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**DMS oxidation coupled to biomass production by a marine *Flavobacterium***

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**ABSTRACT**

Dimethylsulfide (DMS) is an important climatically-active gas, which in the sea is produced primarily by microbial metabolism of the algal compatible solute dimethylsulfoniopropionate. Laboratory growth of *Bacteroidetes* with DMS resulted in its oxidation to dimethylsulfoxide, but only in the presence of glucose. It was hypothesized that electrons liberated from sulfur oxidation were used to augment biomass production.

The gas dimethylsulfide (DMS) is regarded as an important volatile sulfur species because in the atmosphere its oxidation products are postulated to influence large-scale climate regulation through enhanced cloud formation (5). The production of DMS from marine sources has been calculated to represent a significant (ca. 50-84 %) proportion of its total global production (2, 30). While terrestrial DMS production is primarily industrial in origin, marine DMS comes principally from microbial breakdown of the algal compatible solute, dimethylsulfoniopropionate (DMSP) (20). However, only a relatively small fraction of the total DMS produced escapes to the atmosphere (3, 10, 21), with the majority being turned over by biological processes in a matter of days (e.g. 0.6-4.6 days, see (21)).

At present, the primary processes driving DMS removal are believed to be photochemical oxidation (15) and bacterial consumption (21). While microbial utilisation of DMS has been demonstrated in several groups of C<sub>1</sub>-utilising marine organisms, such as *Methylophaga* (8, 25, 27), it is still unclear how important these organisms are to the removal of DMS in the surface ocean. Instead, a growing body of evidence shows that under certain circumstances much of the DMS removed (e.g. 81-93 %; (7)) is not consumed for carbon or sulfur by bacterial cells, but instead ends up in a pool of dissolved non-volatile sulfur, composed of dimethylsulfoxide (DMSO) and sulfate (7, 22, 27, 32). Therefore, there is still uncertainty about the processes responsible for the removal of DMS from the surface ocean, and this needs to be resolved if we are to be able to predict DMS concentrations as a result of changing environmental conditions.

### **DMS is oxidised to DMSO by diverse members of the *Bacteroidetes***

The oxidation of DMS to DMSO by bacteria was first demonstrated during photoautotrophic growth of purple sulfur bacteria (14, 28, 31). Later work showed that DMS oxidation also occurs during chemoheterotrophic bacterial growth (17). While DMS oxidation has been suggested as a likely property of many marine bacteria due to the ubiquitous occurrence of DMS in the ocean (14), surprisingly few bacteria typical of productive surface ocean waters have been shown to oxidize DMS. *Sagittula stellata* E-37 is an exception (12), as it is a member of the *Roseobacter*-clade that can be abundant in productive surface waters (4). Importantly, the work of Vila-Costa and colleagues (27) implicated a wider range of bacteria to be capable of DMS turnover. They identified *Methylophaga* predominantly in DMS-enriched mesocosm experiments conducted using Sargasso Sea and Gulf of Mexico seawater, but they also detected *Alphaproteobacteria* and several members of the *Flavobacteria* (*Bacteroidetes*), whose abundances were stimulated by DMS enrichment.

It was during work with the cultivable bacteria isolated from the DMSP-producing dinoflagellate, *Scrippsiella trochoidea* CCAP 1134/1, that we demonstrated that a wide range of these isolates were capable of DMS oxidation (D. Shenoy, A. D. Hatton, D. H. Green *et al.*, in preparation). Notably, the rates of DMS oxidation were typically higher for the *Bacteroidetes* isolates. We reasoned that, as

*Flavobacteria* are ubiquitous and can comprise up to ~40 % of total bacterial abundance in surface waters (1, 9, 11), this group of organisms could be important to the turnover of DMS.

To test this hypothesis, we examined five *Bacteroidetes* strains (Table 1), four isolated from the dinoflagellate *Scrippsiella trochoidea* CCAP 1134/1, and *Leeuwenhoekiella blandensis* MED 217 isolated from Mediterranean seawater (23), to establish whether DMS oxidation was a common phenotype of marine *Bacteroidetes*. The isolates were initially grown to stationary phase in ZM/10 broth, a dilute yeast extract/peptone seawater medium (13), then washed and suspended in 1.5 ml basal seawater medium supplemented with Fe-EDTA and vitamins (12) and sealed in 6 ml crimp-top glass vials with PTFE lined stoppers, to which ca. 1.5  $\mu$ M DMS was added in the presence or absence of 5 mM glucose. All samples were incubated in the dark (ca. 21°C) to minimise photochemical oxidation of DMS. Samples were removed periodically for DMS and DMSO analysis by pulse flame photometric gas chromatography (GC) as described previously (15, 16).

The results showed that after 72 hr incubation the *Bacteroidetes* strains had removed between 46-97 % of the DMS, but only in the presence of glucose (Table 1). If glucose was omitted, relatively little (0-14 %) of the DMS was removed. On average, ~90 % of the DMS removed could be quantitatively accounted for as DMSO (Table 1). Thus, diverse members of the *Bacteroidetes* (*Flavobacteria* and *Sphingobacteria*) are capable of DMS removal via its oxidation to DMSO. However, as glucose presence was essential for a substantive amount of the DMS to be oxidised, this suggested that this process was potentially co-metabolism. Following Dalton and colleagues' re-definition of co-metabolism (6), we hypothesize that glucose, as the "growth substrate", is providing the reducing equivalents necessary for the oxidation of DMS, the "non-growth substrate".

### **DMS oxidation is contingent on an available source of glucose**

To examine the requirement of glucose for DMS oxidation, we tested varying glucose concentrations on DMS removal and DMSO formation by one strain of *Flavobacteria*, DG1233. This strain was chosen as it grew well on glucose in basal seawater medium, and it was typical of other *Flavobacteria* in our collection. The methodology was as above, but glucose concentrations were varied between 0-5 mM. This experiment showed that glucose concentrations  $\geq 250 \mu$ M had a significant effect (Students *t*-test,  $\alpha = 0.01$ ) on the rate and amount of DMS oxidised to DMSO (Fig. 1). Lower concentrations of glucose ( $\leq 25 \mu$ M) had relatively little effect on the total amount of DMS oxidised. For these lower glucose concentrations, it was noted that after 24 hr, all growth ( $A_{540nm}$ ) had ceased (data not shown). This was assumed to be due to the complete utilisation of glucose as the sole available carbon source, and thus, we infer there were insufficient reducing equivalents available for DMS oxidation. The overall interpretation was that glucose, possibly as an available source of reducing equivalents, was essential for DMS co-metabolism by strain DG1233.

### DMS oxidation can be coupled to biomass production

The oxidation of DMS to DMSO will yield 2 electrons, which can be coupled to protein and biomass formation in purple sulphur bacteria (14, 28, 31). As *Bacteroidetes* belong to a separate phylum, we sought to understand whether they could also couple electron flow from DMS oxidation to biomass production. To test this question we grew DG1233 in 1.5 ml basal medium containing 5 mM glucose in crimped 6 ml serum vials as above, and supplemented each bottle with DMS at concentrations ranging between 0-34 mM. At time points up to 96 hr, triplicate vials of each DMS treatment were sacrificed for DMSO measurement, cell turbidity and total protein assay. Samples for DMSO analysis were frozen immediately and stored (-20°C) until analyzed by GC as above. Cell turbidity was measured immediately using a UV-Vis spectrometer ( $A_{540\text{nm}}$ ), and the bacterial cells were harvested by centrifugation (13,000 x g for 30 min), suspended in sterile deionised water and re-centrifuged to collect the washed cells. The cell pellets were then stored frozen (-20°C) until protein was assayed using the BCA protein assay (26).

Growth of DG1233 with increasing concentrations of DMS resulted in increasing amounts of DMSO being formed (Fig. 2A), and from this data, the apparent saturation constant ( $K_m$ ) and  $V_{\text{max}}$  were calculated to be 70  $\mu\text{M}$  and 1.2  $\mu\text{M hr}^{-1}$  respectively. Remarkably, this  $K_m$  is orders of magnitude higher than the typical range of seawater DMS concentrations of around 2-10 nM (19). This estimated  $K_m$  was of comparable magnitude to the 38  $\mu\text{M}$  reported for DMS oxidation by the purple sulphur bacterium *Thiocapsa roseopersicina* M1 (28), but was appreciably higher than 2  $\mu\text{M}$  reported for another strain of *T. roseopersicina* M11 (18). These variable enzymatic efficiencies may reflect the involvement of different enzyme systems, or it may reflect organisms adaptation to niches of differing DMS concentrations, which are potentially high on or around algal surfaces and particles (24).

Simultaneous measurement of total cell protein and turbidity showed that the amount of biomass formed increased with increasing DMS concentrations  $\geq 338 \mu\text{M}$  (Fig. 2B-C). The individual rates of protein and biomass formation over the 96 hr period for DMS concentrations  $\geq 338 \mu\text{M}$  were all significantly greater than both the no DMS and 11  $\mu\text{M}$  DMS treatments (Student's *t*-test,  $\alpha = 0.01$ ; data not shown). Regression analysis of the rates of DMSO and protein formation, with the latter normalized to remove the contribution of glucose to protein formation, were used to estimate the growth augmentation derived from DMS oxidation (Fig. 3). This was calculated to be 0.138  $\mu\text{g protein ml}^{-1}$  per  $\mu\text{M}$  DMSO formed. This is ca. 5 to 25-fold higher than the previously reported growth yields for purple sulfur bacteria (18, 28, 31), and implies that DMS oxidation can be a readily available source of energy by DG1233.

Overall, this study suggests that DMS co-metabolism is likely to be an important auxiliary energy source for *Flavobacteria*, and be widespread amongst other *Bacteroidetes*. However, this study clearly cannot infer the significance DMS co-metabolism has on bacterial growth and energetics in the field as the concentrations tested here were orders of magnitude higher than the 2-10 nM seawater DMS concentrations these organisms are typically exposed to. Nevertheless, *Flavobacteria* are an

ecologically important lineage of bacteria in productive waters, where they are frequent colonizers of both living and decaying particulate organic matter (1, 9, 11). Therefore, it is highly likely that this association with organic-rich material will bring *Flavobacteria* into contact with elevated concentrations of both DMS and labile organic matter. The result of which can be the co-metabolism of DMS generating an electron flow that can be utilized for cellular energetics, such as biomass formation. Furthermore, as DMS is ubiquitous throughout the world's surface ocean, the observations of this study suggest that DMS oxidation could be an important mechanism by which marine bacteria provision their cellular energy requirements.

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## REFERENCES

1. **Abell, G. C. J., and J. P. Bowman.** 2005. Ecological and biogeographic relationships of class Flavobacteria in the Southern Ocean. *FEMS Microbiol Ecol* **51**:265-277.
2. **Andreae, M. O.** 1990. Ocean-atmosphere interactions in the global biogeochemical sulphur cycle. *Mar Chem* **30**:1-29.
3. **Archer, S. D., F. J. Gilbert, P. D. Nightingale, M. V. Zubkov, A. H. Taylor, G. C. Smith, and P. H. Burkill.** 2002. Transformation of dimethylsulphoniopropionate to dimethyl sulphide during summer in the North Sea with an examination of key processes via a modelling approach. *Deep-Sea Res II* **49**:3067-3101.
4. **Buchan, A., J. M. Gonzalez, and M. A. Moran.** 2005. Overview of the marine *Roseobacter* lineage. *Appl Environ Microbiol* **71**:5665-77.
5. **Charlson, R. J., J. E. Lovelock, M. O. Andreae, and S. G. Warren.** 1987. Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. *Nature* **326**:655-661.
6. **Dalton, H., D. I. Stirling, and J. R. Quayle.** 1982. Co-metabolism [and discussion]. *Phil Trans R Soc Lond B* **297**:481-496.
7. **del Valle, D. A., D. J. Kieber, and R. P. Kiene.** 2007. Depth-dependent fate of biologically-consumed dimethylsulfide in the Sargasso Sea. *Mar Chem* **103**:197-208.
8. **deZwart, J. M. M., P. N. Nelisse, and J. G. Kuenen.** 1996. Isolation and characterization of *Methylophaga sulfidovorans* sp nov: An obligately methylotrophic, aerobic, dimethylsulfide oxidizing bacterium from a microbial mat. *FEMS Microbiol Ecol* **20**:261-270.
9. **Fandino, L. B., L. Riemann, G. F. Steward, and F. Azam.** 2005. Population dynamics of *Cytophaga-Flavobacteria* during marine phytoplankton blooms analyzed by real-time quantitative PCR. *Aquat Microbial Ecol* **40**:251-257.
10. **Gabric, A., N. Murray, L. Stone, and M. Kohl.** 1993. Modelling the production of dimethylsulfide during a phytoplankton bloom. *J Geophys Res* **98**:22,805.
11. **Gomez-Pereira, P. R., B. M. Fuchs, C. Alonso, M. J. Oliver, J. E. E. van Beusekom, and R. Amann.** 2010. Distinct flavobacterial communities in contrasting water masses of the North Atlantic Ocean. *Isme J* **4**:472-487.
12. **Gonzalez, J. M., R. P. Kiene, and M. A. Moran.** 1999. Transformation of sulfur compounds by an abundant lineage of marine bacteria in the alpha-subclass of the class Proteobacteria. *Appl Environ Microbiol* **65**:3810-3819.
13. **Green, D. H., L. E. Llewellyn, A. P. Negri, S. I. Blackburn, and C. J. S. Bolch.** 2004. Phylogenetic and functional diversity of the cultivable bacterial community associated with the paralytic shellfish poisoning dinoflagellate *Gymnodinium catenatum*. *FEMS Microbiol Ecol* **47**:345-357.

14. **Hanlon, S. P., R. A. Holt, G. R. Moore, and A. G. McEwan.** 1994. Isolation and characterization of a strain of *Rhodobacter sulfidophilus*: A bacterium which grows autotrophically with dimethylsulphide as electron donor. *Microbiology* **140**:1953-1958.
15. **Hatton, A. D.** 2002. Influence of photochemistry on the marine biogeochemical cycle of dimethylsulphide in the northern North Sea. *Deep-Sea Res II* **49**:3039-3052.
16. **Hatton, A. D., G. Malin, A. G. McEwan, and P. S. Liss.** 1994. Determination of dimethyl sulfoxide in aqueous solution by an enzyme-linked method. *Anal Chem* **66**:4093-4096.
17. **Horinouchi, M., T. Yoshida, H. Nojiri, H. Yamane, and T. Omori.** 1999. Oxidation of dimethyl sulfide by various aromatic compound oxygenases from bacteria. *Biotechnol Lett* **21**:929-933.
18. **Jonkers, H. M., M. Jansen, M. Van der Maarel, and H. Van Gemerden.** 1999. Aerobic turnover of dimethyl sulfide by the anoxygenic phototrophic bacterium *Thiocapsa roseopersicina*. *Arch Microbiol* **172**:150-156.
19. **Kettle, A. J., M. O. Andreae, D. Amouroux, T. W. Andreae, T. S. Bates, H. Berresheim, H. Bingemer, R. Boniforti, M. A. J. Curran, and G. R. DiTullio.** 1999. A global database of sea surface dimethylsulfide (DMS) measurements and a procedure to predict sea surface DMS as a function of latitude, longitude, and month. *Global Biogeochem Cycles* **13**:399-444.
20. **Kiene, R. P.** 1990. Dimethyl sulfide production from dimethylsulfoniopropionate in coastal seawater samples and bacterial cultures. *Appl. Environ. Microbiol.* **56**:3292-3297.
21. **Kiene, R. P., and T. S. Bates.** 1990. Biological removal of dimethyl sulphide from sea water. *Nature* **345**:702-705.
22. **Kiene, R. P., and L. J. Linn.** 2000. The fate of dissolved dimethylsulfoniopropionate (DMSP) in seawater: tracer studies using <sup>35</sup>S-DMSP. *Geochimica et Cosmochimica Acta* **64**:2797-2810.
23. **Pinhassi, J., J. P. Bowman, O. I. Nedashkovskaya, I. Lekunberri, L. Gomez-Consarnau, and C. Pedros-Alio.** 2006. *Leeuwenhoekiella blandensis* sp. nov., a genome-sequenced marine member of the family Flavobacteriaceae. *Int J Syst Evol Microbiol* **56**:1489-1493.
24. **Scarratt, M., G. Cantin, M. Levasseur, and S. Michaud.** 2000. Particle size-fractionated kinetics of DMS production: where does DMSP cleavage occur at the microscale? *J Sea Res* **43**:245-252.
25. **Schäfer, H.** 2007. Isolation of *Methylophaga* spp. from marine dimethylsulfide-degrading enrichment cultures and identification of polypeptides induced during growth on dimethylsulfide. *Appl Environ Microbiol* **73**:2580-2591.
26. **Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk.** 1985. Measurement of protein using bicinchoninic acid. *Anal Biochem* **150**:76-85.
27. **Vila-Costa, M., D. A. del Valle, J. M. Gonzalez, D. Slezak, R. P. Kiene, O. Sanchez, and R. Simo.** 2006. Phylogenetic identification and metabolism of marine dimethylsulfide-consuming bacteria. *Environ Microbiol* **8**:2189-2200.
28. **Visscher, P. T., and H. van Gemerden.** 1991. Photo-autotrophic growth of *Thiocapsa roseopersicina* on dimethylsulphide. *FEMS Microbiol Lett* **81**:247-250.
29. **Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole.** 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* **73**:5261-7.
30. **Watts, S. F.** 2000. The mass budgets of carbonyl sulfide, dimethyl sulfide, carbon disulfide and hydrogen sulfide. *Atmospheric Environment* **34**:761-779.
31. **Zeyer, J., P. Eicher, S. G. Wakeham, and R. P. Schwarzenbach.** 1987. Oxidation of dimethylsulfide to dimethylsulfoxide by phototrophic purple bacteria. *Appl Environ Microbiol* **53**:2026-2032.
32. **Zubkov, M. V., B. M. Fuchs, S. D. Archer, R. P. Kiene, R. Amann, and P. H. Burkil.** 2002. Rapid turnover of dissolved DMS and DMSP by defined bacterioplankton communities in the stratified euphotic zone of the North Sea. *Deep-Sea Res II* **49**:3017-3038.

TABLE 1. DMS oxidation by marine *Bacteroidetes* isolates<sup>a</sup>.

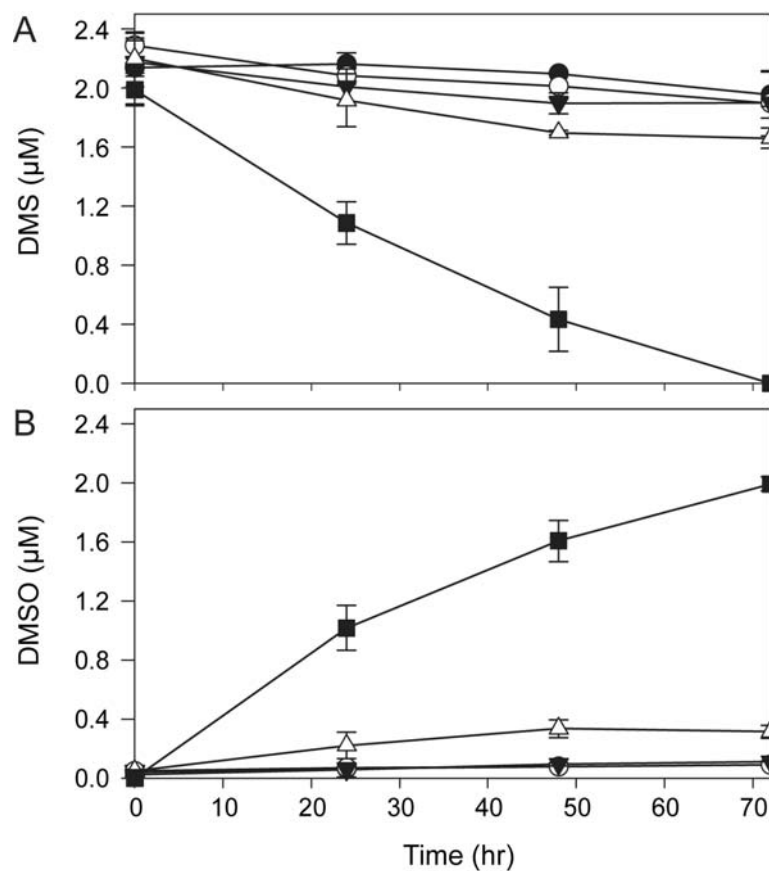
<i>Bacteroidetes</i> Strains <sup>b</sup>		No Glucose		Glucose	
		% DMS	% DMSO	% DMS	% DMSO
		Removed <sup>c</sup>	Formed <sup>d</sup>	Removed <sup>c</sup>	Formed <sup>d</sup>
<i>Flavobacteria; Flavobacteriaceae</i>					
<i>Muricauda</i>	DG1233	0	–	89	99.9
<i>Arenibacter</i>	DG1238	13.9	54.4	97.4	100
<i>Leeuwenhoekiella</i>	MED217	1.4	100	84.4	98.1
<i>Sphingobacteria; Flammeovirgaceae</i>					
<i>Reichenbachiella</i>	DG1232	0	–	46.3	70.3
<i>Roseivirga</i>	DG1392	5.8	100	85.8	100

<sup>a</sup> Strains were incubated with ~1.5  $\mu$ M DMS and with or without 5 mM glucose.

<sup>b</sup> Taxonomy is based on the affiliation of 16S rRNA gene sequences as determined by the RDP II Classifier programme (29). Accession numbers for strains DG1232-DG1392 are: DQ486479, DQ486480, DQ486485 and DQ486489.

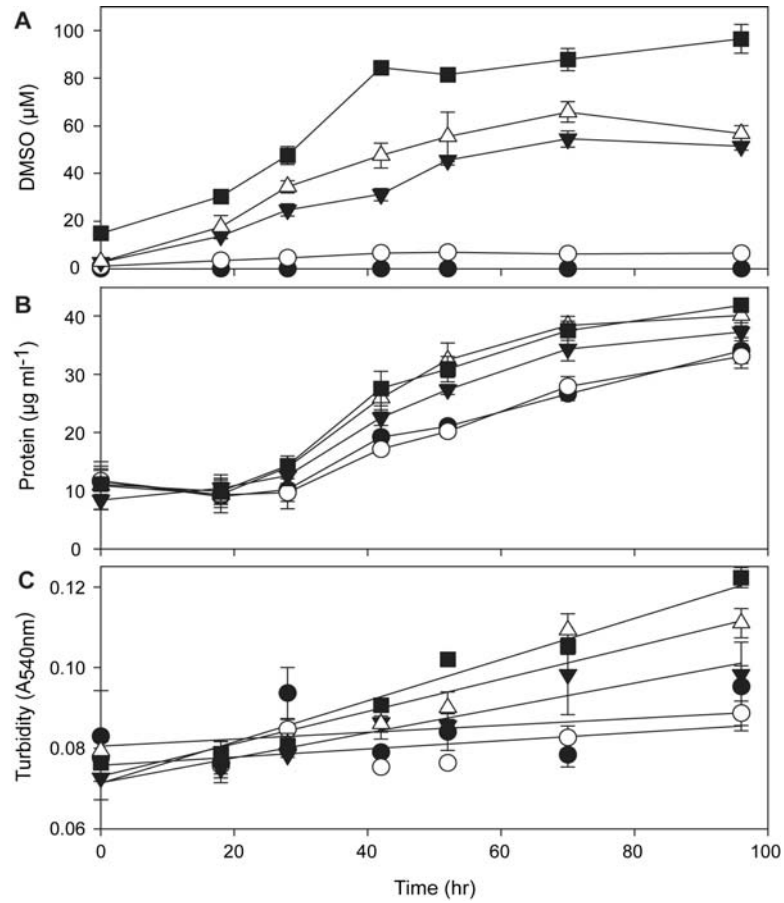
<sup>c</sup> Percentage of DMS removed after 72 hr in the presence or absence of 5 mM glucose.

<sup>d</sup> Percentage of DMS removed that was quantitatively recovered as DMSO.

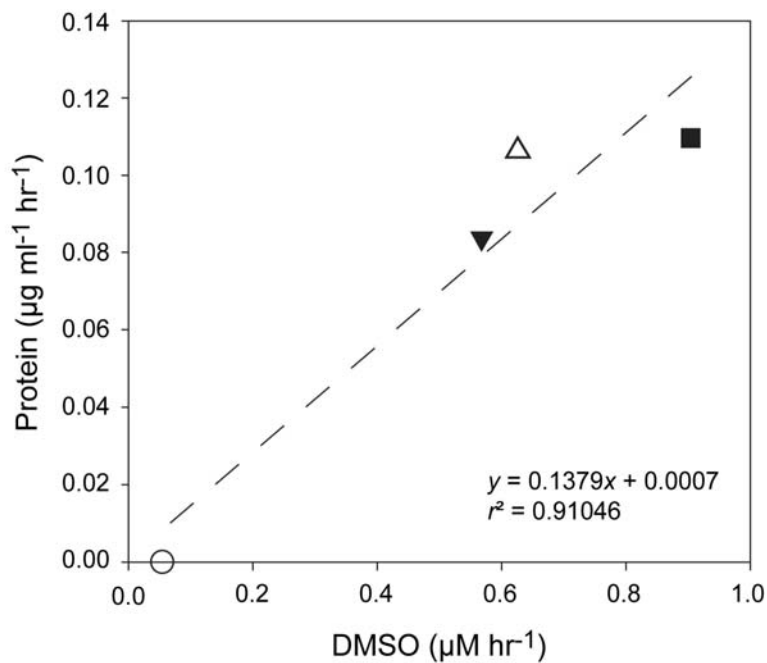


**FIG. 1.** DMS oxidation by DG1233 in the presence of increasing concentrations of glucose. (A) DMS removal and (B) concomitant DMSO formation at each glucose concentration over the period of 72 hr of incubation in the dark. (●) No glucose, (○) 1 μM glucose, (▼) 25 μM glucose, (△) 250 μM glucose, (■) 5 mM glucose. Error bars denote 1 SD of the mean of triplicate data points.





**FIG. 2.** Biomass production is coupled to DMSO production. DMSO (A), protein (B) and cell turbidity (C) formation by strain DG1233 when growing with 5 mM glucose and increasing DMS concentrations. (●) No DMS, (○) 11  $\mu\text{M}$  DMS, (▼) 338  $\mu\text{M}$  DMS, (△) 3.4 mM DMS, (■) 34 mM DMS. Error bars denote 1 SD of the mean of triplicate data points.



**FIG. 3.** DMSO and protein rate formation by DG1233 grown with 5 mM glucose and increasing DMS concentrations. Rates were calculated across 96 hr. Protein rates were normalized to remove the contribution of glucose to protein formation by subtracting the rate of protein formation of cells grown with 'No DMS' from cells grown with DMS. (○) 11 µM DMS, (▼) 338 µM DMS, (△) 3.4 mM DMS, (■) 34 mM DMS.