP_767858.1) and B18274 (NP_772381.1), Mycobacterium tuberculosis H37Rv (NP_217755.1), Maricallis maris MCS10 (YP_757811.1), Sulfovulvum sp. NBC37-1 (YP_001358232.1) and Ste14p from Saccharomyces cerevisiae (AAA16840.1) were aligned with ClustalW using the Bioinformatics analysis engines detailed in Supplementary Table 1.

Radiation resistance tests. Growth inhibition were recorded.

Growth curves of P. deceptionis M17 wild type and J566 strains were plotted for differing conditions including temperature (0 and 28 °C), salinity (NaCl 1–6%) and the absence and presence of 2 mM Met or 0.5 mM MesH. Growth was estimated by measuring the OD600 of the cultures at different time points by spectrophotometer UV-1800 (Shimadzu).

Oxidative stress tests. For disk inhibition assays, P. deceptionis M17 wild type and J566 strains were grown in Mueller Hinton Broth (Oxoid) at 15 °C for 24 h and cultures adjusted to an OD600 of 0.5. A swab was soaked in the cultures and spread onto Mueller Hinton Agar (Oxoid) plates. Staphylococcus aureus discs (Whatman) containing 10 μl of 100 mM H2O2 or 25 mM paraquat (Sigma Aldrich) were placed on the top of the plates. After an incubation period of 48 h at 15 °C, zones of growth inhibition were recorded.

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**Author contributions**

J.D.T. and O.C. designed and performed the experiments, analysed the data and wrote the paper; A.R.J.C. constructed the *Pseudomonas deceptionensis* M1T genomic library and wrote the paper; E.M. designed the experiments; A.S.L. and Y.F. generated a draft sequence of the genome of *Pseudomonas deceptionensis* M1T; D.K. carried out the bioinformatics analysis of metagenomic data sets. All authors reviewed the manuscript before submission.

**Additional information**

**Accession codes:** Sequence data for the cosmid pBIO2219 (accession code KM030271), the *P. deceptionensis* M1T megL gene (accession code KM030270) and the *P. deceptionensis* M1T DddA protein (accession code AJE75769) have been deposited in the NCBI GenBank database.

**Supplementary Information** accompanies this paper at http://www.nature.com/
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**Competing financial interests:** The authors declare no competing financial interests.

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