Degradation of Dimethyl Disulfide by *Pseudomonas fluorescens* Strain 76

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Strain 76, which was able to utilize dimethyl disulfide (DMDS) as a sole sulfur source, was screened from our microbial collection. It was identified as *Pseudomonas fluorescens* by taxonomical characterization and 16S rDNA sequence analysis. It does not belong to the methylotrophs, because it did not grow on DMDS or other C1 compounds as sole carbon source, and DMDS degradation was not repressed in the presence of glucose, Na$_2$SO$_4$, or nutrient broth. Moreover, it showed high resistance to DMDS by growing in DMDS at concentrations up to 9.04 mM. Based on these findings, strain 76 metabolizes DMDS and has dual physiological roles: sulfur assimilation and degradation. Thus it has advantages as a biological scavenger of DMDS.

**Key words:** *Pseudomonas fluorescens*; dimethyl disulfide degradation

Volatile organic sulfur compounds (VOSCs), e.g., dimethyl disulfide (DMDS), dimethyl sulfide (DMS), and methanethiol (MT), are malodorous compounds widely distributed in the environment. Since VOSCs exceed the odor threshold at low concentrations, they cause serious environmental problems. Therefore, it is necessary to remove VOSCs from the environment. Chemical and physical methods to scavenge VOSCs have been developed and applied.\(^1\) Recently, biological methods of deodorization for VOSCs have been attracting attention, because these methods are performed at low-cost, are easily maintained, are safe, and have low environmental impact.\(^2\)

DMDS is one of the major VOSCs. It is produced in several industrial and biological processes such as the wood-pulp industry,\(^3\) oil refineries, manure and sewer systems,\(^4\) microbial degradation of sulfur-containing amino acids,\(^5\) and several other processes.\(^6\) DMDS is not only malodorous, but also very toxic for all organisms. It exerts the highest insecticidal neurotoxicity through mitochondrial dysfunction in eukaryotic cells.\(^7\) Therefore, development of a biological scavenger for DMDS has been studied.\(^8\) Several DMDS-degrading microorganisms, such as *Thiobacillus thio-

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**Materials and Methods**

**Screening and cultivation conditions.** DMDS-degrading microorganisms were screened from our microbial collection using glucose/DMDS medium. This medium contained 5.0 g of K$_2$HPO$_4$, 1.0 g of NH$_4$Cl, 6.8 mg of FeCl$_3$·6H$_2$O, 0.2 g of MgCl$_2$·6H$_2$O, 10.0 g of glucose as carbon source, and 5.65 mM DMDS as sole sulfur source in 1,000 ml of distilled water. This medium and nutrient broth (NB) medium (Nissui Pharmaceutical Co., Ltd., Tokyo) were used in screening and cultivating DMDS degrading microorganisms. The final pH of the media was adjusted to 7.0. Both media were autoclaved at 121°C for 15 min. Screening and cultivation were performed at 30°C with shaking at 130 rpm with a butyl rubber plug to prevent sulfide volatilization. To detect growth of the microorganisms in the cultures, culture turbidity was measured at 660 nm.

**Identification of strain 76.** The results of taxonomical characterization for strain 76 are summarized in Table 1. Strain 76 was initially characterized using API 20NE.
strips (BioMerieux, Marcy, l’Etoile, France). Species identification (IDs) was obtained using the API database. 

A region of the 16S rDNA from strain 76 was amplified and sequenced as previously described. Fluorescent colonies were detected by illuminating the agar plates with UV light (254 nm) on King’s B medium.

**Resting cell reactions.** Pseudomonas fluorescens strain 76 was cultivated in glucose/MT medium supplemented with 0.13 mM MT for 2 d. Cells were harvested by centrifugation at 10,000 × g for 10 min at 4°C, washed twice with 0.85% NaCl, and resuspended in 0.85% NaCl. The resting cell reaction was performed in the same solution.

**Detection of DMDS metabolism intermediates of strain 76.** Strain 76 had several unique features of DMDS utilization: (i) it could not utilize DMDS as sole carbon source, although it utilized DMDS as a sulfur source, and (ii) it showed high resistance to DMDS. Hence, we attempted to identify a metabolic pathway for DMDS in strain 76. After aerobic cultivation of strain 76 at 30°C, microorganisms, strain 76 showed the highest growth rate on glucose/DMDS medium consisting of 1% glucose as carbon source and 5.65 mM DMDS as sole sulfur source, although Na₂SO₄ is a better sulfur source than DMDS (Fig. 1). Strain 76 showed a high resistance to DMDS, and it grew to concentrations of up to 9.04 mM DMDS, although other reported DMDS-degrading microorganisms have been screened below 2 mM DMDS, and their degradability was examined at concentrations below 5 mM. On the other hand, strain 76 did not belong to the methylotrophs, because it did not utilize DMDS (Fig. 1) or other C₁ compounds, e.g., methanol, formaldehyde or formic acid, as sole carbon source (data not shown). Since all DMDS-utilizing microorganisms reported to date have been methylotrophs, we thought that strain 76 had unique DMDS metabolism features. Hence, we selected it as a model organism to study its utilization of DMDS further.

First, strain 76 was identified taxonomically and biochemically. From the results of the taxonomical characterization, it belongs to the genus Pseudomonas (Table 1). The partial 16S rDNA sequence of strain 76 displayed significant homology to those of Pseudomonas fluorescens biotype F (99.4%) (Fig. 2). Moreover, strain 76 formed fluorescent colonies that were detected by illuminating the agar plates with UV light (254 nm) on King’s B medium. These findings and the results of the identification test with the API 20NE system led us to conclusion that strain 76 belonged to P. fluorescens.

**Results and Discussion**

**Screening and identification of DMDS-degrading microorganism strain 76**

Eight microorganisms were selected from our laboratory microbial collection, which contained 88 strains, and utilized DMDS as sole sulfur source. Among these microorganisms, strain 76 showed the highest growth rate on glucose/DMDS medium consisting of 1% glucose as carbon source and 5.65 mM DMDS as sole sulfur source, although Na₂SO₄ is a better sulfur source than DMDS (Fig. 1). Strain 76 showed a high resistance to DMDS, and it grew to concentrations of up to 9.04 mM DMDS, although other reported DMDS-degrading microorganisms have been screened below 2 mM DMDS, and their degradability was examined at concentrations below 5 mM. On the other hand, strain 76 did not belong to the methylotrophs, because it did not utilize DMDS (Fig. 1) or other C₁ compounds, e.g., methanol, formaldehyde or formic acid, as sole carbon source (data not shown). Since all DMDS-utilizing microorganisms reported to date have been methylotrophs, we thought that strain 76 had unique DMDS metabolism features. Hence, we selected it as a model organism to study its utilization of DMDS further.

**DMDS metabolic pathway of P. fluorescens strain 76**

Strain 76 had several unique features of DMDS utilization: (i) it could not utilize DMDS as sole carbon source, although it utilized DMDS as a sulfur source, and (ii) it showed high resistance to DMDS. Hence, we attempted to identify a metabolic pathway for DMDS in strain 76. After aerobic cultivation of strain 76 at 30°C
for 4 d, metabolic intermediates of DMDS in the headspace of the culture were detected by gas chromatography. Although DMDS was not degraded in a culture without cells at 4 d (data not shown), MT was detected from a culture incubated for 4 d by gas chromatography (Fig. 3). These findings suggest that strain 76 metabolizes DMDS to MT.

Next, strain 76 was cultivated on glucose/MT medium containing MT as sole sulfur source. It utilized MT as sole sulfur source, although it did not use it as carbon source, similarly to DMDS (Fig. 4A). Cell growth on MT reached an OD<sub>660</sub> of 1.8 after 48 h (Fig. 4A), higher than that grown on DMDS. Moreover, we attempted to detect the intermediates of MT metabolism using resting cells. After incubation of resting cells on MT, a decrease in MT in the reaction mixture and generation of formaldehyde were detected, and 45% of MT was consumed by the resting cells after 8 h (Fig. 4B). These results suggest that strain 76 metabolizes DMDS to formaldehyde via MT. The amount of formaldehyde formed was not consistent with that of MT consumed, suggesting further oxidation of the formaldehyde formed to lower metabolites, as in the formaldehyde oxidation pathway, such as form-
aldehyde detoxification of the methyloptroph. 16) Judging by these results, the DMDS metabolic pathway of strain 76 was similar to that of *Thiobacillus thioparus* E6. However, strain 76 utilized DMDS and MT as sole sulfur sources, although *T. thioparus* E6 utilized those compounds as sole carbon source. Moreover, strain 76 is a heterotroph, since it can utilize glucose as a carbon source. This might be advantageous in obtaining high cell yields of the strain at pre-cultivation. Therefore, strain 76, which has a DMDS-metabolic pathway, should be useful for applied biological deodorization in several industrial processes, *e.g.*, the wood-pulp industry, oil refineries, manure and sewer systems, and so on.

**DMDS Degradation of Strain 76 Was Not Repressed by Other Carbon and Sulfur Sources**

DMDS degradation of strain 76 was not repressed in spite of the presence of glucose, although strain 76 did not utilize DMDS as a carbon source. In addition, DMDS degradation was also unaffected by the presence of nutrient broth (data not shown). This suggests that DMDS degradation cannot be repressed by easily assimilable carbon sources such as glucose. Next, we investigated the repression of DMDS degradation by an inorganic sulfur source, Na$_2$SO$_4$. When strain 76 was cultivated on a medium containing both DMDS and 5.65 mM Na$_2$SO$_4$ as sulfur sources, MT was accumulated in the culture fluid up to 216 µM after 80 h (Fig. 5). Hence, we concluded that DMDS degradation of strain 76 was not repressed by Na$_2$SO$_4$, as it was not by glucose.

In this study, we isolated *P. fluorescens* strain 76, which had the ability to utilize DMDS as sole sulfur source but not as sole carbon source. Strain 76 metabolized DMDS via MT, and it showed high resistance to DMDS. Since DMDS is a highly toxic compound for all organisms, organisms must scavenge DMDS in order to survive in DMDS-containing habitats. DMDS degradation of strain 76 was not repressed in the presence of glucose, Na$_2$SO$_4$, or even nutrient broth. Therefore, we think that strain 76 can degrade DMDS in some habitats containing DMDS, regardless of the presence of other carbon or sulfur compounds, and that the DMDS-degrading pathway of strain 76 might function mainly in the degradation of DMDS, as well as the supply of DMDS as a sulfur source.

Strain 76 is advantageous as a tool for biological deodorization, since (i) it shows high resistance to DMDS, and (ii) its DMDS-degrading pathway was not repressed in the presence of glucose, Na$_2$SO$_4$, or nutrient broth. Based on these facts, it is possible that strain 76 can be applied to biological deodorization of DMDS.

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References