



That familiar pong on the beach is down to dimethylsulfide (DMS) produced by microbes. **Andy Johnston** shows that the molecular biology behind its formation is amazingly complex and diverse.

# On the microbial genetics of seaside smells

▲ Kynance Cove, Cornwall. Ian Atherton, SGM

Ever walk along the shore, taking in that tangy aroma? Ever wonder how ocean birds, such as Shearwaters find their lunch and dinner over the featureless wastes of the Atlantic? Or why seals poke their noses above water, taking in the air? Ever ask how clouds form over the oceans? Many questions, but just one answer – dimethylsulfide (DMS), a gas with many influences and one, like the others in this issue, which is made by microbial action.

The great savant, James Lovelock, realised 40 years ago that DMS is the pre-dominant form of sulfur that escapes from the seas to the air and thence back to land, thus completing

the global sulfur cycle. (Bizarrely, the received wisdom of that time was that the culprit was hydrogen sulfide, a gas with a very different aroma!)

We now know that around 50 million tonnes of DMS emerge from the oceans and their margins, but this is just a 'drop in the ocean' (around 10 %) of the total annual production. The most important effect of DMS is that it is oxidized in the air to form sulfates, which in turn form cloud condensation nuclei which affect local weather and, perhaps, world climate. Thus, one of those earlier questions is answered, but to address the others, we must know the provenance of the starting material for DMS production.

## The source material, dimethylsulfoniopropionate

The substrate for DMS production is dimethylsulfoniopropionate (DMSP), with around one billion tonnes of this metabolite being cycled each year. As if this were not enough, its catabolic legacy, as DMS, further adds to its importance – yet how many of us are familiar with this compound? DMSP is among the most abundant intracellular molecules in the myriads of single-celled phytoplankton, including many haptophytes, whose forebears formed, among other places, the White Cliffs of Dover. It also occurs in macroalgal seaweeds and in a few known land plants. DMSP is a compatible solute, protecting cells against UV, oxidative and osmotic challenges, but there is still some debate about its precise role(s). What is clear, though, is that microbes catabolize DMSP in various ways – on a massive scale, and with global consequences.

As far back as 1956, a red algal seaweed, *Polysiphonia*, that makes DMSP, was shown to catabolize it, via an enzyme called DMSP lyase that would also generate acrylate plus a proton. Indeed, many plankton that make DMSP can also degrade

it when stressed, perhaps liberating the resultant DMS and acrylate as defence or signalling molecules. However, the majority of DMSP catabolism is mediated by marine bacteria, some of which can grow on it as their sole carbon source. These include strains of the *Alpha-* (various *Roseobacter* spp.), *Beta-* (*Alcaligenes*) and *Gammaproteobacteria* (e.g. *Vibrio*).

Bacteria degrade DMSP in at least two very different ways. In one, it is demethylated, the thiol products being used as sources of both carbon and sulfur. The other general mechanism releases DMS, which acts as a chemoattractant beacon for those seabirds, seals and other animals that eat plankton, or their cohabiting fish and crustaceans – and, for us, it contributes to that sentient seaside smell.

Despite its importance, and the ease of cultivating many bacteria that degrade DMSP, not one gene involved in the process had been identified until 2006. However, recent studies have begun to provide some genetic insights, although, in reality they set more questions on topics that range from enzymology to evolution.

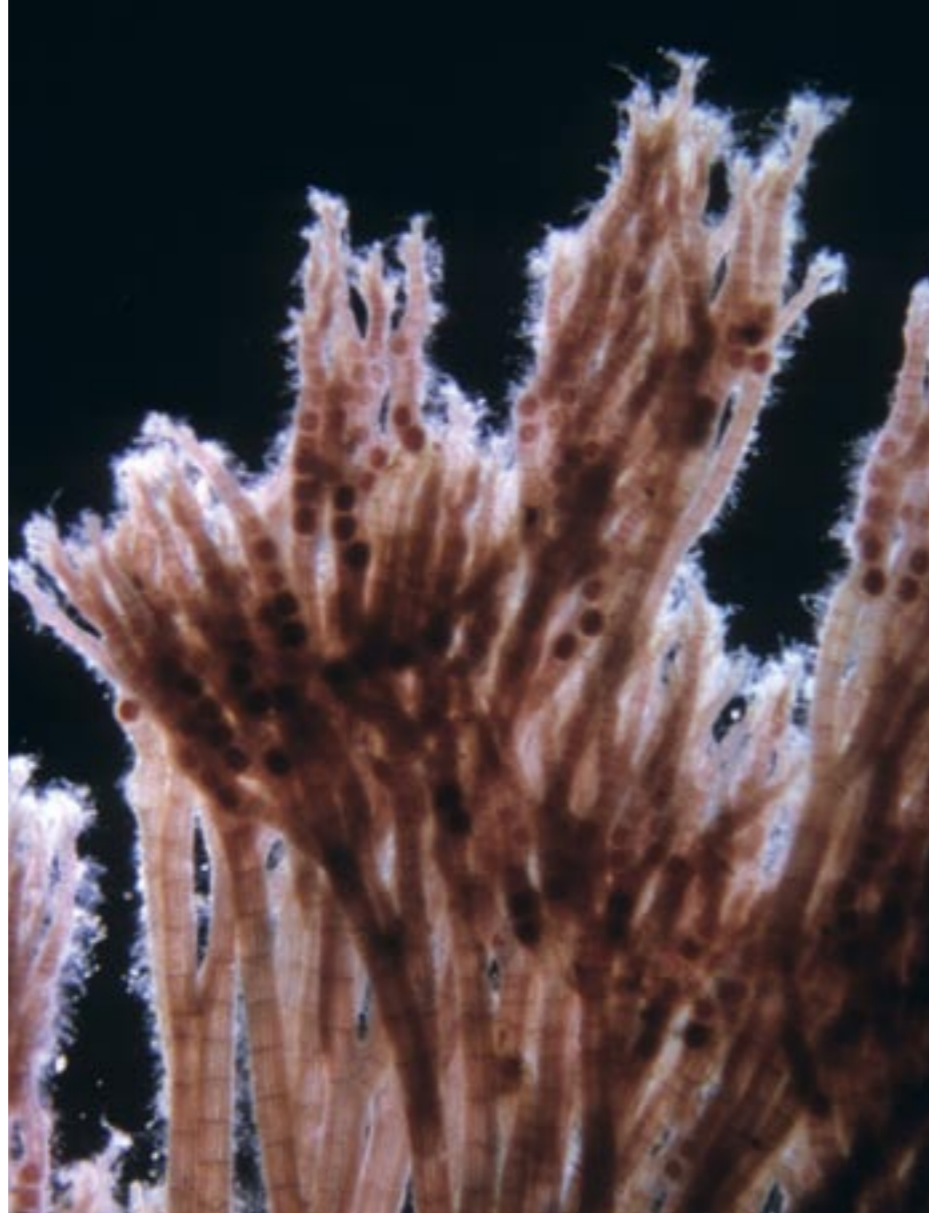
### The *dmd* and *ddd* genes for bacterial catabolism of DMSP

The first reported gene for DMSP catabolism was *dmdA*, which encodes DMSP demethylase, the initial step in the demethylation pathway. Found by Mary Ann Moran's lab in a strain of the abundant *Roseobacter* clade of the marine *Alphaproteobacteria*, close homologues of *DmdA* occur in other marine alphaproteobacteria, including the SAR11 bacterium *Pelagibacter ubique*, the most populous organism on the planet.

Then, the first of the *ddd* (DMSP-dependent DMS) genes was found in the gammaproteobacterium *Marinomonas*, isolated from roots of the grass *Spartina*, one of the few angiosperms known to make DMSP. A single cloned gene, called *dddD*, was enough to confer to *Escherichia coli* the ability to make DMS when grown on DMSP – as detected by the evocative aroma from the Petri dishes. However, the *DddD* polypeptide was not the expected DMSP lyase, but was an acyl-CoA transferase that could add CoA to DMSP. The predicted DMSP-CoA product is unstable, and could spontaneously release DMS, plus acryloyl CoA, with the latter being further catabolized for growth on DMSP as carbon source.

Strikingly, *DddD* was lacking from the deduced proteomes of several bacteria that are known to make DMS from DMSP and whose genomes had been sequenced. So, there must be different ways to make DMS, something that had been implied by work by Duane Yoch some time ago.

One such 'alternative' system is specified by the *dddL* gene, which was identified in *Sulfitobacter*, a *Roseobacter*-type marine bacterium. The *DddL* polypeptide, which had no homologues with known function, may be the long-predicted DMSP-lyase, since *E. coli* that expresses *dddL* makes acrylate plus DMS.



▲ Close-up of tetraspores of the red alga seaweed *Polysiphonia nigrescens* from Devon. D.P. Wilson / FLPA-images.co.uk

▼ Salt grass (*Spartina alterniflora*), one of the few angiosperms known to make DMSP. John Bova / Science Photo Library



### Gene sampling of the oceans from the comfort of one's laptop

Thanks to the industrial-scale sequencing of the partial genomes of uncultured marine bacteria, done largely by Craig Venter's Global Ocean Survey and other projects supported by the Moore Foundation, one can survey any given gene in the seemingly astronomical list of genes in these metagenomes. Of the genes mentioned above, *dmdA* is the clear 'winner' with hundreds of hits in these marine gene databases, in keeping with the importance of demethylation at a global level and the finding of *dmdA* in *P. ubique*. The *dddL* and *dddD* genes rather trail behind, with nine and six apiece, but even these relatively low numbers represent trillions of total *dddD* and *dddL* genes, in bacteria strewn around the planet's oceans. Of course, numbers are not everything, and the levels of expression and activities of the enzymes will also contribute to the fluxes through the different pathways.

### Rampant gene transfer – among unexpected bedfellows

The *ddd* genes are prone to widespread horizontal gene transfer (HGT), in some cases to totally unexpected organisms. *dddL* is the least promiscuous, being found only among occasional strains of the Order *Rhodobacterales*, though even here, there was one nice surprise. Going back to Cornelius van Neil's studies in the 1930s, *Rhodobacter sphaeroides* is something of a lab rat for studies on bacterial photosynthesis, motility and bioenergetics, but it had never been suspected of making DMS. However, of three strains of *R. sphaeroides* sequenced in Sam Kaplan's lab, two had a close *DddL* homologue and one did not. Gratifyingly, their DMS-producing phenotypes were entirely as predicted from their genomes.

The other gene, *dddD* is much more adventurous. It was not totally surprising that *DddD* also occurs in other, known DMS-emitting marine bacteria such as *Sagittula*. Even here though, *Sagittula* is an alphaproteobacterium, so presumably it acquired (or donated) *dddD* by HGT from/to the rather distantly related gammaproteobacterium *Marinomonas*. But, wholly unexpected was the presence of *DddD* in some terrestrial bacteria, which interact with angiosperm roots. These included some strains of root-colonizing *Burkholderia* (*Betaproteobacteria*) and a highly unusual strain (NGR234) of *Rhizobium*, which forms nitrogen-fixing root nodules on many different legumes and even (uniquely) on some non-legumes. Perhaps these root-dwelling bacteria associate with some, unknown, angiosperms that, like *Spartina*, make DMSP.

### What next?

These are early days, but it seems likely that there will be good progress in elucidating the biochemistry of the entirety

of the various DMSP catabolic pathways. However, this article started with rhetorical questions, and ends with real ones, prompted by these tantalizing glimpses into the genetics of DMS production. Why do bacteria use the DMS-producing pathway in the first place, when demethylation would allow them to recoup all the C and S, and not see much of it drift off into the seas and the skies as DMS? This is particularly pertinent to strains that contain both demethylation and DMS-emitting pathways. How and why do they 'choose' which system to use under any given set of environmental circumstances? What are the constraints (if any) to HGT of the *ddd* genes. And finally, there are still many environments such as corals and the tissues of some invertebrate animals, like clams, that are awash with DMSP but whose microbiology has not been examined, certainly not at a molecular genetic level. What other surprises lie in wait in the deeps?

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### Further reading

- Charlson, R., Lovelock, J., Andreae, M. & Warren, S. (1987). Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. *Nature* 326, 655–661.
- Curson, A.R.J., Rogers, R., Todd, J.D., Brearley, C.A. & Johnston, A.W.B. (2008). Molecular genetic analysis of a dimethylsulfoniopropionate lyase that liberates the climate-changing gas dimethylsulfide in several marine  $\alpha$ -proteobacteria and *Rhodobacter sphaeroides*. *Environ Microbiol* 10, 757–767.
- Howard, E.C., Henriksen, J.R., Buchan, A. & others (2006). Bacterial taxa that limit sulfur flux from the ocean. *Science* 314, 649–665.
- Lovelock, J.E., Maggs, R.J. & Rasmussen, R.A. (1972). Atmospheric dimethyl sulphide and the natural sulphur cycle. *Nature* 237, 452–453.
- Simó, R. (2001). Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological and evolutionary links. *Trends Ecol Evol* 16, 287–294.
- Stefels, J., Steinke, M., Turner, S., Malin, G. & Belviso, S. (2007). Environmental constraints on the production and removal of the climatically active gas dimethylsulphide (DMS) and implications for ecosystem modelling. *Biogeochemistry* 83, 245–275.
- Todd, J.D., Rogers, R., Li, Y.G. & others (2007). Structural and regulatory genes required to make the gas dimethyl sulfide in bacteria. *Science* 315, 666–669.
- Vallina, S.M. & Simó, R. (2007). Strong relationship between DMS and the solar radiation dose over the global surface ocean. *Science* 315, 506–508.