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

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Abstract

The volatile compound dimethylsulphide (DMS) is important in climate regulation, the sulphur cycle and signalling to higher organisms. Microbial catabolism of the marine osmolyte dimethylsulphoniopropionate (DMSP) is thought to be the major biological process generating DMS. Here we report the discovery and characterisation of the first gene for DMSP-independent DMS production in any bacterium. This gene, *mddA*, encodes a methyltransferase that methylates methanethiol (MeSH) and generates DMS. MddA functions in many taxonomically diverse bacteria including sediment-dwelling pseudomonads, nitrogen-fixing bradyrhizobia and cyanobacteria, and mycobacteria, including the pathogen *Mycobacterium tuberculosis.* The *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.

Introduction

Globally, microbes, and particularly bacteria in the oceans and their margins, drive the production of dimethylsulphide (DMS), generating ~3 x 10^{14} g of this gas *per annum*[1] through biotransformations of organosulphur molecules[2,3]. Much of the DMS produced is either catabolised by bacteria or photochemically oxidised[4]. However, ~10% escapes into the atmosphere, making it the most abundant biogenically derived form of sulphur transferred from the sea to the air[4]. DMS oxidation products can act as cloud condensation nuclei, aiding cloud formation and affecting atmospheric chemistry[2,5]. This in turn can influence climate regulation[5], although its significance has recently been questioned[6]. DMS is also a signalling molecule used by some seabirds, crustaceans and marine mammals as a foraging cue[7].

Marine DMS is produced predominantly as a result of microbial catabolism of the algal osmolyte dimethylsulphoniopropionate (DMSP) through the action of DMSP lyase enzymes[3,8]. However, there are microbial DMSP-independent pathways generating DMS that are not limited to marine environments, such as the enzymatic degradation of S-methyl-methionine, dimethyl sulphoxide (DMSO), or methoxyaromatic compounds[9]. Also, many varied anoxic environments produce DMS at levels (e.g. 1-44 nM)[10] that can be in excess of those described for the upper marine water column[11], possibly through microbial methylation of methanethiol (MeSH)[9,12], a product of hydrogen sulphide (H_2S) methylation, or methionine (Met) catabolism. These environments include freshwater lake sediments[10,13], saltmarsh sediments[14], cyanobacterial mats[15] and peat bogs, the latter of which cover 4 million km^2 worldwide and can emit ~6 µmol DMS m^{-2} day^{-1} [12].

The ability to generate MeSH from sulphide and/or Met under oxic conditions is common in heterotrophic bacteria from many diverse environments[16], but to our knowledge there are no reports of aerobic bacteria generating DMS by MeSH methylation reactions in any environment. Thus, prior to this study, the significant levels of DMS produced by non-marine environments were apportioned solely to anaerobic microbes. Here we identify bacteria producing DMS, independent of DMSP, under aerobic conditions, and characterise the first gene for the production of DMS from MeSH in these bacteria.

Results

DMS production by *Pseudomonas deceptionensis* M1^T

We noted that *Pseudomonas deceptionensis* M1^T, a new species isolated from Antarctic marine sediment[17], produced DMS from minimal media containing magnesium sulphate as the only sulphur source (Table 1). *P. deceptionensis* M1^T does not synthesise DMSP, nor does it catabolise DMSP with its genome lacking any known genes for DMSP catabolism[3,8]. Therefore, *P. deceptionensis* M1^T has a novel DMSP-independent DMS production pathway.

In *P. deceptionensis* M1^T, Met and MeSH are precursors for DMS since both these molecules significantly enhanced (4- to 7-fold) DMS production, with MeSH also being produced from Met (Table 1). The *P. deceptionensis* M1^T genome contains a homologue 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^T *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 *mdd* gene(s) responsible for the conversion of MeSH to DMS, a genomic library of *P. deceptionensis* $M1^T$ 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* $M1^T$ itself (Table 1). Using transposon mutagenesis, a Tn*5lac* 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* (strain J566) completely abolished DMS production, without affecting the ability to produce MeSH from Met (Table 1).

DMS and MeSH production from H_2S

Given that it has been shown previously that MeSH is produced from H_2S[16], both *P. deceptionensis* $M1^T$ and *E. coli* expressing *mddA* were tested for MeSH and DMS production in the presence of H_2S, but neither produced increased levels of these gases when compared to controls without the addition of H_2S. This suggests that H_2S is not a significant contributor to DMS production through the MddA pathway in *P. deceptionensis* $M1^T$.

MddA is a membrane methyltransferase

The polypeptide sequence of MddA has no significant sequence identity to any characterised 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, e.g. Ste14p of *Saccharomyces cerevisiae*, which is localised in endoplasmic reticulum membranes, catalyse the S-adenosyl-L-Met (Ado-Met)-dependent carboxyl methylation of proteins[18]. MddA is also predicted to be a membrane protein containing 4-6 membrane-spanning helices (Supplementary Table 1). As with Ste14p, *in vitro* Mdd activity in cell extracts of both *P. deceptionensis* $M1^T$ and *E. coli* expressing MddA (16200 ± 2130 and 1750 ± 291 pmol DMS min^{-1} mg $protein^{-1}$ respectively) required Ado-Met as methyl donor for MeSH to be converted to DMS. Also, subcellular fractionation of *P. deceptionensis* $M1^T$ showed that Mdd activity was only seen in the membrane fraction (6170 ± 1370 pmol DMS·min^{-1}·mg $protein^{-1}$).

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* $M1^T$, 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 characterised in any organism or which have no known connection to sulphur metabolism (Supplementary Fig. 1). Of the pseudomonads lacking *mddA*, e.g. *Pseudomonas putida* and *Pseudomonas*

psychrophila, those tested showed no detectable levels of DMS production (Supplementary Table 3).

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 ~50% amino acid sequence identity to *P. deceptionensis* $M1^T$ MddA are present in multiple species of the actinobacteria *Gordonia*, *Rhodococcus* and *Mycobacterium*, including the pathogens *M. tuberculosis* and *M. avium*, Rhizobiales members including N_2-fixing *Mesorhizobium* and *Bradyrhizobium* (two distinct forms, ca. 50% identical to each other, exist in many, e.g. *B. diazoefficiens* USDA 110 Blr1218 and Blr5741), and N_2-fixing cyanobacteria, including *Cyanothece*, *Pseudanabaena* and *Nodosilinea* species (Fig. 2, Fig. 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 (Fig. 2, Fig. 3, and Supplementary Table 2).

It is known that some mycobacteria that contain *mddA* produce DMS, including *M. bovis* BCG[19], but no soil-dwelling *Bradyrhizobium* or cyanobacteria have previously been reported to produce DMS. We found that *B. diazoefficiens* USDA 110 and *Cyanothece* sp. ATCC 51142 made DMS when grown in minimal media and that this 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[13]. The genes encoding the MddA polypeptides from *B. diazoefficiens* USDA 110 (blr1218 and blr5741, Rhizobiales), *M. tuberculosis* H37Rv (Actinomycetales), and *Cyanothece* 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. diazoefficiens* USDA 110 (Supplementary Table 3), perhaps explaining why this widely studied strain produces ~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 datasets from a range of environments (Supplementary Table 4). MddA protein-encoding genes ($E<e^{-30}$) 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 ≤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.

Potential roles of the MddA pathway

P. deceptionensis $M1^T$ cannot use DMS, or its precursors Met and MeSH, as a carbon source. It also cannot 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. diazoefficiens* 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. diazoefficiens* 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[20]. 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[3]. 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[7] and in some environments may play a major role in structuring bacterial communities[21].

Many heterotrophic bacteria, including *P. putida* DS1 (ref. [22]), oxidise DMS to DMSO, with some coupling this reaction to adenosine triphosphate (ATP) synthesis and increased biomass production[23,24]. *P. deceptionensis* M1^T also generated DMSO, likely via oxidation of DMS because estimates of DMSO production by the wild type strain (0.64 pmol DMSO min^{-1} mg protein^{-1}) were 7-fold higher than the *mddA* mutant. Oxidation of DMS to DMSO may form part of an antioxidant cascade protecting against oxidative stress[25]. However, in *P. deceptionensis* M1^T it is unlikely that the MddA pathway has a role in protection against oxidative stress or energy production since the *mddA* mutant showed no growth deficiency when compared to the wild type under all tested conditions, including oxidative stress, and the expression of the *mddA* gene was also unaffected by oxidative stress.

Another function for MeSH-dependent DMS production in these bacteria could be in regulating levels of Met in the cell. If Met levels in the cell were to become too high,

conversion to MeSH, and then subsequently DMS through the action of MddA, would provide a pathway for the removal of excess Met without leading to an accumulation of the potentially harmful MeSH[26]. However, while still possible, this idea is not supported by the fact that *mddA* transcription was unaffected by Met (see above) or that the *mddA* mutant showed no decreased tolerance to MeSH, at least under our conditions.

Discussion

The *mddA* gene encodes a methyltransferase that catalyses the conversion of MeSH into DMS and represents the first gene characterised for DMSP-independent DMS production in any bacterium. Although the role of the Mdd pathway in bacteria remains unclear, it is possible, given the distribution of *mddA* in taxonomically diverse bacteria and in varied environments, that this pathway may play a significant role in the global biogenic sulphur cycle. Also, the relative abundance of *mddA* in terrestrial metagenomes, rather than marine metagenomes, indicates that DMS produced from non-marine, and critically non-DMSP, sources may play a more significant role in global DMS production than previously thought.

Methods

Bacterial strains and culturing

Strains and plasmids used in this study are shown in Supplementary Tables 7 and 8. *Pseudomonas deceptionensis* M1^T, *Pseudomonas* sp. GM41, *Pseudomonas fragi* DSM 3456, *Pseudomonas psychrophila* DSM 17535, *Pseudomonas putida* ATCC 12623 and *Escherichia coli* strains were grown in LB (complete) and M9 (minimal) media[27] for 48 h at 28ºC and overnight at 37ºC, respectively. *Bradyrhizobium diazoefficiens* USDA 110 and *Rhizobium*

leguminosarum J391 were grown in TY (complete) and Y (minimal) media[28] for 48 h at 28ºC. *Cyanothece* sp. ATCC 51142 was grown in ATCC 1047 media[29,30] at 26ºC under constant light conditions (50 μE m^{-2} s^{-1}) for one week.

Sole carbon source growth tests

A culture of *P. deceptionensis* $M1^T$ in M9 was washed three times with M9 without any carbon source and inoculated into fresh M9 media containing no carbon source, 10 mM glycerol or 5 mM DMSP (made in house[31]), 5 mM Met (Sigma Aldrich), 2 mM DMSO (Sigma Aldrich), 1 mM DMS (Sigma Aldrich) or 1 mM MeSH (Sigma Aldrich) as sole carbon source. Cultures were incubated at 28ºC for 5 days and growth was estimated by measuring the OD_{600} of the cultures with a spectrophotometer UV-1800 (Shimadzu).

Sole sulphur source growth tests

Cultures of *P. deceptionensis* $M1^T$ in M9 were washed three times with M9 without sulphur and inoculated into fresh M9 media containing no sulphur or $MgSO_4$, DMSP, Met, DMSO, DMS or MeSH as sole sulphur source at 100 μM concentrations. Cultures were incubated at 28ºC for 5 days and growth was estimated by measuring the OD_{600} 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 *P. deceptionensis* $M1^T$ J564 using helper plasmid pRK2013 (ref. [32]); or by electroporation into *P. deceptionensis* $M1^T$ J564 using a BIO-RAD MicroPulser™ machine as in Tombolini *et al*.[33]. Bacterial genomic DNA was isolated using a Promega genomic preparation kit. Routine restriction digestions, ligations, Southern blotting and hybridizations were performed essentially as in Downie *et al*.[34]. The oligonucleotide primers used for molecular cloning

were synthesised by Eurofins Genomics and are detailed in Supplementary Table 9. Sequencing of plasmids and PCR products was done by the dideoxy chain termination method by Eurofins Genomics.

Sequencing of *P. deceptionensis* M1^T genomic DNA

Generation of a draft genome sequence of *P. deceptionensis* M1^T is in progress. High molecular weight *P. deceptionensis* M1^T genomic DNA was extracted using a Qiagen genomic tip 100/G and Qiagen buffer set (Qiagen GmbH, 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 selection of 450-500-bp fragments from a 2% agarose gel. The Ion Sphere Particles (ISPs) 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 Ion 316™ chip v2 and the Ion PGM™ Sequencing 400 Kit. The generated fastq file containing the resulting sequences was trimmed using Galaxy (https://usegalaxy.org/). Ends were trimmed to remove all bases with quality scores less than 50 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 analyzed with Rapid Annotation using Subsystems Technology (RAST)[35], Artemis v14.0.0 (http://www.sanger.ac.uk/Software/Artemis) and BioEdit[36] 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 clone 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* M1^T

For the targeted mutagenesis of the *megL* gene in *P. deceptionensis* M1^T, a 0.5 kb internal PCR fragment of *megL* (Supplementary Table 9) was cloned into the suicide plasmid pBIO1879[37] and transformed into *P. deceptionensis* M1^T 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* M1^T J565 were confirmed by PCR.

Construction of a *P. deceptionensis* M1^T genomic library

P. deceptionensis M1^T genomic DNA was used to generate a genomic library in the cosmid pLAFR3[38] as detailed in Curson *et al.*[39]. Approximately 12,000 primary clones were obtained in *E. coli* 803[40]. A sample of 10 clones were characterised by restriction digestion and found to contain 25-35 kb inserts that all differed from each other.

Screening of the *P. deceptionensis* M1^T genomic library

The *P. deceptionensis* M1^T genomic library was mobilised *en masse* by triparental conjugation into *R. leguminosarum* J391[41]. Approximately 250 transconjugants, that arose on TY medium containing tetracycline (5 µg ml^{-1}) and streptomycin (400 µg ml^{-1}), were inoculated into 300 µl Y medium supplemented with 0.5 mM MeSH in 2 ml gas-tight vials (Alltech Associates) and were screened for DMS production, see below.

Mutation of the *mddA* gene in *P. deceptionensis* M1^T

The cosmid pBIO2219, which contains *mddA* and its surrounding region of *P. deceptionensis* M1^T genomic DNA, was mutagenised with Tn*5lacZ*. pBIO2219 was transformed into *E. coli* strain A118[42], then this transformant strain was used as the donor in a conjugational cross with *R. leguminosarum* J391. Derivatives of pBIO2219 with a Tn*5lacZ* insertion in the cosmid were obtained by plating the cross on medium containing kanamycin (200 µg ml^{-1}),

streptomycin (400 µg ml^{-1}) and tetracycline (5 µg ml^{-1}). Individual colonies were inoculated into 300 µl Y medium supplemented with 0.5 mM MeSH, as above, and were screened for those derivatives that no longer produced DMS. The Tn*5lacZ* insertion in pBIO2220 was precisely located within the *mddA* gene by sequencing. The pBIO2220 cosmid was transformed into *P. deceptionensis* $M1^T$ J564 and then the *mddA*::Tn*5lacZ* mutation was introduced into the *P. deceptionensis* $M1^T$ J564 genome by marker exchange, by conjugating in pPH1JI[43] (gentamycin resistant), the P1 group plasmid, to eliminate the pLAFR3-based cosmid, as in Downie *et al*.[34]. Mutants were selected by their resistance to gentamicin (5 µg ml^{-1}), kanamycin (20 µg ml^{-1}) and sensitivity to tetracycline and were confirmed by PCR and Southern hybridisation.

Analysis of volatile organic sulphur compounds and DMSO

P. deceptionensis $M1^T$ wild type and J566 were grown on tryptone soya agar (Oxoid) slants in 20 ml gas chromatography headspace vials (Chromlab) for 72 h at 15ºC. Volatile organic sulphur compounds (VOSCs) and DMSO produced were identified by solid-phase microextraction (SPME) using a carboxen-polydimethylsiloxane fiber (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 x 0.32 mm, Agilent Technologies).

DMSP production and consumption assays

P. deceptionensis $M1^T$ was grown in M9 media in the presence and absence of 1 mM DMSP at 28ºC for 24 h. The culture was then pelleted and the supernatant was analyzed for DMSP versus buffer control samples via the addition of 10 M NaOH to dilutions in gas-tight 2 ml vials (12 × 32 mm, Alltech Associates) and the subsequent liberation of DMS was quantified by gas chromatography, as below.

Assays of microbial DMS and MeSH production

To measure DMS and MeSH production in pseudomonads, *R. leguminosarum, B. diazoefficiens* and *Cyanothece* sp. ATCC 51142, each strain was first grown overnight (one 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 H_2S. For *Cyanothece* sp. ATCC 51142, 300 µl of cultures were harvested and resuspended in the same volume of fresh media in sealed 2 ml vials and incubated overnight at 26°C, as above, before being assayed for DMS and MeSH production**.** All other bacterial cultures were adjusted to an OD_{600} of 0.3 and the cells were diluted 10-fold into 300 µl of minimal media supplemented with the additives as indicated above and incubated overnight at 28°C in 2 ml vials before assaying for DMS**.** DMS and MeSH production in the headspace of vials was quantified by gas chromatography, using a flame photometric detector (Agilent 7890A GC fitted with a 7693 autosampler) and a HP-INNOWax 30 m x 0.320 mm column (Agilent Technologies J&W Scientific) capillary column. The detection limits for DMS and MeSH were 0.15 nmol and 4 nmol respectively. An eight point calibration curve of DMS 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 $protein^{-1}$.

MddA activity assays in *P. deceptionensis* M1^T cell lysates

P. deceptionensis M1^T was grown in LB media for 24 h at 28 ºC 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 x 10 s) using an ultrasonic processor VC50 sonicator (Jencons) 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 $M1^T$ was grown in LB media for 24 h at 28 ºC. After that period, 2 ml aliquots were fractionated into the periplasmic, cytoplasmic and membrane fractions using a PeriPreps Periplasting Kit (Epicentre) as in Curson *et al.*[45]. 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 of the vials 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 OD_{600} of 0.8. The cells were diluted 10-fold into 300 µl of M9 media containing 100 µM Isopropyl β-D-1-thiogalactopyranoside (IPTG) and 0.5 mM MeSH or 1 mM H_2S in 2 ml vials. Vials were incubated at 28°C for 18 hours and then DMS, MeSH, and protein concentrations were assayed as above.

***E. coli* cell lysate activity assays**

For cell lysate experiments, *E. coli* BL21 containing cloned *mddA* genes were grown to an OD_{600} of 0.4 then induced with 100 µM IPTG and grown for 18 hours 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 x 10s) with an ultrasonic processor VC50 sonicator (Jencons). The cleared supernatant was incubated with 1 mM Ado-Met and 1 mM MeSH in vials for 20 minutes before being analysed for DMS, MeSH and protein content, as above.

Transcriptional assays of *mddA* in *P. deceptionensis* M1^T

The sequence upstream of the *P. deceptionensis* M1^T *mddA* gene containing the predicted promoter region was cloned into the *lacZ* reporter plasmid pBIO1878[46] (Supplementary Tables 8 and 9). The resulting construct pBIO2232 was transformed into *P. deceptionensis* M1^T 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 H_2O_2 or 300 µM paraquat. Transformants were also incubated at 4 ºC, 15 ºC and 28 ºC; in rich versus minimal media, sulphur-limited (M9 lacking $MgSO_4$); 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.*[47].

P. deceptionensis M1^T wild type and J566 growth curves

Growth curves of *P. deceptionensis* M1^T wild type and J566 strains were plotted for differing conditions including temperature (0°C and 28°C), salinity (NaCl 1-6%) and absence and presence of 2 mM Met or 0.5 mM MeSH. Growth was estimated by measuring the OD_{600} of the cultures at different time points using a spectrophotometer UV-1800 (Shimadzu).

Oxidative stress tests

For disk inhibition assays, *P. deceptionensis* M1^T wild type and J566 strains were grown in Mueller Hinton Broth (Oxoid) at 15ºC for 24 h and cultures adjusted to an OD_{600} of 0.3. A swab was soaked in the cultures and spread onto Mueller Hinton Agar (Oxoid) plates. Sterile 6 mm filter discs (Whatman) containing 10 µl of 100 mM H_2O_2 or 25 mM paraquat (Sigma Aldrich) were placed on the top of the plates. After an incubation period of 48 h at 15ºC, zones of growth inhibition were recorded.

Minimal inhibitory concentration assays with *P. deceptionensis* $M1^T$ wild type and J566 strains were performed by the broth microdilution method described by Wiegand *et al.*[48] using H_2O_2 ranging from 0.4 – 100 mM or Paraquat (Sigma Aldrich) ranging from 0.1 – 25 mM. OD_{600} of the wells was measured with a Synergy HT microplate reader (BioTek) after an incubation of 48 h at 15ºC.

Bioinformatics analysis

The protein sequence of *P. deceptionensis* $M1^T$ 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 Package[49] using sequences with at least 40 % amino acid identity to the MddA protein of *P. deceptionensis* $M1^T$ provided by BLAST[50]. The neighbour-joining method and the Jones-Taylor-Thornton (JTT) model of amino acid substitution were used, with 1000 bootstrap replications.

MddA protein sequences from *P. deceptionensis* $M1^T$ (AJE75769), *Pseudomonas* sp. GM41 (WP_008148420.1), *Cyanothece* sp. ATCC 51142 (YP_001803274.1), *B. diazoefficiens* USDA 110 Blr1218 (NP_767858.1) and Blr5741 (NP_772381.1), *Mycobacterium tuberculosis* H37Rv (NP_217755.1), *Maricaulis maris* MCS10 (YP_757811.1), *Sulfurovum* sp. NBC37-1 (YP_001358232.1) and Ste14p from *Saccharomyces cerevisiae* (AAA16840.1) were aligned with ClustalW using the MEGA6 Package[49] and analyzed for conserved domains using GeneDoc[51] v2.5.010.

Ratified protein sequences of MddA (from distinct bacterial clades; Fig. 2 and Fig. 3) were used as query sequences to perform BLASTP[50] searches against peptide databases created from metagenome sequences downloaded from CAMERA and MG-RAST (information on the metagenomes is provided in Supplementary Table 4). The number of unique hits to MddA were normalised to the number of unique RecA sequences in the metagenomes in

order to estimate the abundance of *mddA* in each metagenome. The number of unique RecA sequences in each metagenome was obtained using 120 RecA sequences as probes[52] (Supplementary Table 6). A similar strategy was used for the DddD, L, P, Q, and W sequences using ratified protein sequences as queries (Supplementary Table 6).

The publically available gene expression datasets for *B. diazoefficiens* USDA 110 and *Mycobacterium tuberculosis* H37Rv were analysed for conditions that resulted in significant changes in expression for the *mddA* gene. The *B. diazoefficiens* datasets were analysed using GEOR2 (http://www.ncbi.nlm.nih.gov/geo/geo2r). The *Mycobacterium tuberculosis* H37Rv datasets were analysed using the TB expression database (http://www.tbdb.org/tbdbPages/expressionData.shtml). Significant changes were judged as those where *mddA* was shown to be up- or downregulated >2-fold in ≥2/3 of the replicates, and that were consistent at multiple time points.

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Author contributions

J.D.T. and O.C. designed and performed experiments, analysed data and wrote the paper; A.R.J.C. constructed the *Pseudomonas deceptionensis* $M1^T$ genomic library and wrote the paper; E.M. designed experiments; A.S.L. and Y.F. generated a draft sequence of the genome of *Pseudomonas deceptionensis* $M1^T$; D.K. carried out bioinformatics analysis of metagenomic datasets. All authors reviewed the manuscript before submission.

Additional information

Accession codes: Sequence data for the cosmid pBIO2219 (accession: KM030271),the *P. deceptionensis* $M1^T$ *megL* gene (accession: KM030270) and the *P. deceptionensis* $M1^T$ MddA protein (accession: AJE75769) are deposited in Genbank.

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Figure legends

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

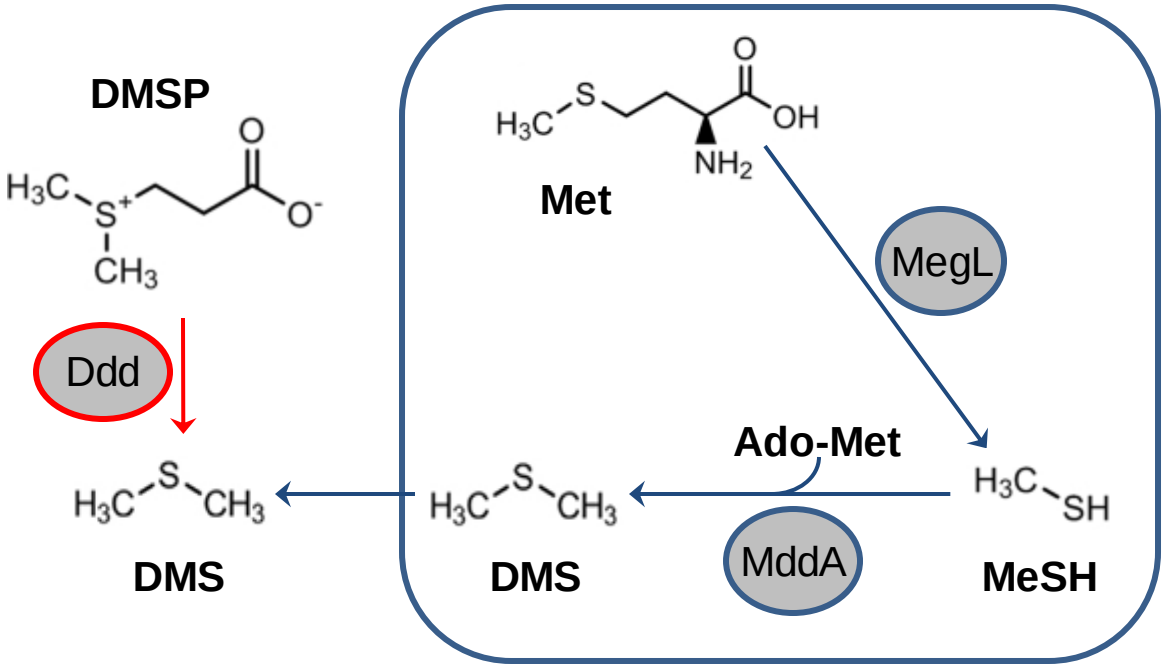
Figure 2. ClustalW alignment of representative bacterial MddA polypeptides and the *Saccharomyces cerevisiae* Ste14p polypeptide. Residues that are identical or have similar properties are highlighted in red, orange or yellow if they are conserved in all 9, at least 7 or at least 5 polypeptides, respectively. Residues with similar properties that are conserved in all 8 bacterial MddA sequences are marked with asterisks below. The position of the Tn*5lacZ* insertion in the *Pseudomonas deceptionensis* $M1^T$ *mddA* mutant J566 is marked with an 'X' below. Species names with the MddA polypeptide accession codes shown in brackets are: *Cyanothece* sp. ATCC 51142 (YP_001803274), *Mycobacterium tuberculosis* H37Rv (NP_217755), *Bradyrhizobium diazoefficiens* USDA 110 (Blr1218; NP_767858.1), *Bradyrhizobium diazoefficiens* USDA 110 (Blr5741, NP_772381.1), *Pseudomonas* sp. GM41 (WP_008148420), *Pseudomonas deceptionensis* $M1^T$ (AJE75769), *Maricaulis maris* MCS10 (YP_757811.1), *Sulfurovum* sp. NBC 37-1 (YP_001358232.1). Species names marked with a hash indicate those MddA sequences whose activity has been ratified in *Escherichia coli*. The *Saccharomyces cerevisiae* Ste14p accession is AAA16840.1.

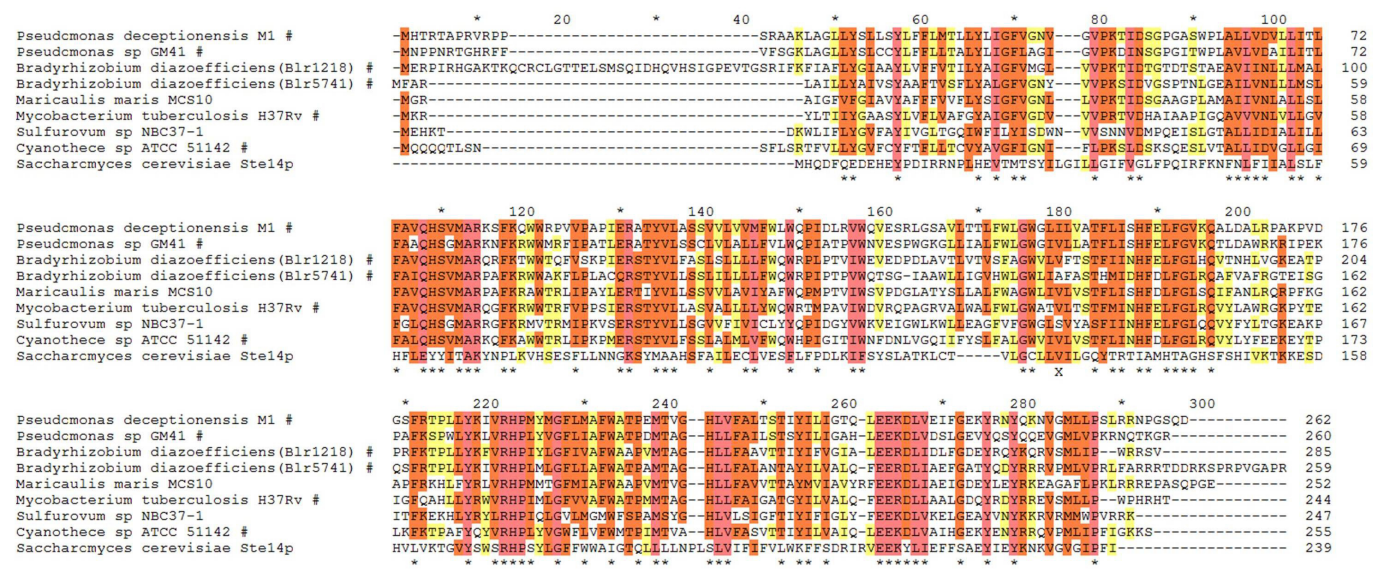
Figure 3. Molecular phylogenetic analysis of MddA proteins. Bacterial strains, their taxonomy and the accession codes of bacterial MddA proteins that have >40% amino acid identity to *Pseudomonas deceptionensis* $M1^T$ MddA are shown. Sequences with >57% identity to each other that are in bacteria of 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 methanethiol-dependent dimethylsulphide production to *Escherichia coli* are written in purple. The two MddA proteins of *Bradyrhizobium diazoefficiens* USDA110 (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 ≥50% (based on 1000 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.

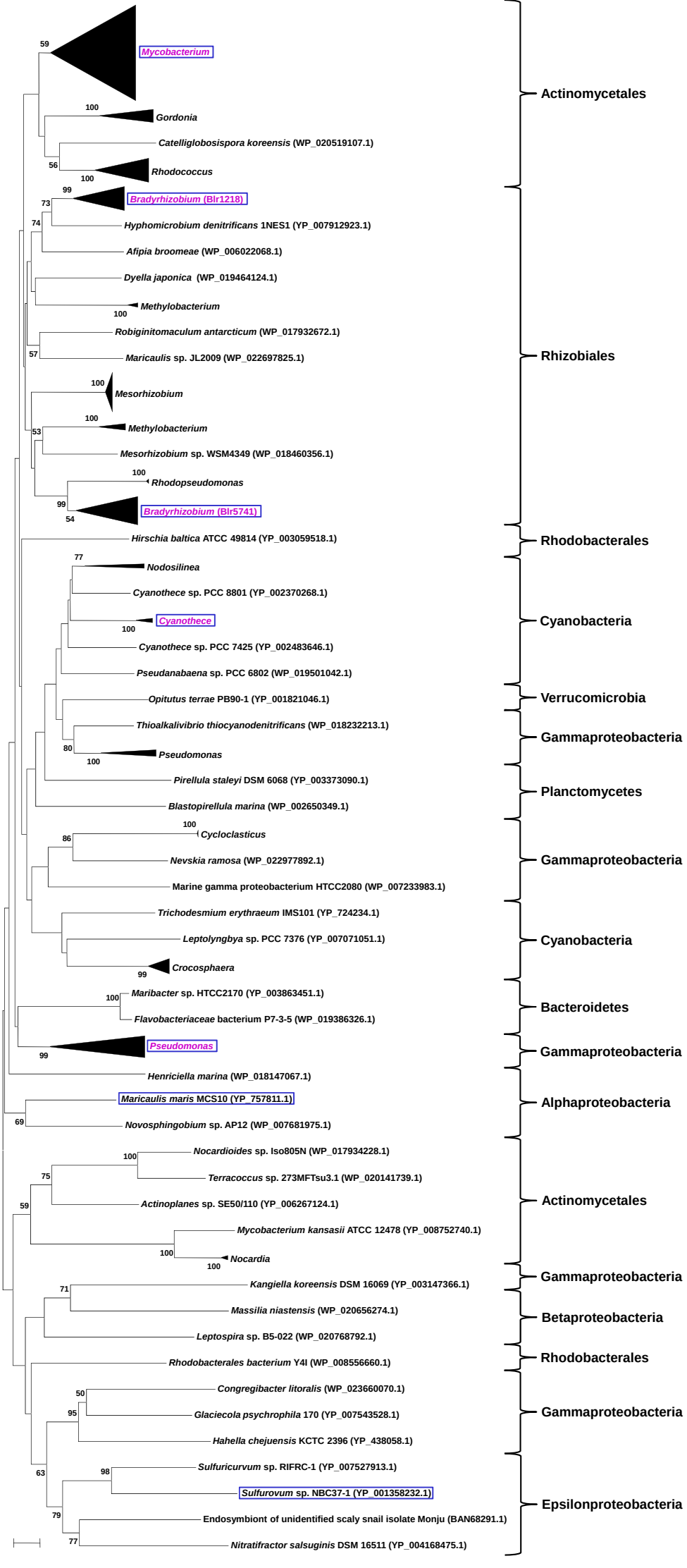
Figure 4. Comparison of normalised values of *mddA* and *dddDPQWL* sequences in different environmental metagenomes. Details of metagenomes are in Supplementary Table 4 and the number of unique hits (Supplementary Table 5) were normalised to the number of unique RecA sequences in the metagenome and are presented as percentage of the total RecA sequences. Peptide databases were downloaded from metagenome sequences available from CAMERA and MG-RAST. RecA and Ddd probe sequences are detailed in Supplementary Table 6.

Strain	Medium additive	pmol DMS min^{-1} mg $protein^{-1}$	pmol MeSH min^{-1} mg $protein^{-1}$
Pseudomonas deceptionensis $M1^T$ wild type	None	7.65 ± 0.56	ND
	DMSP	6.99 ± 1.42	ND
	Methionine	55.22 ± 5.00	1433.47 ± 326.01
	MeSH	34.42 ± 5.13	ND
P. deceptionensis $M1^T$ *megL* mutant(J565)	None	ND	ND
	Methionine	ND	ND
	MeSH	51.55 ± 3.10	ND
P. deceptionensis $M1^T$ *mddA* mutant (J566)	None	ND	ND
	Methionine	ND	4926.16 ± 685.68
	MeSH	ND	ND
Rhizobium leguminosarum J391 pBIO2219 (*P. deceptionensis* $M1^T$ *mddA* region cloned)	MeSH	57.72 ± 2.65	ND
R. leguminosarum J391 pBIO2220 (pBIO2219 mutated in *mddA*)	MeSH	ND	ND
Escherichia coli BL21 pBIO2223 (*mddA* of *P. deceptionensis* $M1^T$ cloned)	MeSH	414.07 ± 88.06	ND
Bradyrhizobium diazoefficiens USDA110	None	385.00 ± 38.17	ND
	MeSH	596.79 ± 32.82	ND
Cyanothece sp. ATCC 51142	None	5.69 ± 0.23	ND
	MeSH	7.53 ± 0.07	ND

Table 1. The DMS and MeSH produced by key microbial strains used in this study. 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 averages of three biological replicates with the standard deviations being shown. ND, not detected.


DMSP
Met
MegL
Ddd
Ado-Met
MddA
DMS
DMS
MeSH


Pseudcmonas deceptionensis M1 #
Pseudcmonas sp GM41 #
Bradyrhizobium diazoefficiens(Blr1218) #
Bradyrhizobium diazoefficiens(Blr5741) #
Maricaulis maris MCS10
Mycobacterium tuberculosis H37Rv #
Sulfurovum sp NBC37-1
Cyanothece sp ATCC 51142 #
Saccharcmyces cerevisiae Ste14p


Mycobacterium
Gordonia
Catelliglobosispora koreensis (WP_020519107.1)
Rhodococcus
Actinomycetales
Bradyrhizobium (Blr1218)
Hyphomicrobium denitrificans 1NES1 (YP_007912923.1)
Afipia broomeae (WP_006022068.1)
Dyella japonica (WP_019464124.1)
Methylobacterium
Robiginitomaculum antarcticum (WP_017932672.1)
Maricaulis sp. JL2009 (WP_022697825.1)
Mesorhizobium
Methylobacterium
Mesorhizobium sp. WSM4349 (WP_018460356.1)
Rhodopseudomonas
Bradyrhizobium (Blr5741)
Rhizobiales
Hirschia baltica ATCC 49814 (YP_003059518.1)
Rhodobacterales
Nodosilinea
Cyanothece sp. PCC 8801 (YP_002370268.1)
Cyanothece
Cyanothece sp. PCC 7425 (YP_002483646.1)
Pseudanabaena sp. PCC 6802 (WP_019501042.1)
Cyanobacteria
Opitutus terrae PB90-1 (YP_001821046.1)
Verrucomicrobia
Thioalkalivibrio thiocyanodenitrificans (WP_018232213.1)
Pseudomonas
Gammaproteobacteria
Pirellula staleyi DSM 6068 (YP_003373090.1)
Blastopirellula marina (WP_002650349.1)
Planctomycetes
Cycloclasticus
Nevskia ramosa (WP_022977892.1)
Marine gamma proteobacterium HTCC2080 (WP_007233983.1)
Gammaproteobacteria
Trichodesmium erythraeum IMS101 (YP_724234.1)
Leptolyngbya sp. PCC 7376 (YP_007071051.1)
Crocosphaera
Cyanobacteria
Maribacter sp. HTCC2170 (YP_003863451.1)
Flavobacteriaceae bacterium P7-3-5 (WP_019386326.1)
Bacteroidetes
Pseudomonas
Gammaproteobacteria
Henriciella marina (WP_018147067.1)
Maricaulis maris MCS10 (YP_757811.1)
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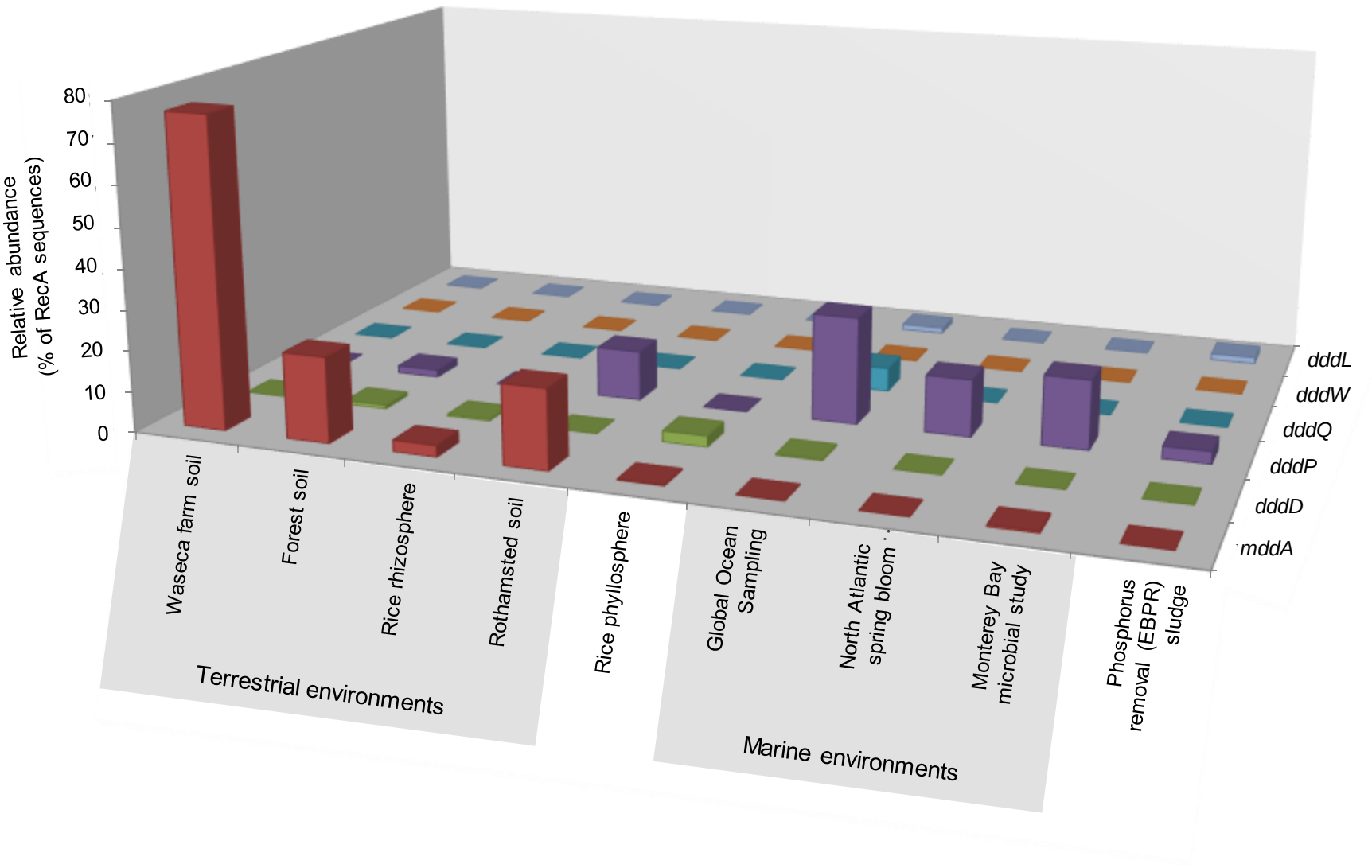
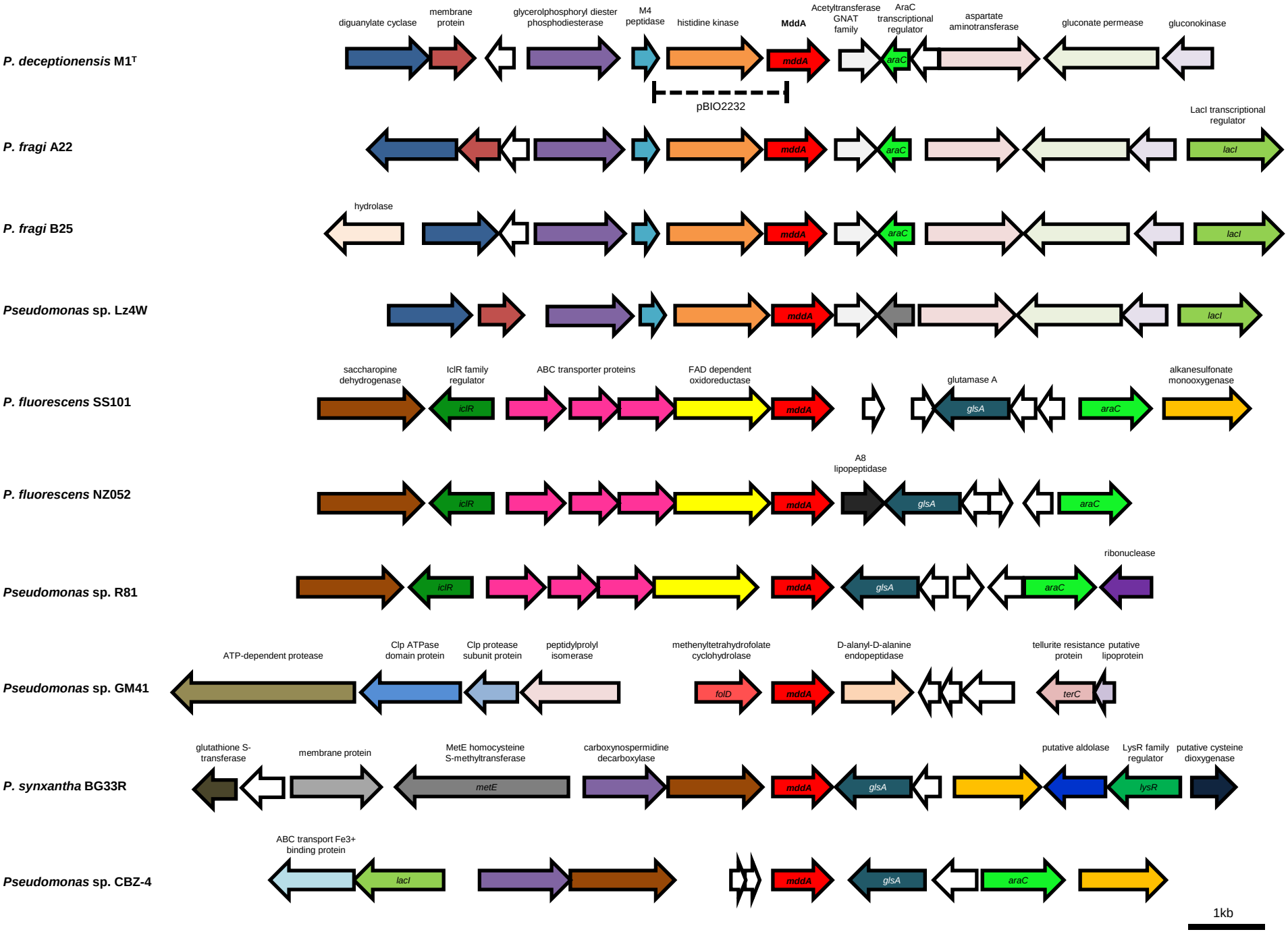
Alphaproteobacteria
Nocardioides sp. Iso805N (WP_017934228.1)
Terracoccus sp. 273MFTsu3.1 (WP_020141739.1)
Actinoplanes sp. SE50/110 (YP_006267124.1)
Mycobacterium kansasii ATCC 12478 (YP_008752740.1)
Nocardia
Actinomycetales
Kangiella koreensis DSM 16069 (YP_003147366.1)
Gammaproteobacteria
Massilia niastensis (WP_020656274.1)
Betaproteobacteria
Leptospira sp. B5-022 (WP_020768792.1)
Rhodobacterales bacterium Y4I (WP_008556660.1)
Rhodobacterales
Congregibacter litoralis (WP_023660070.1)
Glaciecola psychrophila 170 (YP_007543528.1)
Hahella chejuensis KCTC 2396 (YP_438058.1)
Gammaproteobacteria
Sulfuricurvum sp. RIFRC-1 (YP_007527913.1)
Sulfurovum sp. NBC37-1 (YP_001358232.1)
Endosymbiont of unidentified scaly snail isolate Monju (BAN68291.1)
Nitratifractor salsuginis DSM 16511 (YP_004168475.1)
Epsilonproteobacteria


Relative abundance
(% of RecA sequences)
80
70
60
50
40
30
20
10
0
Waseca farm soil
Forest soil
Rice rhizosphere
Rothamsted soil
Rice phyllosphere
Global Ocean Sampling
North Atlantic spring bloom
Monterey Bay microbial study
Phosphorus removal (EBPR) sludge
Terrestrial environments
Marine environments
dddL
dddW
dddQ
dddP
dddD
mddA


P. deceptionensis M1T
diguanylate cyclase
membrane protein
glycerolphosphoryl diester phosphodiesterase
M4 peptidase
histidine kinase
MddA
Acetyltransferase GNAT family
AraC transcriptional regulator
aspartate aminotransferase
gluconate permease
gluconokinase
pBIO2232
P. fragi A22
LacI transcriptional regulator
P. fragi B25
hydrolase
Pseudomonas sp. Lz4W
P. fluorescens SS101
saccharopine dehydrogenase
IclR family regulator
ABC transporter proteins
FAD dependent oxidoreductase
glutamase A
alkanesulfonate monooxygenase
P. fluorescens NZ052
A8 lipopeptidase
Pseudomonas sp. R81
ribonuclease
Pseudomonas sp. GM41
ATP-dependent protease
Clp ATPase domain protein
Clp protease subunit protein
peptidylprolyl isomerase
methenyltetrahydrofolate cyclohydrolase
D-alanyl-D-alanine endopeptidase
tellurite resistance protein
putative lipoprotein
P. synxantha BG33R
glutathione S-transferase
membrane protein
MetE homocysteine S-methyltransferase
carboxynospermidine decarboxylase
putative aldolase
LysR family regulator
putative cysteine dioxygenase
Pseudomonas sp. CBZ-4
ABC transport Fe3+ binding protein
1kb

Supplementary Figure 1. The *mddA* gene and surrounding genes of *Pseudomonas* strains. The genomic regions of *P. deceptionensis* M1^T (KM030271), *P. fragi* A22 (GCA_000250595), *P. fragi* B25 (PRJNA200017), *Pseudomonas* sp. Lz4W (PRJNA193784), *P. fluorescens* SS101 (PRJNA181712), *P. fluorescens* NZ052 (PRJNA200137), *Pseudomonas* sp. R81 (PRJNA176384), *Pseudomonas* sp. GM41 (PRJNA171667), *P. synxantha* BG33R (PRJNA167316) and *Pseudomonas* sp. CBZ-4 (PRJNA199902) containing six genes either side of *mddA* are shown. Genes are colour-coded and predicted protein products are detailed above the arrows. Known genes are labelled in italics within arrows; genes that encode hypothetical proteins are shown as white arrows; regulatory genes are shaded green with the class of their protein product detailed in the arrows. The region of *P. deceptionensis* M1^T cloned in pBIO2232 is shown as a dashed line. Scale bar indicates 1 kb of genomic DNA.

Software	Transmembrane helices	Helix 1	Helix 2	Helix 3	Helix 4	Helix 5	Helix 6
TMPred (European Molecular Biology Network, Swiss node)	5	21-42 (2348)	59-81 (1552)	100-118 (1841)	135-154 (2727)	ND	208-227 (1286)
MPex (University of California)	6	21-42 (2.74)	57-75 (0.15)	99-120 (1.02)	135-157 (1.83)	183-203 (1.88)	206-227 (1.61)
Optimum Antigen (GenScript)	4	20-42	57-79	100-117	132-154	ND	ND
TMHMM Server v. 2.0 (CBS, Denmark)	4	20-42	57-79	100-117	132-154	ND	ND
Predict Protein (Technical University of Munich)	5	21-42 (0.56)	60-77 (0.74)	101-118 (0.79)	136-154 (0.62)	194-218 (0.69)	
TOP Pred (Insitute Pasteur)	5	24-44 (2.17)	56-76 (1.49)	100-120 (1.96)	135-155 (2.27)	ND	206-226 (1.69)
PRED-TMR2: Prediction of Transmembrane regions in proteins (Univesity of Athens)	5	25-42	57-75	100-117	135-154	196-215	
PSIPRED V2.3 (University College London)	6	17-41 (30.04)	59-82 (30.97)	96-117 (21.73)	133-157 (23.18)	185-204 (12.45)	207-227 (23.84)
Split Server: Membrane Protein Secondary Structure Prediction Server (University of Split, Croatia)	6	17-50	64-92	101-130	138-165	188-205	209-230
SACS MEMSAT2 Transmembrane Prediction (University of California, San Diego)	5	18-42 (5.33)	59-75 (3.57)	100-117 (4.89)	131-154 (5.10)	ND	206-223 (2.73)

Supplementary Table 1. **Transmembrane domain predictions for *Pseudomonas deceptionensis* MddA.** The bioinformatics programs and their hosting institute, shown in brackets, are in column 1. The numbers of predicted transmembrane spanning helices are shown in column 2 and the locations of the individual domains in columns 3, 4, 5, 6, 7 and 8 with their respective confidence levels shown in brackets. The confidence results for TMPred (in row 1) are score values where >500 is considered significant; MPex (in row 2) are apparent free energy difference values; Predict Protein (in row 5) are total score values; TOP Pred (in row 6) are score values where values above 1 are judged as certain; PSIPRED (in row 8) are score values; SACS MEMSAT2 (in row 10) are score values. Columns coloured in light grey represent MddA transmembrane helices predicted by all 10 programs. Boxes coloured in dark grey represent predicted transmembrane helices that span a part of MddA where the MPex, PSIPRED, Split Server and SACS MEMSAT2 programs predict two transmembrane domains. ND, not detected.

Strain	Accession Number	Amino acid identity (%)
Pseudomonas fragi	WP_010655917.1	71
Pseudomonas sp. Lz4W	WP_003440799.1	71
Pseudomonas fragi	WP_016782104.1	69
Pseudomonas sp. GM41 (2012)	WP_008148420.1	62
Pseudomonas fluorescens SS101	WP_003191715.1	58
Pseudomonas fluorescens	WP_016977171.1	57
Pseudomonas sp. CBZ-4	WP_017738966.1	57
Pseudomonas fluorescens EGD-AQ6	WP_010209907.1	56
Mesorhizobium sp. L2C089B000	WP_023806743.1	56
Mesorhizobium	WP_023685403.1	56
Mesorhizobium	WP_023698505.1	56
Mesorhizobium	WP_023687132.1	56
Mesorhizobium	WP_023692088.1	56
Mesorhizobium sp. L103C105A0	WP_023834474.1	56
Mesorhizobium sp. LNJC395A00	WP_023740700.1	56
Mesorhizobium	WP_023732105.1	56
Mesorhizobium sp. L2C054A000	WP_023821026.1	56
Mesorhizobium sp. LNJC391B00	WP_023747554.1	56
Mesorhizobium	WP_023709882.1	56
Pseudomonas synxantha BG33R	WP_005787686.1	55
Robiginitomaculum antarcticum	WP_017932672.1	55
Mesorhizobium	WP_023754096.1	55
Mesorhizobium sp. L103C120A0	WP_023830035.1	55
Mesorhizobium sp. LSJC285A00	WP_023668444.1	55
Mesorhizobium	WP_023702030.1	55
Mesorhizobium sp. L2C066B000	WP_023818971.1	55
Mesorhizobium sp. LNJC384A00	WP_023749729.1	55
Mesorhizobium	WP_023723279.1	55
Bradyrhizobium sp. ORS 278	YP_001202883.1	54
Bradyrhizobium sp. YR681	WP_008143861.1	54
Bradyrhizobium sp. STM 3809	WP_008960188.1	53
Dyella japonica	WP_019464124.1	53
Bradyrhizobium sp. S23321	YP_005449834.1	53
Methylobacterium sp. 4-46	YP_001773117.1	53
Methylobacterium sp. WSM2598	WP_018263339.1	53
Mesorhizobium sp. WSM4349	WP_018460356.1	53
Maricaulis sp. JL2009	WP_022697825.1	52
Mycobacterium sp. JDM601	YP_004522856.1	52
Mycobacterium avium	WP_019734943.1	52
Bradyrhizobium japonicum USDA 6	YP_005608857.1	52
Bradyrhizobium sp. STM 3843	WP_008972832.1	52
Cyanothece sp. PCC 7425	YP_002483646.1	52
Mycobacterium rhodesiae NBB3	YP_004998641.1	52
Bradyrhizobium diazoefficiens USDA 110 Blr5741	NP_772381.1	51
Bradyrhizobium sp. ORS 375	WP_009027485.1	51
Bradyrhizobium sp. BTAi1	YP_001243154.1	51
Bradyrhizobium japonicum	WP_018643406.1	51
Maricaulis maris MCS10	YP_757811.1	51
Methylobacterium mesophilicum	WP_010686508.1	51
Bradyrhizobium japonicum	WP_018316310.1	51
Bradyrhizobium oligotrophicum S58	YP_007516458.1	51

Methylobacterium nodulans ORS 2060	YP_002500169.1	51
Rhodopseudomonas palustris CGA009	NP_948178.1	51
Rhodopseudomonas palustris TIE-1	YP_001992155.1	51
Hyphomicrobium denitrificans 1NES1	YP_007912923.1	51
Mycobacterium yongonense 05-1390	YP_008189949.1	51
Bradyrhizobium sp. S23321	YP_005448171.1	50
Bradyrhizobium sp. YR681	WP_008135454.1	50
Bradyrhizobium japonicum	YP_005613467.1	50
Bradyrhizobium diazoefficiens USDA 110 Blr1218	NP_767858.1	50
Opitutus terrae PB90-1	YP_001821046.1	50
Bradyrhizobium sp. ORS 285	WP_006613592.1	50
Cyanothece sp. CCY0110	WP_008274188.1	50
Cyanothece sp. ATCC 51142	YP_001803274.1	50
Mycobacterium sp. 360MFTsu5.1	WP_020102131.1	50
Mycobacterium chubuense NBB4	YP_006451809.1	50
Bradyrhizobium sp. WSM1253	WP_007602238.1	50
Methylobacterium sp. WSM2598	WP_018261391.1	50
Maribacter sp. HTCC2170	YP_003863451.1	50
Mycobacterium intracellulare MOTT-64	YP_005350001.1	50
Mycobacterium indicus pranii MTCC 9506	YP_006731206.1	50
Mycobacterium intracellulare MOTT-02	YP_005344743.1	50
Mycobacterium parascrofulaceum	WP_007168108.1	50
Nodosilinea nodulosa	WP_017302056.1	49
Cyanothece sp. PCC 8801	YP_002370268.1	49
Mesorhizobium sp. WSM4349	WP_018455518.1	49
Bradyrhizobium sp. CCGE-LA001	EJZ35341.1	49
Flavobacteriaceae bacterium P7-3-5	WP_019386326.1	49
Bradyrhizobium sp. WSM471	WP_007605132.1	49
Mesorhizobium sp. WSM4349	WP_018458172.1	49
Mycobacterium chubuense NBB4	YP_006454919.1	49
Thioalkalivibrio thiocyanodenitrificans	WP_018232213.1	49
Novosphingobium sp. AP12	WP_007681975.1	49
Mycobacterium rhodesiae	WP_005147178.1	49
Mycobacterium canettii CIPT 140060008	YP_004746669.1	49
Mycobacterium canettii CIPT 140070010	YP_007265965.1	49
Hirschia baltica ATCC 49814	YP_003059518.1	49
Mycobacterium kansasii ATCC 12478	YP_008751780.1	49
Afipia broomeae	WP_006022068.1	49
Mycobacterium fortuitum	WP_003882934.1	49
Mycobacterium kansasii ATCC 12478	YP_008750971.1	49
Mycobacterium intracellulare ATCC 13950	YP_005339590.1	49
Mycobacterium tuberculosis	WP_017351950.1	49
Mycobacterium chubuense NBB4	YP_006453936.1	48
Nodosilinea nodulosa	WP_017302039.1	48
Bradyrhizobium sp. WSM471	WP_007612160.1	48
Pseudanabaena sp. PCC 6802	WP_019501042.1	48
Bradyrhizobium sp. WSM1253	WP_007599504.1	48
Mycobacterium colombiense	WP_007773112.1	48
Mycobacterium avium	WP_009978727.1	48
Mycobacterium avium	WP_023880924.1	48
Mycobacterium avium	WP_023868154.1	48
Mycobacterium avium 104	YP_883345.1	48
Mycobacterium avium	WP_023860909.1	48
Mycobacterium avium	WP_023885067.1	48

Mycobacterium avium subsp. *paratuberculosis* K-10	NP_962285.1	48
Mycobacterium avium	WP_003874574.1	48
Mycobacterium tuberculosis H37Rv	NP_217755.1	48
Mycobacterium tuberculosis	WP_021083469.1	48
Trichodesmium erythraeum IMS101	YP_724234.1	48
Mycobacterium vanbaalenii PYR-1	YP_953759.1	48
Mycobacterium avium	WP_023874735.1	48
Mycobacterium tuberculosis str. Haarlem/NITR202	YP_007959233.1	48
Gordonia rhizosphera	WP_006336261.1	47
Mycobacterium sp. MCS	YP_640649.1	47
Mycobacterium xenopi	WP_003918788.1	47
Gordonia rubripertincta	WP_005199332.1	47
Pseudomonas sp. EGD-AK9	WP_021444986.1	47
Mycobacterium smegmatis JS623	YP_007290726.1	47
Rhodococcus sp. EsD8	WP_006930258.1	47
Mycobacterium tuberculosis	WP_017351997.1	47
Gordonia alkanivorans	WP_006359338.1	46
Gordonia amicalis	WP_006434827.1	46
Gordonia namibiensis	WP_006865748.1	46
Marine gamma proteobacterium HTCC2080	WP_007233983.1	46
Mycobacterium abscessus	WP_005101647.1	46
Rhodobacterales bacterium Y4I	WP_008556660.1	46
Mycobacterium marinum	WP_020732150.1	46
Mycobacterium marinum M	YP_001849620.1	46
Mycobacterium marinum	WP_020727267.1	46
Rhodococcus ruber	WP_017679400.1	46
Rhodococcus sp. P14	WP_010593087.1	46
Pseudomonas pseudoalcaligenes	WP_003456133.1	46
Catelliglobosispora koreensis	WP_020519107.1	46
Endosymbiont of unidentified scaly snail isolate Monju	BAN68291.1	46
Mycobacterium ulcerans Agy99	YP_906384.1	46
Pirellula staleyi DSM 6068	YP_003373090.1	46
Leptospira sp. B5-022	WP_020768792.1	46
Uncultured marine bacterium HOT0_07D09	ABL97530.1	46
Gordonia alkanivorans CGMCC 6845	ETA07490.1	45
Henriciella marina	WP_018147067.1	45
Mycobacterium liflandii 128FXT	YP_007367935.1	45
Actinoplanes sp. SE50/110	YP_006267124.1	45
Rhodococcus ruber	WP_003936334.1	45
Blastopirellula marina	WP_002650349.1	45
Rhodococcus opacus	WP_005252698.1	45
Rhodococcus jostii RHA1	YP_701623.1	45
Rhodococcus	WP_009474334.1	45
Crocosphaera watsonii	WP_021836637.1	45
Leptolyngbya sp. PCC 7376	YP_007071051.1	44
Pseudomonas resinovorans NBRC 106553	YP_008102526.1	44
Crocosphaera watsonii	WP_021832840.1	44
Crocosphaera watsonii	WP_007311317.1	44
Crocosphaera watsonii	WP_021831992.1	44
Crocosphaera watsonii	WP_007306127.1	44
Congregibacter litoralis	WP_023660070.1	44
Rhodococcus imtechensis	WP_007300507.1	43
Rhodococcus wratislaviensis	WP_005570936.1	43
Rhodococcus opacus	WP_005254096.1	43

Rhodococcus opacus B4	YP_002778539.1	43
Nevskia ramosa	WP_022977892.1	43
Hahella chejuensis KCTC 2396	YP_438058.1	42
Nocardioides sp. Iso805N	WP_017934228.1	42
Sulfurovum sp. NBC37-1	YP_001358232.1	42
Terracoccus sp. 273MFTsu3.1	WP_020141739.1	42
Glaciecola psychrophila 170	YP_007543528.1	42
Crocosphaera watsonii	WP_021836638.1	42
Nocardia asteroides	WP_019047562.1	41
Nocardia sp. WT7	AFI64491.1	41
Cycloclasticus pugetii	WP_020162742.1	41
Cycloclasticus sp. P1	YP_006838697.1	41
Cycloclasticus sp. PY97M	WP_016390811.1	41
Kangiella koreensis DSM 16069	YP_003147366.1	40
Nitratifractor salsuginis DSM 16511	YP_004168475.1	40
Mycobacterium kansasii ATCC 12478	YP_008752740.1	40
Sulfuricurvum sp. RIFRC-1	YP_007527913.1	40
Massilia niastensis	WP_020656274.1	40

Supplementary Table 2. Sequences used in the molecular phylogenetic analysis of MddA proteins. Bacterial strains, accession numbers of their MddA-like proteins, and % amino acid identity to *Pseudomonas deceptionensis* MddA are shown. Those cases where the cloned *mddA* genes were shown experimentally to confer methanethiol-dependent dimethylsulphide production to *Escherichia coli* are written in purple. The two MddA proteins of *Bradyrhizobium diazoefficiens* USDA110 (Blr1218 and Blr5741) are shown. MddA sequences that were aligned in Fig. 2 are in blue boxes.

Strain	Medium additive	pmol DMS min^{-1} $mg\ protein^{-1}$	pmol MeSH min^{-1} $mg\ protein^{-1}$
Pseudomonas fragi DSM 3456	None	12.95 ± 5.71	ND
	MeSH	21.77 ± 2.56	ND
Pseudomonas sp. GM41	None	2.61 ± 0.07	ND
	MeSH	35.09 ± 4.84	ND
Pseudomonas putida ATCC 12623	None	ND	ND
Pseudomonas psychrophila DSM 17535	None	ND	ND
Escherichia coli BL21	MeSH	ND	ND
E. coli BL21 pBIO2224 (*mdd*A of *Pseudomonas* sp. GM41 cloned)	MeSH	198.24 ± 40.01	ND
E. coli BL21 pBIO2225 (*mddA* [blr1218] of *B. diazoefficiens* USDA110 cloned)	MeSH	78.13 ± 6.76	ND
E. coli BL21 pBIO2227 (*mddA* [blr5741] of *B. diazoefficiens* USDA110 cloned)	MeSH	536.46 ± 52.96	ND
E. coli BL21 pBIO2229 (*mdd*A of *Mycobacterium tuberculosis* H37Rv cloned)	MeSH	65.46 ± 2.55	ND
E. coli BL21 pBIO2231 (*mdd*A of *Cyanothece* sp. ATCC 51142 cloned)	MeSH	46.59 ± 13.64	ND

Supplementary Table 3. The DMS and MeSH produced by key microbial strains used in this study. 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 averages of three biological replicates with the standard deviations being shown. ND, not detected.

Metagenome/Project	Accession Number	Biome	Location	Total number of sequences	Database
Rothamsted soil	4453247.3	Temperate grasslands	Rothamsted, UK	1166789	MG-RAST
Global Ocean Sampling	CAM_PROJ_GOS	Marine	Various	41146566	CAMERA
Forest soil	4446153.3	Soil	Luquillo Experimental Forest soil, Puerto Rico	689464	MG-RAST
Rice rhizosphere	4449956.3	Soil	Los Banos, Philippines	1072868	MG-RAST
Rice phyllosphere	4450328.3	Phyllosphere (plant-associated habitat)	Los Banos, Philippines	2293878	MG-RAST
Waseca Farm soil	CAM_PROJ_FarmSoil	Soil	Minnesota, USA	183297	CAMERA
North Atlantic spring bloom	CAM_PROJ_BATS	Marine	Sargasso Sea	3005827	CAMERA
Monterey Bay microbial study	CAM_PROJ_Monterey Bay	Coastal	California, USA	2528595	CAMERA
Phosphorus removal (EBPR) sludge	CAM_PROJ_EBPRSludge	Sludge	Brisbane, Australia	313657	CAMERA

Supplementary Table 4. Information on metagenomes used in this study. Peptide sequences were downloaded from metagenomes listed on either CAMERA or MG-RAST databases.

Metagenome	MddA	DddD	DddP	DddQ	DddW	DddL	RecA
Farm soil	13	0	0	0	0	0	17
Forest soil	23	1	2	0	0	0	109
Rice rhizosphere	6	1	0	0	0	0	208
Rothamsted soil	30	0	19	0	0	0	153
Rice phyllosphere	1	8	0	0	0	0	293
Global Ocean Sampling	3	12	810	182	3	43	3064
North Atlantic spring bloom	0	0	14	0	0	0	99
Monterey Bay microbial study	1	0	33	0	0	0	193
Phosphorus removal (EBPR) sludge	0	0	2	0	0	1	65

Supplementary Table 5. Ddd and MddA homologues in selected metagenomes. Number of unique proteins with $E<e^{-30}$ to reference Ddd and MddA proteins (see Methods) retrieved from the metagenomes detailed in Supplementary Table 4.

Accession	Protein name	Pathway	Taxonomic group	Genus	Protein size (amino acids)
ACB15385.1	RecA	Control	Actinobacteria	*Bifidobacterium*	267
ACT64224.1	RecA	Control	Actinobacteria	*Brevibacteriaceae*	186
AAD12743.1	RecA	Control	Actinobacteria	*Corynebacteriaceae*	376
CAA41395.1	RecA	Control	Actinobacteria	*Mycobacteriaceae*	790
AAT70029.1	RecA	Control	Actinobacteria	*Propionibacteriaceae*	348
ABB29469.1	RecA	Control	Actinobacteria	*Streptomycetaceae*	376
ABD34619.1	RecA	Control	Alphaproteobacteria	*Anaplasma*	357
EAS48674.1	RecA	Control	Alphaproteobacteria	*Aurantimonas*	357
AAR00257.1	RecA	Control	Alphaproteobacteria	*Azospirillum*	369
ABW82568.1	RecA	Control	Alphaproteobacteria	*Bradyrhizobiaceae*	167
ABI98676.1	RecA	Control	Alphaproteobacteria	*Bradyrhizobium*	183
ABR27811.1	RecA	Control	Alphaproteobacteria	*Candidatus* Pelagibacter	289
ABS70974.1	RecA	Control	Alphaproteobacteria	*Daeguia*	183
AAC60438.1	RecA	Control	Alphaproteobacteria	*Gluconacetobacter*	348
AAA73517.1	RecA	Control	Alphaproteobacteria	*Gluconobacter*	344
ABC59514.1	RecA	Control	Alphaproteobacteria	*Granulibacter*	351
BAB47705.1	RecA	Control	Alphaproteobacteria	*Mesorhizobium*	365
EAR49467.1	RecA	Control	Alphaproteobacteria	*Oceanicola*	358
AAB51561.1	RecA	Control	Alphaproteobacteria	*Paracoccus*	356
ACH86213.1	RecA	Control	Alphaproteobacteria	*Phyllobacterium*	186
CAA44346.1	RecA	Control	Alphaproteobacteria	*Rhizobium/Agrobacterium* group	361
ABW91115.1	RecA	Control	Alphaproteobacteria	*Rhodobaca*	183
CAA57673.1	RecA	Control	Alphaproteobacteria	*Rhodobacter*	355
ABY73125.1	RecA	Control	Alphaproteobacteria	*Rickettsieae*	362
AAV95306.1	RecA	Control	Alphaproteobacteria	*Ruegeria*	347
ABW82534.1	RecA	Control	Alphaproteobacteria	*Shinella*	161
ACH86226.1	RecA	Control	Alphaproteobacteria	*Sinorhizobium/Ensifer* group	193
AAY81982.1	RecA	Control	Alphaproteobacteria	*Wolbachieae*	304
ABR40036.1	RecA	Control	Bacteroidetes	*Bacteroides*	347
EAP87785.1	RecA	Control	Bacteroidetes	*Croceibacter*	337
EAQ51475.1	RecA	Control	Bacteroidetes	*Leeuwenhoekiella*	336
ABR43209.1	RecA	Control	Bacteroidetes	*Parabacteroides*	350
AAC44506.1	RecA	Control	Bacteroidetes	*Prevotella*	340
AAB82716.1	RecA	Control	Bacteroidetes	*Rhodothermus*	119
ABX83446.1	RecA	Control	Betaproteobacteria	*Acidovorax*	243
ABX26205.1	RecA	Control	Betaproteobacteria	*Alcaligenaceae*	71
CAI08046.1	RecA	Control	Betaproteobacteria	*Aromatoleum*	346
AAM68121.1	RecA	Control	Betaproteobacteria	*Bordetella*	296
AAU08168.1	RecA	Control	Betaproteobacteria	*Burkholderia*	356

AAK01164.1	RecA	Control	Betaproteobacteria	*Cupriavidus*	353
YP_002794795.1	RecA	Control	Betaproteobacteria	*Laribacter*	354
AAB49199.1	RecA	Control	Betaproteobacteria	*Neisseria*	274
EEO31005.1	RecA	Control	Betaproteobacteria	*Oxalobacter*	353
AAU84856.1	RecA	Control	Betaproteobacteria	*Pandoraea*	248
ABX83452.1	RecA	Control	Betaproteobacteria	*Verminephrobacter*	239
AAW39122.1	RecA	Control	Chloroflexi	*Dehalococcoides*	337
AAA74947.1	RecA	Control	Chlamydiae	*Chlamydia*	352
AAP98719.1	RecA	Control	Chlamydiae	*Chlamydophila*	353
AAA75282.1	RecA	Control	Cyanobacteria	*Arthrospira*	365
CAO90208.1	RecA	Control	Cyanobacteria	*Microcystis*	355
AAA88636.1	RecA	Control	Cyanobacteria	*Synechococcus*	348
ACB87024.1	RecA	Control	Deltaproteobacteria	*Bacteriovorax*	171
ACN15826.1	RecA	Control	Deltaproteobacteria	*Desulfobacterium*	347
AAP15354.1	RecA	Control	Deltaproteobacteria	*Desulfuromonas*	151
AAP15351.1	RecA	Control	Deltaproteobacteria	*Desulfuromusa*	179
AAS19285.1	RecA	Control	Deltaproteobacteria	Environmental samples	138
AAS19284.1	RecA	Control	Deltaproteobacteria	Environmental samples	138
AAS19283.1	RecA	Control	Deltaproteobacteria	Environmental samples	146
AAS19282.1	RecA	Control	Deltaproteobacteria	Environmental samples	138
AAS19281.1	RecA	Control	Deltaproteobacteria	Environmental samples	136
AAS19280.1	RecA	Control	Deltaproteobacteria	Environmental samples	138
ABR01055.1	RecA	Control	Deltaproteobacteria	*Geobacter*	172
AAP15361.1	RecA	Control	Deltaproteobacteria	*Geothermobacter*	165
AAP15364.1	RecA	Control	Deltaproteobacteria	*Malonomonas*	114
AAP15363.1	RecA	Control	Deltaproteobacteria	*Pelobacter*	217
BAA21330.1	RecA	Control	Deinococcus-thermus	*Deinococcus*	363
AAK15321.1	RecA	Control	Deinococcus-thermus	*Thermus*	340
AAA62510.1	RecA	Control	Environment	Environmental samples	319
BAB62717.1	RecA	Control	Epsilonproteobacteria	*Campylobacter*	345
AAC43379.1	RecA	Control	Epsilonproteobacteria	*Helicobacter*	347
AHG52898.1	RecA	Control	Firmicutes	*Bacillus*	293
AAS42719.1	RecA	Control	Firmicutes	*Bacillus cereus* group	343
EDK33487.1	RecA	Control	Firmicutes	*Clostridium*	363
EEU78491.1	RecA	Control	Firmicutes	*Enterococcus*	348
ABM68013.1	RecA	Control	Firmicutes	*Epulopiscium*	267
ABY20679.1	RecA	Control	Firmicutes	*Lactobacillus*	208
AAA25216.1	RecA	Control	Firmicutes	*Lactococcus*	365
ACA83089.1	RecA	Control	Firmicutes	*Leuconostoc*	381
AAN15817.1	RecA	Control	Firmicutes	*Listeria*	348
ABC96757.1	RecA	Control	Firmicutes	*Pasteuria*	93

ABR57176.1	RecA	Control	Firmicutes	*Staphylococcus*	349
CAA78955.1	RecA	Control	Firmicutes	*Streptococcus*	388
ABD94349.1	RecA	Control	Firmicutes	*Thermoanaerobacter*	181
AAN46112.1	RecA	Control	Firmicutes	*Virgibacillus*	157
AAZ07710.1	RecA	Control	Firmicutes	*Weissella*	101
ACY40642.1	RecA	Control	Fusobacteria	*Leptotrichia*	239
AAC27115.1	RecA	Control	Gammaproteobacteria	*Acinetobacter*	349
AAB41053.1	RecA	Control	Gammaproteobacteria	*Aeromonas*	353
ABL95925.1	RecA	Control	Gammaproteobacteria	*Aggregatibacter*	165
ACB78181.1	RecA	Control	Gammaproteobacteria	*Aliivibrio*	271
EJF22224.1	RecA	Control	Gammaproteobacteria	*Citrobacter*	354
ACJ20036.1	RecA	Control	Gammaproteobacteria	*Coxiella*	343
ABR13876.1	RecA	Control	Gammaproteobacteria	*Dickeya*	171
ACZ26522.1	RecA	Control	Gammaproteobacteria	*Enterobacter*	239
ACZ26521.1	RecA	Control	Gammaproteobacteria	*Erwinia*	279
CAQ33031.1	RecA	Control	Gammaproteobacteria	*Escherichia*	353
AAX87645.1	RecA	Control	Gammaproteobacteria	*Haemophilus*	354
CAA39097.1	RecA	Control	Gammaproteobacteria	*Legionella*	348
AAA91766.1	RecA	Control	Gammaproteobacteria	*Pantoea*	354
AAK03901.1	RecA	Control	Gammaproteobacteria	*Pasteurella*	354
CAB56783.1	RecA	Control	Gammaproteobacteria	*Pectobacterium*	342
ABA46365.1	RecA	Control	Gammaproteobacteria	*Photobacterium*	276
CAB56804.1	RecA	Control	Gammaproteobacteria	*Proteus*	325
AAB16921.1	RecA	Control	Gammaproteobacteria	*Pseudomonas*	355
ABG34236.1	RecA	Control	Gammaproteobacteria	*Salmonella*	353
CAB56806.1	RecA	Control	Gammaproteobacteria	*Shigella*	353
ACH91009.1	RecA	Control	Gammaproteobacteria	*Stenotrophomonas*	182
CAA43657.1	RecA	Control	Gammaproteobacteria	*Vibrio*	376
ACR61179.1	RecA	Control	Gammaproteobacteria	*Vibrionaceae*	264
BAC24381.1	RecA	Control	Gammaproteobacteria	*Wigglesworthia*	331
AAW76195.1	RecA	Control	Gammaproteobacteria	*Xanthomonas*	373
AAD32599.1	RecA	Control	Gammaproteobacteria	*Xenorhabdus*	358
ABM05840.1	RecA	Control	Gammaproteobacteria	*Yersinia*	153
AAL57321.1	RecA	Control	Spirochetes	*Borrelia*	356
AAA97557.1	RecA	Control	Spirochetes	*Borrelia burgdorferi* group	365
AAL01117.1	RecA	Control	Spirochetes	*Leptospira*	387
AAB59011.1	RecA	Control	Tenericutes	*Acholeplasma*	295
AAF69794.1	RecA	Control	Tenericutes	*Candidatus* Phytoplasma	336
AAL86741.1	RecA	Control	Tenericutes	*Mycoplasma*	351
AAC44644.1	RecA	Control	Tenericutes	*Spiroplasma*	77
YP_776185	DddD	DMSP lyase	Betaproteobacteria	*Burkholderia*	832

WP_005858710	DddD	DMSP lyase	Alphaproteobacteria	*Sagittula*	836
ACV84065	DddD	DMSP lyase	Gammaproteobacteroa	*Halomonas*	836
AAV94883	DddQ	DMSP lyase	Alphaproteobacteria	*Ruegeria*	201
WP_009814827	DddQ1	DMSP lyase	Alphaproteobacteria	*Roseovarius*	202
EAP76001.1	DddQ2	DMSP lyase	Alphaproteobacteria	*Roseovarius*	196
WP_005978225	DddQ	DMSP lyase	Alphaproteobacteria	*Ruegeria*	192
ECW91654	DddQ	DMSP lyase	Environment	Environmental samples	189
EBP74803	DddQ	DMSP lyase	Environment	Environmental samples	193
ECX82089	DddQ	DMSP lyase	Environment	Environmental samples	197
AAV93771	DddW	DMSP lyase	Alphaproteobacteria	*Ruegeria*	152
YP_351475	DddL	DMSP lyase	Alphaproteobacteria	*Rhodobacter*	232
ADK55772	DddL	DMSP lyase	Alphaproteobacteria	*Sulfitobacter*	213
YP_001185042	DddY	DMSP lyase	Gammaproteobacteria	*Shewanella*	414
ADT64689	DddY	DMSP lyase	Betaproteobacteria	*Alcaligenes*	401
WP_009813101	DddP	DMSP lyase	Alphaproteobacteria	*Roseovarius*	446
YP_167522	DddP	DMSP lyase	Alphaproteobacteria	*Ruegeria*	393
EYB22087	DddP	DMSP lyase	Eukaryotic Fungi	*Fusarium*	451
AEQ39091	DddP	DMSP lyase	Gammaproteobacteria	*Oceanimonas*	448
AEQ39103	DddP	DMSP lyase	Gammaproteobacteria	*Oceanimonas*	416

Supplementary Table 6. Accession numbers, taxonomy and length of protein sequences used as probes against the metagenomic datasets.

Strain	Description	Reference or source
Bradyrhizobium diazoefficiens USDA110	Wild type strain	Dr. A. Gates, University of East Anglia (UK)
Cyanothece sp. ATCC 51142	Wild type strain	ATCC bacteria collection (USA)
Escherichia coli 803	Used for all routine work	Wood[1]
E. coli BL21	Strain with T7 polymerase used to express cloned *mddA* genes	Invitrogen
E. coli A118	Strain containing chromosomal copy of Tn*5lac* (tet^R and kan^R)	Simon *et al*.[2]
Pseudomonas deceptionensis $M1^T$	Wild type strain	Carrión *et al.*[3]
Pseudomonas fragi DSM 3456	Wild type strain	DSMZ bacteria collection (Germany)
Pseudomonas sp. GM41	Wild type strain	Dr. A. Pelletier, Oak Ridge National Laboratory (USA)
Pseudomonas psychrophila DSM 17535	Wild type strain	DSMZ bacteria collection (Germany)
Pseudomonas putida ATCC 12623	Wild type strain	ATCC bacteria collection (USA)
Rhizobium leguminosarum J391	Wild type strain (str^R)	Young *et al*.[4]
J564	*P. deceptionensis* $M1^T$ spontaneous rif^R resistant mutant	This study
J565	J564 derivative mutated in *megL* gene by pBIO2221 mutagenesis (kan^R, rif^R and $spec^R$)	This study
J566	J564 derivative mutated in *mddA* gene by marker exchange using pBIO2220 (gm^R, kan^R and rif^R)	This study

Supplementary Table 7. Strains used in this study.

Plasmid	Description	Application	Reference or source
pET21a	*E. coli* T7 expression vector (amp^R)	Used to clone *mddA*	Novagen
pLAFR3	Wide host range cosmid (tet^R)	Used to generate the *P. deceptionensis* genomic library	Friedman *et al.*[5]
pPH1JI	Plasmid of IncP-1 incompatibility group (gm^R)	Plasmid used in marker exchange	Hirsch & Beringer[6]
pRK2013	Helper plasmid containing *mob* and *tra* genes (kan^R)	Mobilisating plasmid used in triparental mattings	Figurski & Helsinki[7]
pBIO1878	Wide host-range *lac* reporter plasmid	Used to make *mddA-lac* fusion	Todd *et al.*[8]
pBIO1879	Suicide plasmid	Vector used in the generation of a $megL^-$ mutant	Todd *et al.*[9]
pBIO2219	pLAFR3 based clone containing *P. deceptionensis* $M1^T$ *mddA* gene and surrounding genomic DNA	Cosmid that conferred MeSH-dependent DMS production to *R. leguminosarum*	This study
pBIO2220	Derivative of pBIO2219 containing Tn*5lacZ* inserted in *mddA* gene	Cosmid used to create a *P. deceptionensis* $M1^T$ $mddA^-$ strain by marker exchange	This study
pBIO2221	Derivative of pBIO1879 containing a 0.5 kb internal fragment of the *P. deceptionensis* $M1^T$ *megL* gene	Used for mutagenesis of the *megL* gene in *P. deceptionensis*	This study
pBIO2223	pET21a clone containing the *P. deceptionensis* $M1^T$ *mddA* gene	Used to express *P. deceptionensis* $M1^T$ MddA protein in *E. coli* BL21	This study
pBIO2224	pET21a clone containing the *Pseudomonas* sp. GM41 *mddA* gene	Used to express *Pseudomonas* sp. GM41 MddA protein (WP_008148420) in *E. coli* BL21	This study
pBIO2225	pET21a clone containing the *B. diazoefficiens* USDA110 *mddA* gene (blr1218)	Used to express *B. diazoefficiens* USDA110 MddA protein (NP_767858.1) in *E. coli* BL21	This study
pBIO2227	pET21a clone containing the *B. diazoefficiens* USDA110 *mddA* gene (blr5741)	Used to express *B. diazoefficiens* USDA110 MddA protein (NP_772381.1) in *E. coli* BL21	This study
pBIO2229	pET21a clone containing the *M. tuberculosis* H37Rv *mddA* gene	Used to express *M. tuberculosis* H37Rv MddA protein (NP_217755) in *E. coli* BL21	This study
pBIO2231	pET21a clone containing the *Cyanothece* sp. ATCC 51142 *mddA* gene	Used to express *Cyanothece* sp. ATCC 51142 MddA protein (YP_001803274) in *E. coli* BL21	This study
pBIO2232	Derivative of pBIO1878 containing promoter of the *P. deceptionensis* $M1^T$ *mddA*	Used to assay *mddA* transcription in *P. deceptionensis* $M1^T$	This study

Supplementary Table 8. Plasmids used in this study.

Primer	Sequence (5'–3')	Use
lacZ	GCCAGCTGGCGAAAGGGGGATGTGC	Map Tn*5lacZ* insertion in pBIO2220
*mddANde*I	CGGATCCCATATGCACACCCGAACCGCCCCG	Generate pBIO2223, pBIO2234; confirm J566 mutation
*mddAEco*RI	GCGAATTCTCAGTCCTGGGAACCCGGG	Generate pBIO2223; confirm J566 mutation
*mddABam*HI	GCGGATCCGTCCTGGGAACCCGGGTTGCG	Generate pBIO2234, pBIO2237
PMdd*Xba*F	CCTCTAGAACTCCTACGGCAAACTGGTTTATGAAG	Generate pBIO2232
PMdd*Nsi*R	CCAATGCATGGTGATCAGCAACACATCCACCAG	Generate pBIO2232
blr1218*Nhe*I	GCATGGCTAGCATGGAACGTCCGATCCGAT	Generate pBIO2225
blr1218*Eco*RI	CGGAATTCCTAAACTGACCGGCGCCAGG	Generate pBIO2225
blr5741*Nde*I	GATGGATCATATGTTCGCGCGCCTCGCAATC	Generate pBIO2227
blr5741*Eco*RI	CGGAATTCTCATCGGGGTGCTCCAACG	Generate pBIO2227
GM41*Nde*I	CGGATCCCATATGAATCCTCCTAACCGGAC	Generate pBIO2224
GM41*Eco*RI	GCGAATTCTCAGGCACTGTGGGTGCC	Generate pBIO2224
CyanoMddNde	CGGATCCCATATGCAGCAGCAACAAACCTTG	Generate pBIO2231
CyanoMddEco	GGAATTCCTGCCTTATCTAACCAGC	Generate pBIO2231
*megLEco*RI	CGGAATTCCATCGTCAACCGCACGTTGTATGG	Generate pBIO2221
*megLXba*I	GCTCTAGAGGGGTGATCCTGCAACATCTGC	Generate pBIO2221
*megLHind*III	CCCAAGCTTATTGAAATGACCGATGGCCCTC	Confirm J565 mutation
*megLEco*RI2	CGGAATTCGGTCGTTGCCGAATATCAGC	Confirm J565 mutation

Supplementary Table 9. Primers used in this study.

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