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Comparative Physiology of Dimethyl Sulfide Production by Dimethylsulfoniopropionate Lyase in *Pseudomonas doudoroffii* and *Alcaligenes* sp. Strain M3A

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Dimethylsulfoniopropionate (DMSP) lyase enzymatically cleaves DMSP, an algal metabolite, to produce acrylate, a proton, and dimethyl sulfide (DMS), the most abundant volatile sulfur compound emitted from oceans. The physiology of DMS production by DMSP lyase was studied in vivo in an *Alcaligenes*-like organism, strain M3A, a salt marsh bacterial isolate, and in a marine strain, *Pseudomonas doudoroffii*. Enzymes from both strains were induced at optimum rates by 1 mM DMSP and vigorous aeration. *P. doudoroffii* was very sensitive to continued aeration and lost activity rapidly; the enzyme was more stable when aeration ceased. In addition to DMSP, acrylate and several of its analogs acted as inducers of DMSP lyase in *Alcaligenes* sp. strain M3A but not in *P. doudoroffii*. Turnover of DMSP by *P. doudoroffii* was enhanced by 3.5% NaCl or seawater, whereas the *Alcaligenes* sp. strain M3A enzyme was not salt dependent and salt did not greatly affect its activity. The pH profile showed two peaks of DMSP lyase activity (6.5 and 8.8) for *Alcaligenes* sp. strain M3A and a single peak at pH 8 for *P. doudoroffii*. Enzyme activity in both organisms was inhibited by *methyl*-3-mercaptopropionate and homocysteine. Cyanide, azide and *p*-chloromercuribenzoate inhibited only the *P. doudoroffii* DMSP lyase. The apparent K_m values for DMSP for cell cultures of *Alcaligenes* sp. strain M3A and *P. doudoroffii* were ca. 2 mM and <20 μM, respectively. The differences in the physiology of DMSP metabolism in these two bacterial isolates may enable them to exist in diverse ecological niches.

Dimethylsulfoniopropionate (DMSP), a tertiary sulfonium compound, is produced by many species of phytoplankton (23, 25, 38, 47, 52), macroalgae (8, 19, 24, 43), the salt marsh cordgrass, *Spartina alterniflora*, and other higher plants (12, 20, 34, 42). DMSP is enzymatically degraded by DMSP lyase to dimethyl sulfide (DMS), acrylate, and a proton (7, 14, 49). The production and degradation of DMS, which constitutes 90% of biogenic sulfur gases in marine environments (1), has been a subject of extensive research because of its putative role in increasing the acidity of rainfall (2, 41) and cloud albedo (10), and thus in climate regulation (3, 37, 53). Dissolved DMSP is excreted during algal senescence and lysis (38, 44) and is found in concentrations of 2 to 200 nM in oceanic and nearshore waters (5, 21, 46, 50, 51) and 100 to 200 μM in salt marsh sediments (26). In marine environments DMSP can be degraded to DMS via the DMSP lyase pathway (27–30, 33, 48) or it can be successively demethylated to *methyl*-3-mercaptopropionate (MMPA) and 3-mercaptopropionate (MPA) (31, 32, 48).

DMSP lyase activity has now been detected in several identified species and numerous uncharacterized isolates of bacteria (11, 14, 17, 27, 36, 45, 49, 54), macroalgae (7, 8, 18, 24, 40, 43), and phytoplankton (22, 44). This enzyme has been studied in crude extracts of *Polysiphonia lanosa* (7), a red alga, and *Gyrodinium cohnii* (22), a dinoflagellate. DMSP lyase was first purified from a bacterium, strain M3A, tentatively identified as an *Alcaligenes* species; this microbe was isolated from salt marsh sediment (14). *Pseudomonas doudoroffii*, a well-characterized aerobic marine bacterium (4), was the only one of 15 marine microbes screened which possessed DMSP lyase activity (36). Although it is likely that numerous species of marine bacteria possess DMSP lyase, there is as yet no knowledge of the variation in the physiology of DMS production in these microbes. Both *P. doudoroffii* and *Alcaligenes* sp. strain M3A DMSP lyases have been purified (14, 15), but the physiology of DMS evolution from DMSP has not been described for either species. We report here on a comparative study of the physiology of DMS production by DMSP lyase in *P. doudoroffii* and *Alcaligenes* sp. strain M3A.

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MATERIALS AND METHODS

Growth of cultures. *Alcaligenes* sp. strain M3A (described previously [14]) and *P. doudoroffii* (ATCC 27123) were grown in 50-ml batch cultures in Difco tryptic soy broth (TSB) and TSB supplemented with 3.5% NaCl, respectively. When DMSP lyase-induced cultures of *Alcaligenes* sp. strain M3A were required, cells were grown on a basal salts medium containing acrylate as the carbon and energy source (14). All cultures used in this study were 18 to 24 h old. The protein contents of the cultures were approximately 0.1 and 0.3 mg/ml for *Alcaligenes* sp. strain M3A grown on acrylate and TSB, respectively, and 0.3 mg/ml for *P. doudoroffii* grown on TSB.

Induction of DMSP lyase. The kinetics of DMSP lyase induction in both *P. doudoroffii* and *Alcaligenes* sp. strain M3A with DMSP and the effect of aeration on induction were measured in 38-ml serum bottles containing 5 ml of the appropriate 24-h-old culture grown on TSB. Neutralized stock solutions of DMSP, acrylate, and all the putative inducers and inhibitors (Table 1) were made up at a concentration of 150 mM and tested at a concentration of 5 mM. The molecules tested as inducers of DMSP lyase were either DMSP or acrylate analogs or methylated sulfur compounds; many were onium compounds (6). They were tested on 1-ml aliquots of 24-h cultures of *P. doudoroffii* or *Alcaligenes* sp. strain M3A grown on TSB. The culture containing the putative inducer (or DMSP as the positive control) was incubated on an orbital shaker at 150 rpm; after 6 h of incubation the cell suspensions were spun in a Microfuge for 25 s, resuspended in seawater (for *P. doudoroffii*) or 50 mM phosphate buffer, pH 8 (for *Alcaligenes* sp. strain M3A), and then assayed with 2.5 mM DMSP to determine the extent of induction. If there was no induction of DMSP lyase during this period, the culture was allowed to incubate for 18 h with the potential inducer and rechecked for DMSP lyase activity. To determine the optimum concentration of DMSP or acrylate required for induction of DMSP lyase, cultures of *Alcaligenes* sp. strain M3A and *P. doudoroffii* were incubated as described above with the concentrations of inducers indicated.

To measure the effect of aeration on DMSP lyase induction in these organisms, the 38-ml bottles containing 5 ml of the cultures were incubated at room temperature under static conditions or on orbital shakers maintained at 75, 100,

TABLE 1. Inducers of DMSP lyase in *P. doudoroffii* and *Alcaligenes* sp. strain M3A

Inducer[a]	% Induction with DMSP	
	Strain M3A	*P. doudoroffii*
DMSO	82.2	37.6
DMDS[b]	8.5	46.0
Dimethylglycine	4.9	26.1
Glycine betaine	0.0	20.0
Acrylate	105.7	0.4
Acrylamide	107.4	0.0
Methacrylate	42.3	0.0

[a] 5 mM. The following molecules either had no inducing activity in either organism or had inducing activity that was less 10% of that of DMSP: MPA, MMPA, DMSA, homocysteine, 2-mercaptoacetate, propionate, *S*-methylmethionine, *S*-adenosylmethionine, methionine, and methionine sulfoxide.

[b] DMDS, dimethyl disulfide.

or 125 rpm. The rate of DMSP lyase induction was measured by adding 2.5 mM DMSP as a substrate (for enzyme turnover) to cells removed from the induction medium by centrifugation as described above. The assay for DMSP lyase involves the measurement of the product, DMS, by gas chromatography as described previously (14).

Effect of NaCl on DMSP lyase activity. Aliquots (1 ml) of *P. doudoroffii* and *Alcaligenes* sp. strain M3A cultures preinduced for DMSP lyase activity were spun in a Microfuge and resuspended in dilutions of filtered seawater or 50 mM phosphate buffer, pH 8, supplemented with different concentrations of NaCl.

Determination of pH and temperature optima for DMSP lyase activity. The buffers used to determine the optimum pH for in vivo DMSP lyase activity were 2-(*N*-morpholino)ethanesulfonic acid (MES) (pK_a = 6.09), *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) (pK_a = 7.47), and *N*-([Trishydroxymethyl]methyl)glycine (Tricine) (pK_a = 8.05). Cultures of *P. doudoroffii* and *Alcaligenes* sp. strain M3A with preinduced DMSP lyases were spun in a Microfuge and resuspended in the appropriate buffer. The pH was adjusted upwards in half-point increments from 5 to 9.5, and the cells were allowed to equilibrate with the buffer for 10 min before 2.5 mM DMSP was added. DMSP lyase activity was measured every 5 min over a 30-min period, and maximum rates are presented.

The optimum temperature of DMSP lyase activity in *P. doudoroffii* and *Alcaligenes* sp. strain M3A was determined by incubating preinduced cultures at 5, 25, 37, 45, or 60°C for 10 min, after which DMSP was added and rates were measured.

Effects of inhibitors on DMSP lyase activity. Washed preinduced cultures were incubated with the inhibitors at the concentrations indicated for 15 min, after which 2.5 mM DMSP was added and the rate of DMSP lyase activity was measured. Inhibitors were prepared in 150 mM stock solutions which were neutralized before use, if necessary.

Materials. *P. doudoroffii* ATCC 27123 was obtained from the American Type Culture Collection (Rockville, Md.). DMSP was synthesized according to the method of Chambers et al. (9). Dimethylsulfonioacetate (DMSA) was a generous gift from Jacques Minet (Department of Pharmacy, University of Rennes, Rennes, France). All other chemicals (inducer, inhibitors, and buffers) used here were reagent grade and were obtained from either Aldrich Chemical Corp. Inc. or Sigma Chemical Co., St. Louis, Mo.

RESULTS

Kinetics of DMSP lyase induction. Aeration was required for maximal rates and extent of DMSP lyase induction in both organisms (Fig. 1). The kinetics of induction were measured in vessels of identical geometry and shaker speed. For both cultures, increased aeration led to higher induction rates, which reached an asymptote at shaker speeds between 75 and 100 rpm. At the higher shaker speed the cell suspension was saturated with oxygen (220 μM for seawater to approximately 250 μM for phosphate buffer). Once induced, the *Alcaligenes* sp. strain M3A DMSP lyase was quite stable, with high levels of DMSP lyase activity retained after several days, especially if the culture was not aerated (Fig. 1A). Induced cultures of *P. doudoroffii*, however, retained their enzyme activities for a much shorter time (Fig. 1B). The stability of this activity was inversely correlated to the degree of culture aeration. The *Alcaligenes* sp. strain M3A culture induced much higher levels of DMSP lyase activity in the static culture (43% of maximum) than did *P. doudoroffii* (10% of maximum).

The concentrations of DMSP required to induce DMSP lyase activity to half maximum levels in *Alcaligenes* sp. strain M3A and *P. doudoroffii* were approximately 2×10^{-5} and 3×10^{-4} M, respectively (Fig. 2). Acrylate induced the enzyme in *Alcaligenes* sp. strain M3A to half-maximal levels at approximately 5×10^{-4} M but was ineffective as an inducer in *P. doudoroffii.*

Alternative inducers of DMSP lyase activity. There are several alternative inducers of the *Alcaligenes* sp. strain M3A enzyme (Table 1). Notable among these are acrylate and its analogs acrylamide and methacrylate, which showed levels of induction comparable to that obtained with DMSP. DMSP, however, induced the enzyme much more rapidly in *Alcaligenes* sp. strain M3A than acrylate (data not shown). Dimethyl sulfoxide (DMSO) induced DMSP lyase in both *Alcaligenes* sp. strain M3A and *P. doudoroffii*, but it was more effective in the former. Alternatively, dimethyl disulfide was a more effective

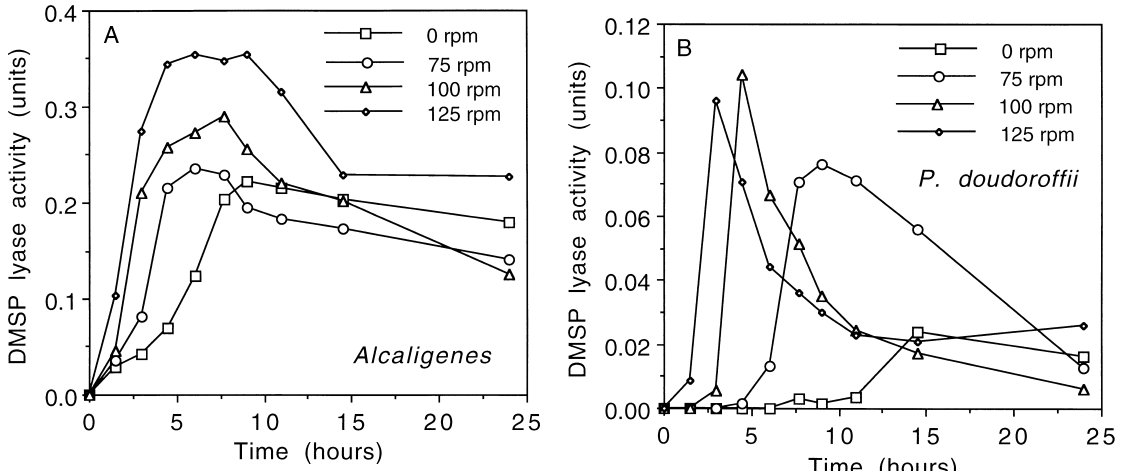

FIG. 1. Effects of aeration on induction and stability of DMSP lyase in *Alcaligenes* sp. strain M3A (A) and *P. doudoroffii* (B). The protein concentration was 0.3 mg/ml for both cultures. One-milliliter aliquots were assayed. One unit of DMSP lyase activity is the amount of cell protein (enzyme) required to produce 1 μmol of DMS in 1 min at 25°C.

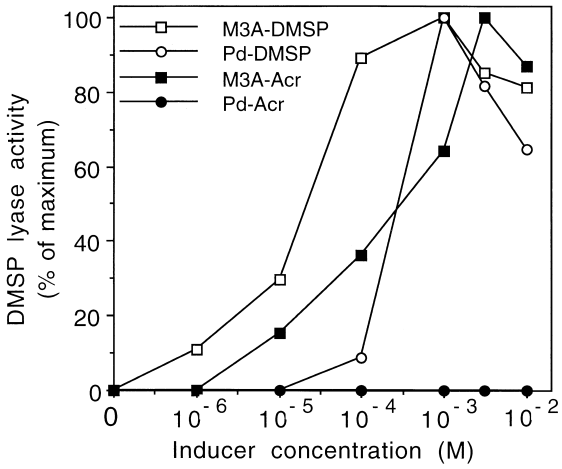

FIG. 2. Effects of DMSP and acrylate (Acr) concentrations on induction of DMSP lyase in *P. doudoroffii* (Pd) and *Alcaligenes* sp. strain M3A. The enzyme was not induced in *P. doudoroffii* even after 24 h in the presence of acrylate. One hundred percent DMSP lyase activities for *P. doudoroffii* and *Alcaligenes* sp. strain M3A represent 0.33 and 1.16 U/mg of cell protein, respectively.

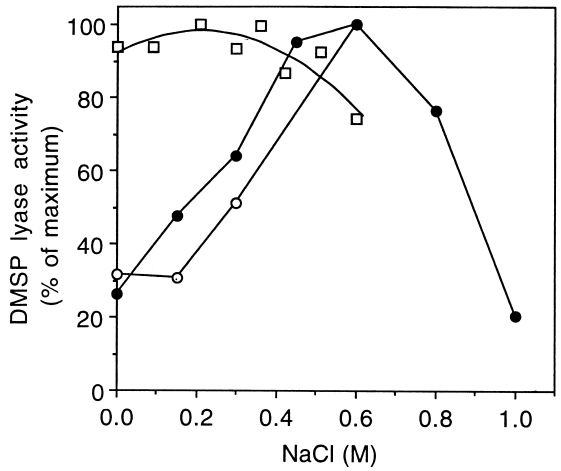

FIG. 3. Effect of sodium chloride concentration on turnover rates of DMSP by DMSP lyase in *P. doudoroffii* and *Alcaligenes* sp. strain M3A. Cultures of *P. doudoroffii* (○) and *Alcaligenes* sp. strain M3A (□) were assayed in dilutions of seawater. DMSP lyase activity in *P. doudoroffii* was also assayed in 50 mM phosphate buffer to which NaCl was added as indicated (●). One hundred percent DMSP lyase activities for *P. doudoroffii* and *Alcaligenes* sp. strain M3A represent 0.27 and 1.3 U/mg of cell protein, respectively.

inducer of the *P. doudoroffii* enzyme. Glycine betaine and dimethylglycine, both DMSP analogs, induced the *P. doudoroffii* enzyme to about 25% but did not serve as inducers in *Alcaligenes* sp. strain M3A. Other close structural analogs of DMSP —*S*-methylmethionine, methionine, *S*-adenosylmethionine, and DMSA—did not induce the enzyme in either organism. When *Alcaligenes* sp. strain M3A was grown on acetate overnight in the presence of DMSO, the enzyme was not induced by it (14).

Kinetic constants of DMSP lyase in vivo. The apparent K_m of DMSP lyase for DMSP determined with cell cultures of *P. doudoroffii* is less than 20 μM DMSP. Maximum rates of DMSP lyase activity were measured at 20 μM, which was our detection limit for DMS. The apparent K_m for the *Alcaligenes* sp. strain M3A DMSP lyase in cell culture was 2 mM (14). The apparent V_{max} values of DMSP lyase in *Alcaligenes* sp. strain M3A and *P. doudoroffii* were 1 and 0.3 μmol of DMS min^{-1} mg of cell $protein^{-1}$, respectively.

Effect of NaCl. The DMSP lyase activity of *P. doudoroffii*, a marine isolate, was dependent on the concentration of NaCl in the reaction mixture. This organism showed maximum DMS production in full-strength seawater or in 50 mM phosphate buffer containing 0.6 M NaCl, the concentration found in seawater (Fig. 3). In the complete absence of NaCl, DMSP lyase activity in this organism was only about 30% of maximum. However, DMSP lyase activity in *Alcaligenes* sp. strain M3A, an estuarine sediment isolate, was not salt dependent but was slightly inhibited (<25%) when NaCl levels reached seawater levels.

pH and temperature optima. Two pH optima were obtained for DMSP lyase activity in cell suspensions of *Alcaligenes* sp. strain M3A, one at pH 6.5 and the other at a pH of 8.8. *P. doudoroffii* showed only one peak of activity at a pH of 8 (Fig. 4). *P. doudoroffii* exhibited optimum DMSP lyase activity at 37°C, whereas *Alcaligenes* sp. strain M3A showed maximal activity between 37 and 45°C but little activity at 60°C (Fig. 5).

Inhibitors of DMSP lyase activity. Of all the potential inhibitors tested, only DMSA, MMPA, methionine, and homocysteine were effective in inhibiting DMSP lyase activity in both *Alcaligenes* sp. strain M3A and *P. doudoroffii* (Table 2). Other DMSP analogs showed lower levels of inhibition. Acrylate, which is an inducer of DMSP lyase in *Alcaligenes* sp. strain M3A, does not inhibit turnover of DMSP in this organism. However, it does inhibit DMSP lyase activity by 25% in *P. doudoroffii*, even though it does not induce the enzyme in this organism. Cyanide, azide, and *para*-chloromercuribenzoate (PCMB), which are potent inhibitors of many enzymes, inhibited DMSP lyase activity in cell suspensions of *P. doudoroffii* but not in *Alcaligenes* sp. strain M3A (Table 3). DMSA, DMSO, *S*-methylmethionine, MMPA, methionine, and methionine sulfoxide were tested as alternative substrates for in vivo DMSP lyase activity in *P. doudoroffii* and *Alcaligenes* sp. strain M3A, but no DMS or methane thiol was detected from these methylated sulfur compounds (data not shown).

DISCUSSION

Although some algae have been reported to possess DMSP lyase (7, 16, 22, 44), there has been speculation that bacteria

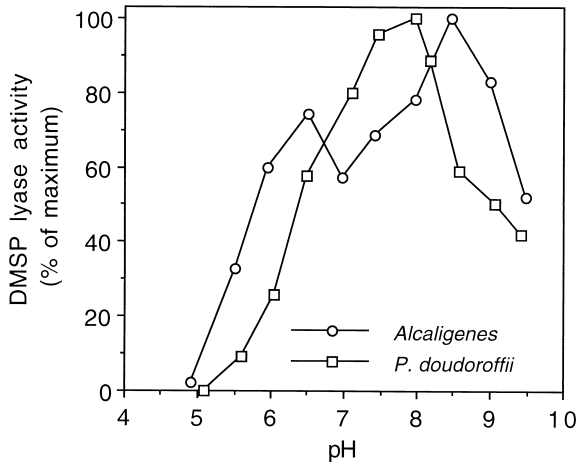

FIG. 4. Effect of pH on DMSP lyase activity in cells of *P. doudoroffii* and *Alcaligenes* sp. strain M3A. One hundred percent DMSP lyase activities for *P. doudoroffii* and *Alcaligenes* sp. strain M3A represent 0.32 and 1.21 U/mg of cell protein, respectively.

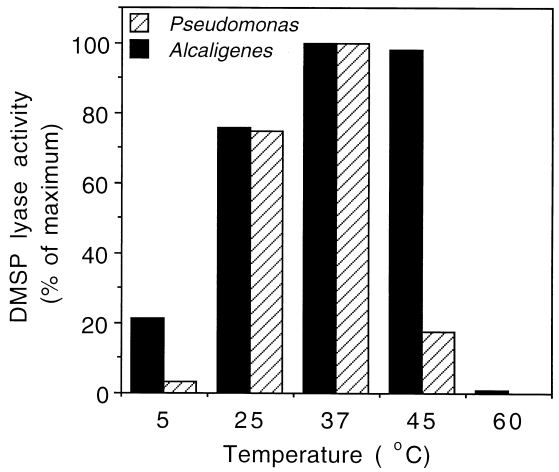

FIG. 5. Effects of temperature on DMSP lyase activity in cells of *P. doudoroffii* and *Alcaligenes* sp. strain M3A. One hundred percent DMSP lyase activities for *P. doudoroffii* and *Alcaligenes* sp. strain M3A represent 0.28 and 1.06 U/mg of cell protein, respectively.

are largely responsible for the production of DMS from dissolved DMSP (13, 44, 54). Considerable amounts of dissolved DMSP are released because of algal senescence and lysis (5, 38, 44) and grazing activity on phytoplankton by zooplankton (13, 54). Although there have been numerous reports of marine bacterial isolates which possess DMSP lyase (see the introduction), the physiology of this process (DMS evolution) has received only minimal attention (35). In order to achieve a better understanding of DMS production from DMSP in the environment, the conditions under which DMSP lyase is produced and its activity is optimized and maintained were investigated in two isolates from marine sources. This study compares the physiology of DMS production from *Alcaligenes* sp. strain M3A and *P. doudoroffii*; the results show differences in induction and kinetic properties and factors affecting substrate turnover which are consistent with the different (estuarine versus marine) habitats from which these two organisms were isolated. Furthermore, the inhibitor and kinetic data, when taken together, indicate a fundamental difference in the mechanism of DMSP uptake between these two species.

TABLE 2. Effects of organosulfur compounds and analogs of DMSP and acrylate as inhibitors of in vivo DMSP lyase activities of *P. doudoroffii* and *Alcaligenes* sp. strain M3A

Inhibitor[a]	% Inhibition	
	Strain M3A	*P. doudoroffii*
DMSA	18	29
S-Methylmethionine	8	17
MMPA	55	90
S-Adenosylmethionine	21	15
Methionine sulfoxide	10	28
Methionine	25	31
Acrylate	0	25
Acrylamide	10	24
Glycine betaine	8	20
Propionate	4	16
Homocysteine	48	80
2-Mercaptoacetate[b]	1	18

[a] 5 mM. DMSO, MPA, methacrylate, and dimethylglycine showed less than 10% inhibition of DMSP lyase in both species.
[b] Thioglycolate.

TABLE 3. Effects of inhibitors on *P. doudoroffii* and *Alcaligenes* sp. strain M3A in vivo DMSP lyase activities

Inhibitor[a] (mM)	% Inhibition	
	Strain M3A	*P. doudoroffii*
Cyanide		
0.2	18	91
1.0	34	100
Azide		
0.2	0	26
1.0	0	24
PCMB		
0.2	0	28
1.0	0	85

[a] Arsenate, 2,4-dinitrophenol, and *N*,*N*′-dicyclohexylcarbodiimide showed no inhibition of DMSP lyase in *Alcaligenes* sp. strain M3A and only ≤10% inhibition in *P. doudoroffii* at 0.2, 1.0, or 5.0 mM.

Alcaligenes sp. strain M3A is a facultative anaerobe (14), while *P. doudoroffii* is a strict aerobe (4). It is not surprising therefore that the *P. doudoroffii* enzyme is not induced to any great extent (by DMSP) in a static culture, unlike the enzyme from the facultative *Alcaligenes* sp. strain M3A strain, which is induced to about 60% of maximum (cf. Fig. 1A and B). These data also show that in vivo the *Alcaligenes* sp. strain M3A activity, once induced, is relatively stable whereas in *P. doudoroffii* DMSP lyase activity is rapidly lost. The loss of activity in *P. doudoroffii* is difficult to explain at present, because, once the cells with activity are disrupted, the activity that is present is very stable and readily purified (15). For example, in buffer containing 5% glycerol the enzyme was unaffected by repeated freezing and thawing for at least 8 months.

DMSP has been detected in coastal and oceanic surface waters at concentrations of 2 to 200 nM (5, 21, 50, 51); however, induction of DMSP lyase in both organisms required at least high micromolar (100 μM) concentrations of DMSP (Fig. 1). These concentrations may be found in microniches of phytoplankton blooms which are known to produce high millimole quantities of DMSP per liter of cell volume (25, 44, 47). Senescence of DMSP-producing phytoplankton results in the release of dissolved DMSP into the water column (5, 38, 44), where it is probably available for bacteria to use as an energy source. We have recently shown that the swim speed of *Alcaligenes* sp. strain M3A is greatly enhanced and tumbling activity is greatly diminished by 10^{-8} M DMSP (55), suggesting that it can respond positively to a DMSP concentration gradient.

A DMSP lyase-producing large rod-shaped bacterium was isolated from a culture of *Oxyrrhis marina*, a dinoflagellate, which was grown on *Dunaliella tertiolecta*, a low-titer DMSP-producing chlorophyte (54). This bacterium showed a 1- to 3-h lag before DMS was produced from 20 μM DMSP, which served as both inducer and substrate. Similar kinetics of induction are exhibited by *Alcaligenes* sp. strain M3A and *P. doudoroffii* (Fig. 1). DMSP lyase in a bacterial isolate, strain LFR, was induced by 20 μM DMSP (35); however, it was not clear from these data (35, 54) if this concentration was sufficient to allow maximal rates of induction.

Unlike *Alcaligenes* sp. strain M3A and other isolates containing DMSP lyase (14, 27, 35), the enzyme from *P. doudoroffii* is not induced by acrylate (Table 1). DMSO induced DMSP lyase in both *P. doudoroffii* and *Alcaligenes* sp. strain

M3A, but to much greater levels in the latter strain. Finally, there appears to be no correlation between the degree of DMSP lyase induction by DMSO and the presence of DMSO reductase in these organisms (15a). In *P. doudoroffii*, DMSO induces DMSO reductase activity, but only moderate levels of DMSP lyase were induced (38% of maximum), whereas DMSO does not induce DMSO reductase in *Alcaligenes* sp. strain M3A, but it does induce fairly high levels of DMSP lyase (82% of maximum).

The pH profiles suggest that DMSP lyase in *Alcaligenes* sp. strain M3A may exist in two forms with optimum activity at pH 6.5 and 8.8. These two pH optima were observed in whole-cell assays (Fig. 4) as well as in crude extracts (15) of *Alcaligenes* sp. strain M3A. However, only the high-pH form of DMSP lyase was observed during its purification (14). The fate of the low-pH form of this enzyme is under investigation. If marine bacteria possess more than one DMSP lyase, the isozyme may carry out the same reaction but possess different kinetic characteristics, thus endowing the organism with the capability to metabolize a range of DMSP concentrations.

Molecules that inhibited DMSP lyase in both organisms in order of descending effectiveness were MMPA, homocysteine, methionine, and DMSA (Table 2). Except for homocysteine (a mercaptan), the others are S-methylated three- or four-carbon carboxylic acid homologs. It is therefore surprising that *S*-methylmethionine, which is also closely related to this group, is not a more effective inhibitor. MMPA is a particularly strong inhibitor of *P. doudoroffii* DMSP lyase; the K_i was approximately 50 μM. MMPA is the primary product of a biochemical pathway in other marine bacteria which competes with DMSP lyase for DMSP in the environment (31, 32), but it is not known if the severe inhibition of DMSP lyase by MMPA has any ecological significance.

The apparent V_{max} of DMSP lyase for DMSP in *Alcaligenes* sp. strain M3A cells (1.1 U/mg) was three times higher than that of *P. doudoroffii* cultures (0.32 U/mg). Apparent V_{max} measurements of other marine bacterial isolates have also been reported (16, 35), with values as low as 0.019 U/mg of cell protein (16). The apparent K_m for DMSP in cell suspensions of *P. doudoroffii* is at least 100 times lower (<20 μM) than that in cell cultures of *Alcaligenes* sp. strain M3A, which is approximately 1.1 mM. Surprisingly, the apparent K_m for DMSP of pure *P. doudoroffii* DMSP lyase is 1.82 mM (15), which is similar to that of the pure *Alcaligenes* sp. strain M3A DMSP lyase (14). Other reports of apparent K_m values for DMSP in bacterial cell culture range from 0.6 μM (36) to 0.5 mM (16).

It is extremely interesting that *P. doudoroffii* DMSP lyase activity in vivo was strongly inhibited by cyanide, PCMB, and azide whereas the activity in *Alcaligenes* sp. strain M3A was relatively unaffected by these molecules (Table 3). Azide also inhibited DMSP lyase in vivo in another marine bacterial isolate, strain 2B-2 (27), and cyanide and PCMB inhibited an algal enzyme in vitro (22). It should be noted that the kinetics of DMSP turnover in vivo comprises the kinetics of both uptake and cleavage. A comparison of kinetic constants and inhibition data for *Alcaligenes* sp. strain M3A and *P. doudoroffii* suggests that *P. doudoroffii* has a site external to the cytoplasm which is sensitive to cyanide, azide, and PCMB, which would explain the lower K_m for DMSP cleavage in vivo. Taken together, these observations suggest that this high-affinity (low apparent K_m), PCMB-sensitive *P. doudoroffii* site may be a DMSP-binding protein and that *Alcaligenes* sp. strain M3A does not possess such a site. A model which illustrates the differences in enzyme location in *Alcaligenes* sp. strain M3A and *P. doudoroffii* and the existence of a putative binding protein in *P. doudoroffii* has been presented elsewhere (15). Since DMSP lyase appears to be intracellular in *P. doudoroffii*, it is premature to speculate about the meaning of the high micromolar levels of DMSP (reported here) required for induction of the enzyme in *Alcaligenes* sp. strain M3A and *P. doudoroffii*. The kinetics of DMSP uptake in these two strains should be compared to consider the significance of environmental concentrations.

In summary, a comparison of the physiologies of DMSP lyases in axenic, marine, bacterial cell cultures shows considerable heterogeneity in induction parameters, factors affecting substrate turnover, and kinetic constants. This diversity may enable bacteria which possess DMSP lyase to inhabit different ecological niches in marine environments where DMSP is found.

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