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Full Length Research Paper

Influence of divalent metal ions on degradation of dimethylsulphide by intact cells of *Thiobacillus thioparus* TK-m

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Dimethylsulphide degradation by intact cells of *Thiobacillus thioparus* TK-m was stimulated by the addition of divalent metal ions (Ca^{2+} > Mg^{2+} > Mn^{2+}). Mixtures of divalent ions were also found to be stimulatory with the exception of the Ca^{2+} + Mn^{2+} combination (Mg^{2+} + Mn^{2+} > Mg^{2+} + Ca^{2+} > Ca^{2+} + Mn^{2+}).

Key words: *Thiobacillus thioparus* Tk-m, dimethyl sulphide, divalent metal ions, degradation.

INTRODUCTION

Methanethiol (methylmercaptan, MM), dimethylsulphide (DMS), dimethyldisulphide (DMDS), and hydrogen sulphide (H_2S) are malodorous compounds which exceed the odour threshold at low concentrations (Leonardos et al., 1969). They are produced by the wood-pulping industry, manure and sewer systems as exhaust gases. These thiols and sulphides constitute health problems as some are known to be toxic to both man and animals at very low concentrations.

The biogenesis of dimethylsulphide provides a principal input of volatile sulphur to the atmosphere. This contribution has significant effects on the sulphur cycle and on global geochemistry (Taylor and Kiene, 1989). DMS is photochemically oxidized in the atmosphere to methanesulphonic and sulphuric acids. These strong acids contribute, along with nitric and organic acids, to the natural acidity of precipitation (Taylor and Kiene, 1989). Recent problems with acid rain have aroused inte-rest in the anthropogenic and natural sources of volatile sulphur compounds. In addition to affecting the pH of precipitation, the emission of DMS has been linked with the regulation of global climate. DMS has been repor-tedly degraded by *Thiobacillus* sp. strain MS1 (Sivela, 1980), *Thiobacillus thioparus* TK-m (Kanagawa et al., 1982; Kanagawa and Kelly, 1986; Kanagawa and Mika-mi, 1989; Tanji et al. 1989; Gould and Kanagawa, 1992), *Hyphomicrobium* sp. strain S (deBont et al., 1981) and *Hyphomicrobium* sp. strain EG (Suylen and Kuenen, 1986; Suylen et al., 1986; Suylen et al., 1987; Smith and Kelly, 1988). The present study ia aimed at determining the influence of divalent metal ions on de-gradation of dimethylsulphide by intact cells of *Thiobacillus thioparus* TK-m.

MATERIALS AND METHODS

Organism

Thiobacillus thioparus TK-m was supplied by Dr. T. Kanagawa (National Institute of Bioscience and Human-Technology, Tsukuba, Japan) and maintained as slants on medium C (Kanagawa and Kelly, 1986).

Culture Media

For all experiments, *T. thioparus* was first grown as batch culture in a liquid version of Medium C (without agar) in Erlenmeyer flasks. The buffer and thiosulphate solutions were sterilized in 10 ml portions in rubber-stoppered test tubes and added aseptically to the rest of the medium when cool (37°C) at intervals of 8 h. The cooled medium was then inoculated with a slant culture of *T. thioparus* and incubated at 30°C in a rotary shaker until almost all the added thiosulphate was consumed.

Scale-up

After growth in batch culture, *T. thioparus* was transferred to a glass fermenter vessel (aeration tube) containing 2 L of sterilized modified version Medium C. The phosphate-carbonate buffer used in the batch culture was replaced with 0.4 M K_2CO_3. Also, 0.4 M $Na_2S_2O_3.5H_2O$ was used as energy source at start-up. The culture in batch culture was added to the fermenter through sterilized Teflon tubes using a micro-pump. The pH of the medium was adjusted to and controlled at 6.8 using the pH meter (Digisense, Cole-Palmer). Utilization of thiosulphate was indicated by a drop in pH and followed by addition of alkali.

Table 1. Summary of culture conditions and specific activities of *T. thioparus* supplemented with divalent metal ions on degradation of DMS.

Culture condition	Specific activity (nMols $Min^{-1}A_{660}0.1$)
1.95 ml phosphate buffer (pH 6.8, 26 mM) + 0.9 ml fresh culture + 300 nMols DMS (0.15 ml of 2 mM solution)	3.3
2.73 ml phosphate buffer + 0.12 ml washed cells + 300 nMols DMS	3.0
2.73 ml Phosphate buffer + 0.12 ml washed cells + 300 nMols DMS	
+ $MgCl_2$: 2.0 mM	3.59
1.0 mM	3.42
0.2 mM	2.80
2.73 ml Phosphate buffer + 0.12 ml washed cells + 300 nMols DMS	
+ $CaCl_2$: 2.0 mM	3.85
1.0 mM	4.83
0.2 mM	4.71
2.73 ml Phosphate buffer (6.5 mM, pH 6.8) + 0.2 mM $CaCl_2$ + 0.12 ml washed cells + 300 nMols DMS	4.29
2.73 ml Phosphate buffer + 0.12 ml washed cells + 300 nMols DMS	
+ $MnSO_4$: 2.0 mM	3.0
1.0 mM	3.0
0.2 mM	2.94
Phosphate buffer + 0.12 ml washed cells + 300 nMols DMS + mixture of divalent metal ions:	
$CaCl_2$ 0.2 mM $MnSO_4$ + 0.2 mM	3.13
mM $CaCl_2$ 2.0 mM $MgCl_2$ + 0.2	4.71
mM $MnSO_4$ 2.0 mM $MgCl_2$ + 0.2	5.23

After consumption of the thiosulphate, growth was now switched to dimethylsulphide (DMS) consumption. Inlet and outlet DMS concen-trations were analysed by injecting the gas into a gas chromatograph using gas-tight micro-syringes. DMS addition was increased when further drop in pH was recorded by the pH meter. To prevent cell death due to addition of excess DMS, the high DMS pump was switched off when outlet gas concentration was high indicating that DMS consumption by the test strain was poor or absent (Kanagawa and Mikami, 1989).

Analytical methods

Growth of the culture in the glass vessel was monitored by measuring the A660 in a Spectrophotometer (101, Hitachi). When sufficiently high turbidity (due to cell growth) was developed, part of the culture (100 to 200 ml) was withdrawn aseptically through the harves-ting port, stored in ice-cooled water and used immediately for the degradation tests.

Another portion (100 to 200 ml) was centrifuged (12,000 x g, 5ºC, 10 mins), washed once with cooled 26 mM potassium buffer (pH 6.8) and resuspended in 3 ml of the same buffer and stored in plastic vials in ice-cooled water.

Chemicals

Dimethylsulphide gas (2 ml L-1 of nitrogen) in 40 L gas tanks was obtained from Seitetsu Chemical Industries Limited, (Osaka, Japan) All other chemicals were of reagent grade.

Biodegradation test

Into phosphoric acid-coated serum vials (68.8 ml) were each placed appropriate volumes of 26 mM potassium phosphate buffer (pH 6.8, Table 1). These were then sealed with Teflon-coated rubber stoppers, and then supplemented with different millimolar concentrations of divalent metal ions in a final reaction volume of 3 ml. To the serum vials were then added about 1 ml of unwashed culture or 0.12 ml of washed resuspended cells (unwashed culture serum vials contained no additives). These were then shaken in a rotary shaker at 30ºC for 5 min. To these vials were then added DMS at an initial concentration of 300 nMoles (0.1mM) and shaken in the rotary shaker at 30ºC. At regular intervals, headspace gas was withdrawn using gas-tight glass syringes and residual gas concentrations measured by injecting 50 - 100 µl of headspace gas into a gas chromatograph (Shimidzu, GC-5A). The method used is essentially a modification of that outlined by Gould and Kanagawa (1992). Summary of reaction conditions are presented in Table 1.

RESULTS AND DISCUSSION

A fresh suspension of strain TK-m previously grown on DMS, oxidized 0.1 mM (300 nMols) DMS in serum vials exhibiting a specific activity of 3.0 nMols Min^{-1} at an A_{660} of 0.1. This was slightly lower than the value of 3.3 exhibited by washed resuspended cells under similar conditions. (Table 1 and Figure 1). It could therefore be inferred that washing removes some factor(s) from the growth medium resulting in potential reduction of activity of the washed cells towards DMS.

Table 1 also shows that the concentration of the buffer used has some influence in determining the specific activity of the test strain towards DMS. At similar conditions but at 26 and 6.5 mM phosphate buffers, specific activities of 4.71 and 4.29 were recorded.

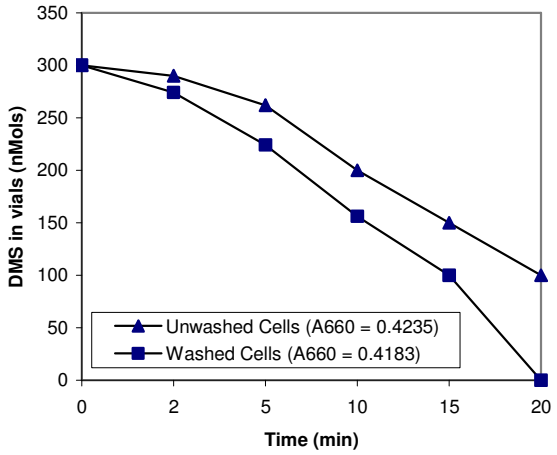

Figure 1. Degradation of DMS by fresh (unwashed) and fresh (washed) cells of *T. thioparus* in 26 mM phosphate buffer (pH 6.8).

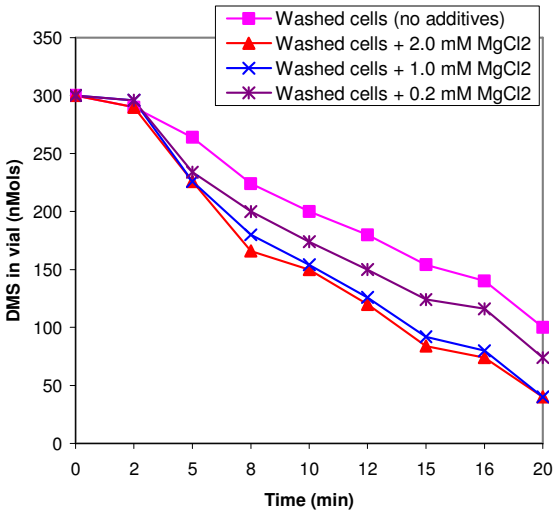

Figure 2. Influence of Mg^{2+} on degradation of DMS by washed cells of *T. thioparus* in 26 mM Phosphate buffer (pH 6.8).

Figure 2 shows the influence of addition of magnesium ions on the degradation of DMS by washed, resuspended cells of *T. thioparus* TK-m. The results indicated that at concentrations between 0.1 and 2.0 mM $MgCl_2$ there was a corresponding increase in activity of the test strain towards DMS. The specific activity values of 3.42 and 3.59 recorded at 1.0 and 2.0 mM $MgCl_2$ tend to suggest that addition of Mg^{2+} to the reaction mixture increased the activity of the washed resuspended cells. This is an indication that removal of metal ions (divalent) from the growth medium by washing was a factor responsible for the potential reduction in activity of the test strain. Whereas suppressive action was recorded with some divalent ions, others like Cu are known to inhibit the test strain at

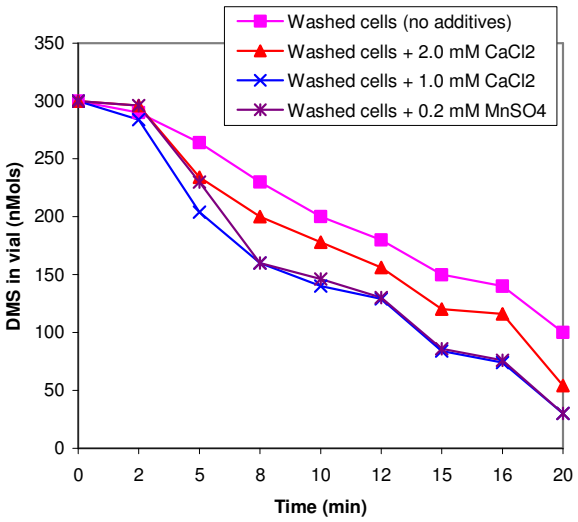

Figure 3. Influence of Ca^{2+} on degradation of DMS by washed cells of *T. Thioparus* in phosphate buffer (26mM, pH 6.8).

0.1 mM concencentration (Gould and Kanagawa, 1992).

Relatively higher activities were recorded with the addition of Ca^{2+} ions to the reaction mixture. Figure 3 shows that a higher specific activity was recorded at $MgCl_2$ concentrations between 0.2 and 1.0 mM. The value at 2.0 mM was lower. This was in contrast to the obser-vation with Mg^{2+} where the highest activity was recorded at 2.0 mM $MgCl_2$.

At the concentrations of $MnSO_4$ tested, Mn^{2+} ions did not seem to influence the activity of the test strain towards DMS. The specific activity values recorded, as shown in Figure 4, were similar to those recorded for fresh un-washed and washed resuspended cells (Table 1). Where-as Mn^{2+} as the sole additive in the reaction mixture did not seem to influence the activity of the test strain, when added in combination with $CaCl_2$ at 0.2 mM concentration each, there was a decrease in activity. This deduction is based on the fact that specific activity of 4.71 nMols Min^{-1} was recorded at a $CaCl_2$ concentration of 0.2 mM. By contrast, an activity of 3.13 was recorded at combination of 0.2 mM each of $MnSO_4$ and $CaCl_2$ (Figure 5).

The apparent "suppressive" action of Mn^{2+} when in combination with other divalent metal ions seems to depend on the nature of the other metal. Figure 5 also shows that whereas Mn^{2+} was suppressive when added to the reaction mixture in combination with Ca^{2+} at 0.2 mM concentration, no such suppressive action was observed with Mg^{2+} (Table 1). The highest activity of the test strain was recorded at a combination of Mg^{2+} and Mn^{2+} ions (5.23 compared to the 3.13 recorded for Ca^{2+} and Mn^{2+} combination). Preliminary tests Cu^{2+} with and Zn^{2+} ions (data not presented) indicated that these divalent ions were slightly inhibitory to the test strain at the concentrations of the salts used (0.1 mM). Whereas limited

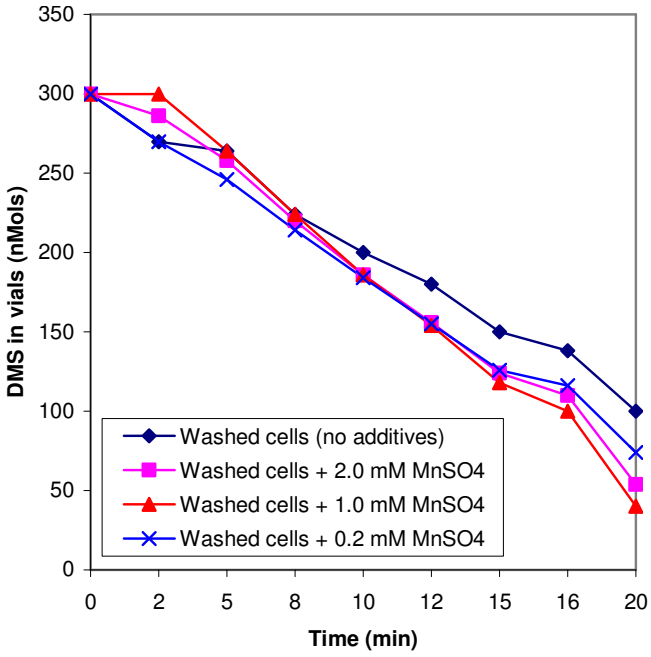

Figure 4. Influence of Mn^{2+} on degradation of DMS by washed cells of *T. thioparus* in phosphate buffer (26 mM, pH 6.8).

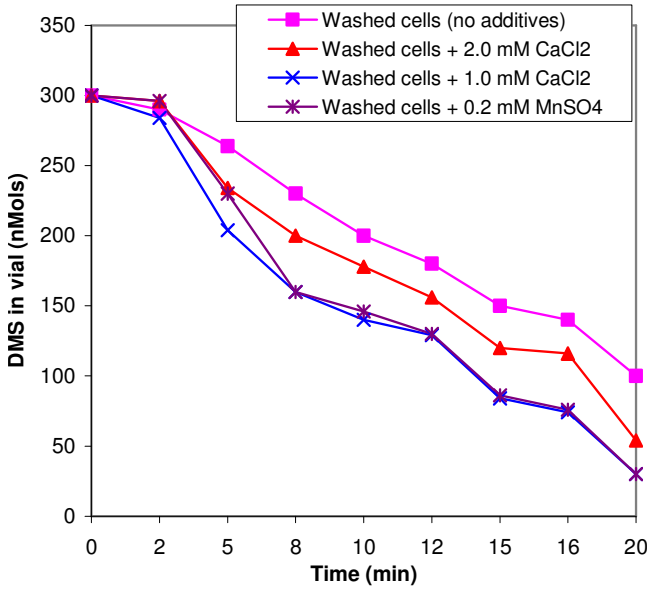

Figure 5. Influence of mixture of divalent ions on degradation of DMS by *T. thioparus* in phosphate buffer (26 mM, pH 6.8).

work has been carried out on the influence of divalent metal ions on the degradation of DMS by *Thiobacillus*, these were not studied in combinations.

In order to better assess the roles of microbes as global consumers of DMS, more knowledge is needed about the biochemical pathways and factors affecting catabolism under aerobic and anaerobic conditions.

Some human activities promote the production of sulphur species (or their precursors) which have undesirable environmental impacts. A well established example is the leaching of mire dumps causing local problems with acidity and metal toxicity.

The global nitrogen cycle has been altered by the use of fertilizers and nitrogen-fixing legumes. Increased nitrogen imputs may also affect the sulphur cycle. Elevated inputs of organic matter into salt marshes, estuaries and near-shore regions will promote anoxia and stimulate sulphate reduction and the sulphur cycle in general, probably resulting in greater releases of volatile sulphur compounds such as DMS. The results of this study show the possible role of metal ions in the degradation of DMS by *Thiobacilli* and other microbes.

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