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SHORT COMMUNICATION

DddW, a third DMSP lyase in a model Roseobacter marine bacterium, *Ruegeria pomeroyi* DSS-3

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***Ruegeria pomeroyi* DSS-3 is a model Roseobacter marine bacterium, particularly regarding its catabolism of dimethylsulfoniopropionate (DMSP), an abundant anti-stress molecule made by marine phytoplankton. We found a novel gene, *dddW,* which encodes a DMSP lyase that cleaves DMSP into acrylate plus the environmentally important volatile dimethyl sulfide (DMS). Mutations in *dddW* reduced, but did not abolish DMS production. Transcription of *dddW* was greatly enhanced by pre-growth of cells with DMSP, via a LysR-type regulator. Close DddW homologs occur in only one other Roseobacter species, and there are no close homologs and only a few related sequences in metagenomes of marine bacteria. In addition to DddW, *R. pomeroyi* DSS-3 had been shown to have two other, different, DMSP lyases, DddP and DddQ, plus an enzyme that demethylates DMSP, emphasizing the importance of this substrate for this model bacterium.**
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A feature of the abundant marine α-proteobacteria known as the Roseobacters is that they catabolize dimethylsulfoniopropionate (DMSP), an anti-stress molecule made in massive amounts ($\sim 10^9$ tons annually) by marine phytoplankton (Kettle *et al.*, 1999). Strikingly, several Roseobacter strains degrade DMSP by more than one mechanism, either demethylating it or cleaving it, in a process that releases the volatile dimethyl sulfide (DMS; González *et al.*, 1999; Newton *et al.*, 2010). DMS has diverse environmental effects; it is a chemoattractant for different marine animals (Seymour *et al.*, 2010) and its oxidation products form cloud condensation nuclei, affecting levels of reflected sunlight (Charlson *et al.*, 1987).

Recent genetic analyses reveal the molecular basis of this metabolic flexibility, as several different enzymes can act on the DMSP substrate. Indeed, some individual bacterial strains have multiple ways to catabolize DMSP. For example, *Ruegeria pomeroyi* DSS-3 contains DmdA, the DMSP demethylase (Howard *et al.*, 2006), plus two genes, *dddQ* and *dddP*, that encode DMSP lyases that cleave DMSP into DMS plus acrylate, although they are in wholly different polypeptide families (Todd *et al.*, 2009, 2011; Kirkwood *et al.*, 2010).

In a microarray study (MK, unpublished) of *R. pomeroyi* genes whose expression was affected by growth in media with 5 mM DMSP, one of the most markedly induced ($\sim$37-fold) was *SPO0453*, as independently noted by Rinta-Kanto *et al.* (2011). The product of this gene, which we term *dddW,* contained a predicted cupin pocket (Figure 1), a widely distributed motif found in many enzymes, where it forms the active site (Dunwell *et al.*, 2004). Such a feature also occurs in the DMSP lyases DddQ (Todd *et al.*, 2011) and DddL (Curson *et al.*, 2008), but both of these are larger ($\sim$22 kDa and $\sim$26 kDa, respectively) than DddW (16.1 kDa), and share no other significant sequence or predicted structural similarities.

To study *dddW*, it was amplified from *R. pomeroyi* genomic DNA, then cloned into the expression vector pET21a. *Escherichia coli* transformants containing the resulting recombinant plasmid produced DMS at a rate of 35 pmol μg protein^{-1} min^{-1} when the substrate DMSP (5 mM) was added to cell-free extracts, compared with a background value in *E. coli* itself of 0.02 pmol μg protein^{-1} min^{-1} (Supplementary methods). This value obtained for the cloned *dddW* is similar to that obtained when the cloned *dddP* and *dddL* genes of *R. pomeroyi* and *Rhodobacter sphaeroides*, respectively, were examined in the same manner (Curson *et al.*, 2008; Todd *et al.*, 2011). On feeding [1-^{14}C]DMSP to cell-free

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Figure 1 Comparison of cupin-like regions of DddL, DddQ and DddW polypeptides. The sequences of the predicted cupin-like motifs of the DddW polypeptides of *Ruegeria pomeroyi* DSS-3 and *Roseobacter* sp. MED193 were compared with those of the DddL DMSP lyases of *Sulfitobacter* sp. EE-36 and *Rhodobacter sphaeroides* 2.4.1 and the DddQ-type lyases of *Ruegeria pomeroyi* DSS-3 and *Roseovarius nubinhibens* ISM. Corresponding gene numbers are underlined. The highly conserved residues in cupins (Dunwell *et al.*, 2004) are indicated in single-letter code above the comparisons. Residues highlighted in black, dark gray or light gray indicate 100%, >80% or >60% conservation, respectively.

extracts of *E. coli* with cloned *dddW*, 100% of the ^{14}C was converted to labeled acrylate after 1-h incubation, confirming that DddW is a *bona fide* 'DMSP lyase'.

To determine its role in *R. pomeroyi*, we made an insertional mutation into *dddW*, using the suicide plasmid pBIO1879 (Supplementary methods). The DddW$^-$ mutant produced DMS from DMSP at a rate ~50% that of wild type, an effect smaller than that seen in a DddQ$^-$ mutant, but similar to that caused by a mutation in *dddP* (Todd *et al.*, 2009, 2011). Thus, DddW, like the other two lyases, contributes to DMSP catabolism in *R. pomeroyi* DSS-3.

To further examine DMSP-dependent regulation of *dddW* expression, we cloned its promoter region into the wide host-range promoter-probe vector pBIO1878, upstream of its *lacZ* reporter. The resulting plasmid, pBIO1945, was mobilized into wild-type *R. pomeroyi* and the transconjugant assayed for β-galactosidase after growth in minimal media that either contained or lacked 5 mM DMSP (Supplementary methods). The *dddW–lacZ* fusion was induced, 10-fold, in the + DMSP media.

Separated by 94 base pairs from the start of *dddW* is a gene, *SPO0454*, which encodes a predicted LysR-type transcriptional regulator. We attempted to mutate *SPO0454* using the same approach as for the insertion into *dddW,* but this was unsuccessful. This may be due to the polar effects of insertions in *SPO0454* on the expression of a downstream gene, *SPO0455*, which is predicted to be co-transcribed with *SPO0454* and which encodes a potentially essential lysyl-tRNA synthetase. We therefore adopted a different approach to show that *SPO0454* regulated *dddW*. First, we conjugated the *dddW–lacZ* fusion plasmid pBIO1945 into the α-proteobacterium *Rhizobium leguminosarum,* which effectively expresses heterologous genes (Young *et al.*, 2006). Into this strain was then transferred pBIO1946, containing intact *SPO0454* including its native promoter, cloned in the wide host-range plasmid pOT2. We then measured the effects of pre-growth in DMSP on *dddW–lacZ* expression (as β-galactosidase activity) in *R. leguminosarum*/pBIO1945 that either contained or lacked the cloned *SPO0454* gene in pBIO1946. In *R. leguminosarum* itself, *dddW–lacZ* was expressed constitutively at a low level in both growth media. However, the presence of *SPO0454* increased β-galactosidase activity ~5-fold, but only when cells were pre-grown with DMSP (Figure 2). Thus, *SPO0454* encodes a transcriptional activator that responds to DMSP. Furthermore, *SPO0454* was auto-regulatory, like other *lysR*-type regulatory genes (Maddocks and Oyston, 2008). This was shown by the behavior in *R. leguminosarum* of an *SPO0454–lacZ* fusion plasmid, termed pBIO1947, that we constructed. This expressed β-galactosidase constitutively in *R. leguminosarum* itself, but was repressed ~5-fold by introducing *SPO0454*, cloned in pBIO1946, irrespective of whether DMSP was in the medium or not (Figure 2).

In addition to its DmdA demethylase (Howard *et al.*, 2006), *R. pomeroyi* DSS-3, remarkably, has three different DMSP lyases, the previously identified DddP and DddQ, and the newly discovered DddW. All these lyases function in *R. pomeroyi*, as mutations in the corresponding genes affect its DMS-emitting phenotype. As judged by the individual mutant phenotypes, in our laboratory conditions, DddW appears to contribute about the same to the flux into DMS production as DddP, but less than DddQ.

Currently, the only other bacterium with a close DddW homolog (65% identical) is *Roseobacter* sp. MED193, whose *dddW* is also transcribed divergently from an *SPO0454* homolog. More poorly conserved homologs (~40% identical to the DddW of *R. pomeroyi* DSS-3) were found in two other Roseobacters, namely *Rhodobacterales bacterium* HTCC2083 and *Citreicella* sp. SE45; nevertheless, DddW is rarer among the deduced proteomes of Roseobacters and other marine bacteria than other Ddd polypeptides (Newton *et al.*, 2010). Consistent with this, there are no very close DddW homologs in marine metagenomic sequences (surveyed in the CAMERA portal at http://camera.calit2.net/), most notably those in the Global Ocean Sampling (GOS) described by Rusch *et al.* (2007). However, four sequences in the GOS had deduced polypeptides with some similarity to DddW (40–50% identical;

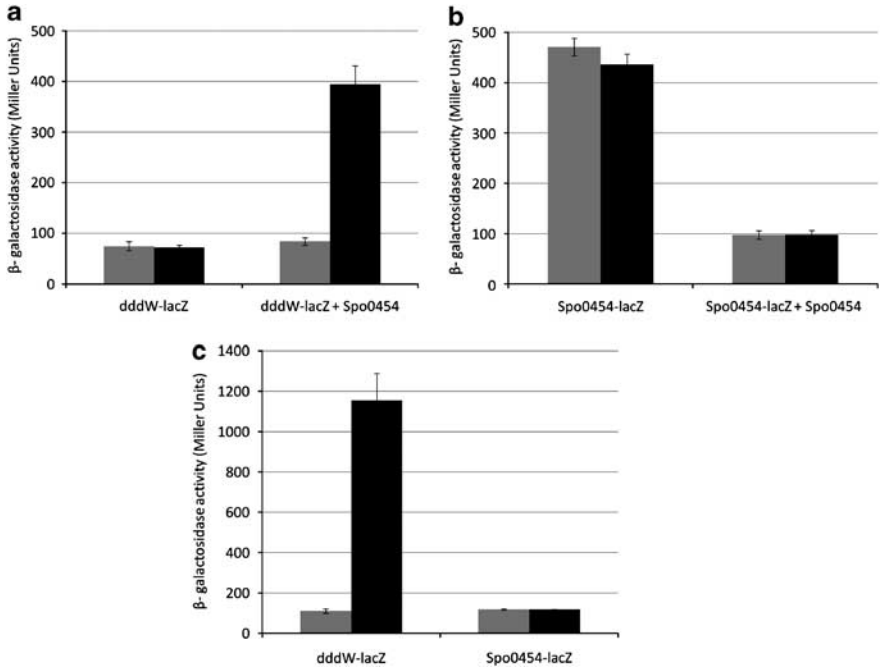

Figure 2 Effects of DMSP and the regulatory *SPO0454* gene on the expression of the *dddW* and *SPO0454* genes of *R. pomeroyi* DSS-3. Cultures of *Rhizobium leguminosarum* strain 3841 (**a**, **b**) or of *Ruegeria pomeroyi* DSS-3 (**c**) and containing either the *dddW–lacZ* fusion plasmid pBIO1945 (**a**, **c**) or the *SPO0454–lacZ* fusion plasmid pBIO1947 (**b**, **c**) were grown in minimal medium that either lacked (gray columns) or contained (black columns) 5 mM DMSP. These strains were assayed in triplicate for β-galactosidase activities, whose values with standard errors are shown in Miller Units. In the *Rhizobium* background, some of the strains with the fusion plasmids also contained pBIO1946, in which the *SPO0454* gene is cloned in the vector pOT2, as indicated.

probability $<e^{-22}$). These were all from the hypersaline lagoon site at Punta Cormorant in Galapagos, the same site at which homologs of a different lyase, DddL, were seen (Curson *et al.*, 2008). However, the ecological significance, if any, of this is unknown. Indeed, given the relatively low level identity of the metagenomic reads and DddW itself, it remains to be confirmed that these correspond to functional DMSP lyases.

Other Roseobacter strains also have multiple DMSP lyases—*Roseovarius nubinhibens,* for example, has two versions of DddQ plus DddP (Todd *et al.*, 2009, 2011). It will be interesting to know if these different enzymes, plus the DmdA demethylase, are particularly adapted to specific environments that vary in the availability of DMSP substrate (see, for example, González *et al.*, 1999) or other factors, such as temperature, pH or the availability of other nutrients. Furthermore, other bacteria have yet other classes of enzymes that release DMS from DMSP (Todd *et al.*, 2007; Curson *et al.*, 2008, 2011), further emphasizing the genetic diversity of this important environmental biotransformation.

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