Isolation and Characterization of a Hydrogen Sulfide-Removing Bacterium, *Pseudomonas* sp. Strain DO-1

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Five microbial strains that removed hydrogen sulfide (H₂S) or methylmercaptan (CH₃SH) gas were newly isolated from soil samples. Strain DO-1, one of the isolates, was identified as a member of *Pseudomonas* sp., and its immobilized cells removed 1 or 10 ppm of H₂S gas within 2 hours. When strain DO-1 was cultured aerobically in a flask containing nutrient broth medium, the deodorizing activity increased, depending on the growth of the culture, and the maximum activity was obtained after 48 hours. Even though the immobilized cells were stored at 4 or 25°C in sealed bottles for 6 months, the deodorizing activity remained. Throughout this study, strain DO-1 removed H₂S gas without preliminary feeding or exposure to sulfur compounds as growth substrates or inducers. These characteristics are advantageous for the deodorization of the malodorous gases surrounding us in daily life.

**Key words:** biological deodorization; sulfur-containing odor; hydrogen sulfide; methylmercaptan; *Pseudomonas*

**Introduction**

Sulfur-containing malodorous gases such as hydrogen sulfide (H₂S) and methylmercaptan (CH₃SH) are emitted from some factories, waste treatment facilities, domestic animal houses, toilets, garbage, etc. Since such gases cause serious environmental problems, techniques to remove them are very important.

Commonly, chemical and physical methods have been used to remove malodorous gases. On the other hand, biological methods of deodorization have recently been attracting attention due to their low-cost, safety, low impact on environment, easy maintenance, etc. So far, various deodorizing microorganisms have been isolated and investigated with the aim of applying their abilities to biological deodorization. For example, *Bacillus* sp. BN53-1, *Hyphomicrobium neptunium* ATCC15444, *Thiobacillus denitrificans* ATCC23642, *T. ferrooxidans* DMS-583, *T. thioparus* DW44, and *Xanthomonas* sp. DY44 have been reported as H₂S-deodorizing microorganisms.

In this study, we newly isolated 5 soil microbial strains that removed H₂S or CH₃SH gas. Then we identified and characterized strain DO-1, one of the isolates.

**Materials and Methods**

**Isolation of soil microorganisms.** Nutrient agar medium for the isolation of soil microorganisms consisted of 0.7% (w/v) Extract Ehrlich (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan), 1.0% peptone (Kyokuto P.I.Co.), 0.3% NaCl, and 2.0% agar in purified water (the pH was adjusted to 7.2 by using 10 N NaOH). One hundred milligrams of a soil sample collected from forests, grasslands, or farms were mixed with 10 ml of sterilized water, and then a loop of the mixture was spread out onto an agar plate. After incubation at 30°C for 24-48 hours, microbial colonies were individually picked up and were transferred onto fresh agar plates. These plates were incubated at 30°C for 24-48 hours.

**Immobilization of microbial cells.** Cultivation of the isolates was done in nutrient broth medium consisting of 0.7% (w/v) Extract Ehrlich (Kyokuto P.I.Co.), 1.0% peptone (Kyokuto P.I.Co.), and 0.3% NaCl in purified water (the pH was adjusted to 7.2 by using 10 N NaOH). One hundred milliliters of the nutrient broth medium in a 500-ml flask was inoculated, and was constantly shaken at 210 rpm for 24-48 hours at 30°C. Then, 100 ml of the culture broth was centrifuged at 10,000 × g for 10 minutes at 4°C, and the harvested cells were resuspended in 100 ml of 0.1 M sodium phosphate buffer (pH 7.0). This suspension was mixed with 100 g of perlite powder as carrier, and then this immobilized cell preparation was used for deodorizing tests. Sterilized cells were obtained by autoclaving at 121°C for 15 min.

**Deodorizing test.** Deodorizing activity of the immobilized cells of the isolate was measured by the decrease of the concentration of malodorous compound as followed. A gas sampling bag (3-liter Tedlar Bag, Type A, Tokyo Garasu Kikai Co., Tokyo, Japan) containing 1.5 g of the immobilized cell preparation was filled with 3 liters of malodorous gas (Fig. 1). The bag was then incubated at room temperature (25 ± 1°C), and the concentration of malodorous compound in the bag was measured. H₂S or CH₃SH gas was used as a malodorous gas, and these gases were generated by a standard gas generator (Permeator, Model PD-1B, Gastec Co., Ayase, Japan) with permeation tubes (No.P-4 and No.P-71-5, Gastec Co.) with the use of air as a dilution gas. The con-
Identification of strain DO-1

Taxonomic studies were done on strain DO-1, and the results were as follows: Gram reaction, negative; cell shape, rod; cell size, 0.4–0.6 µm × 0.8–1.2 µm; motility, positive; flagellar arrangement, polar; flagellar number, 1; poly-β-hydroxybutyrate accumulation, negative; fluorescent pigment, positive; nonfluorescent pigment, negative; yellow colonies, negative; red or orange colonies, negative; violet colonies, negative; oxidase, positive; catalase, positive; arginine dihydrolase, positive; denitrification, negative; growth pH, 3.2–9.5; growth temperature, 8–45°C; growth concentration of NaCl, 0–6%; growth greatly stimulated by or dependent upon cysteine, negative; 1-C compounds as sole carbon source, positive; nitrate used as a nitrogen source, positive; hydrolysis of gelatin, positive; hydrolysis of starch, negative; acid from glucose, positive; utilization-positive, glucose, trehalose, D-ribose, β-alanine, L-arginine, L-serine, L-histidine, betaine, mesoamine, 2,3-butylene glycol; utilization-negative, D-xylene, L-rhamnose, sucrose, fructose, levulinate, D(-)–tartrate, m-hydroxybenzoate, adonitol, mannitol, ethylene glycol.

According to these results, strain DO-1 was identified as a *Pseudomonas* sp. Although these taxonomic characteristics were similar to that of *Pseudomonas aeruginosa*, we could not identify the species, because the taxonomic characteristics of strain DO-1 differed from that of *P. aeruginosa* on the utilization of trehalose and levulinate.

H₂S removal by strain DO-1

The course of the removal of 1 or 10 ppm of H₂S by the immobilized cells of strain DO-1 was investigated. As shown in Fig. 2, H₂S was completely removed within 1–2 hours for each concentration. The sodium phosphate buffer and the pearlite powder that were used to prepare the immobilized cell preparation did not remove H₂S gas under our experimental conditions.

H₂S removal and growth of strain DO-1 in liquid medium

We examined the relation of the progress between the cultivation of strain DO-1 and the ability to remove H₂S. The result shown in Fig. 3 indicated that the deodorizing activity increased depending on the growth of cells in the culture. The maximum activity was obtained at 48 hours, however no great change was observed from 24–72 hours.

Storage stability of immobilized strain DO-1 cells

To investigate the storage stability of the immobilized cell preparation of strain DO-1, it was stored at 4 or 25°C in sealed bottles. The result of periodic examination of the deodorizing activities and the cell densities of the immobilized cell preparation is shown in Fig. 4. The deodorizing activity and the cell density gradually decreased at each temperature. However, approximately 75% of the initial activity still remained even after 6 months for the sample stored at 4°C.

H₂S requirement for strain DO-1 as an inducer

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Table 1. Removal of Hydrogen Sulfide (H₂S) or Methylmercaptan (CH₃SH) by Microbial Isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Removal of:</th>
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<tr>
<td></td>
<td>10 ppm H₂S</td>
</tr>
<tr>
<td>DO-1</td>
<td>+</td>
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<tr>
<td>DO-1033</td>
<td>–</td>
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<tr>
<td>DO-1037</td>
<td>–</td>
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<tr>
<td>E-4</td>
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<td>E-104</td>
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+, removed; –, not removed; NT, not tested.

Deodorizing activities of the immobilized cells of the isolates were measured by deodorizing tests, and the deodorizing microorganisms were selected.
Fig. 2. Course of Hydrogen Sulfide Removal by Immobilized Cells of Strain DO-1.
One point five grams of sample were exposed to 3 liters of 1 ppm (A) or 10 ppm (B) hydrogen sulfide gas in the gas sampling bag. The bag was incubated at room temperature (25 ± 1°C) and removal of hydrogen sulfide was estimated. ■, immobilized-cells of strain DO-1; ○, sterilized immobilized-cells of strain DO-1; △, without cells (sodium phosphate buffer and pearlite powder).

Fig. 3. Removal of Hydrogen Sulfide and Growth of Strain DO-1 in Liquid Medium.
Cells were inoculated into a shaking 500-ml flask containing 100 ml of nutrient broth medium and then incubated at 30°C for 3 days, and the change of cell number and activity to remove hydrogen sulfide were measured. Cell number in the culture medium was measured with the colony forming unit method. Activity of immobilized cells that were prepared from the sample of the culture medium was measured as removal of 1 ppm hydrogen sulfide gas for 30 minutes by the deodorizing test using the gas sampling bag (3 liter). ○, cell number; △, pH; ■, activity.

Fig. 4. Storage Stability of Immobilized Strain DO-1 Cells.
Sealed bottles containing immobilized cell preparation were stored at 4°C (■, ○) or 25°C (■, △) for 6 months, and the change of cell number and activity to remove hydrogen sulfide was measured. Cell number (○, △) was measured with the colony forming unit method. The activity of cells (■, △) was measured as removal of 3 liters of 1 ppm hydrogen sulfide gas for 3 hours by the deodorizing test using the gas sampling bag.

Fig. 5. Effects of the Pre-exposure to Hydrogen Sulfide Gas on the Deodorizing Activity of Strain DO-1.
One point five grams of immobilized cell preparation were exposed to 3 liters of 1 ppm hydrogen sulfide gas for 3 hours in the gas sampling bag, 2 times. Then the activity to remove 2 ppm of hydrogen sulfide was estimated with a deodorizing test. ■, preexposed immobilized-cells; ○, non-preexposed immobilized-cells; △, without cells (sodium phosphate buffer and pearlite powder).

It was investigated whether H₂S gas act as an inducer for the sulfur-deodorizing activity of strain DO-1. First, the immobilized cell preparation of strain DO-1 was exposed to 2 ppm H₂S gas for 3 hours, 2 times. Secondly, the activity to remove 2 ppm of H₂S was estimated. There was no great difference between the pre-exposed cells and the non-pre-exposed cells for the deodorizing activities (Fig. 5).
Discussion

Previously, some isolated sulfur-deodorizing microorganisms were reported. In these studies, concentrations of malodorous compounds were mostly more than 10 ppm (mainly 20–4,000 ppm), because the aims of these studies were deodorizing such high concentrations of malodorous gases emitted from some industrial sources (factories, waste treatment facilities, domestic animal houses, etc.). However, the concentrations of malodorous sulfur-compounds surrounding us in daily life (that is non-industrial sources; toilets, foods, garbage, pets, etc.) is mostly less than 1 ppm, and recently these low concentration of odors were found to cause serious troubles. Not all the microorganisms reported can be used to remove such low concentrations of malodorous gases, because it is known that the microorganisms degrading high concentration of a compound cannot always degrade lower concentrations of it.

Hirano et al. reported a bacterial deodorant aiming to remove H₂S in our daily life, but the concentration of H₂S tested was 20 ppm. There is no information so far available about removing a low concentration (below 1 ppm) of sulfur-containing odor by isolated microorganisms. We isolated microorganisms that can remove 1 ppm of H₂S gas or 0.5 ppm of CH₃SH gas. These microorganisms, including strain DO-1, should be suitable for removal of the low concentration of odors surrounding us in daily life.

On the other hand, some deodorizing microorganisms previously reported required pre-feeding of particular sulfur compound as a growth substrate. For example, some of them required pre-exposure to H₂S gas, and the other one required pre-incubation in a medium containing Na₂S as a growth substrate. Such microorganisms are often difficult to handle to induce, control, and retain their ability of deodorization. So, it seems that these microorganisms are hard to use to remove the odors surrounding us in daily life. We screened of deodorizing microorganisms without first feeding particular sulfur compounds as growth substrates. Our isolates showed their deodorizing abilities without feeding of such compounds.

Some pseudomonads that showed their abilities to degrade environmental contaminants inductively were reported before. Although strain DO-1 showed its ability to remove H₂S constitutively (Fig. 5). This means that strain DO-1 can remove H₂S without a pre-exposing process (or lag time), and can keep their ability without continuous exposure to H₂S. This characteristics seem to be necessary to remove the odors surrounding us in daily life, because most such odors are not emitted continuously.

The deodorizing activity of strain DO-1 was relative to the cell number of the culture (Fig. 3), and the immobilized cells of strain DO-1 retained their deodorizing activity for a long period (Fig. 4). From these results, it can be assumed that strain DO-1 has a mechanism to remove H₂S constitutionally.

Thus, strain DO-1 has the following characteristics:
1. It can remove low concentrations (1 ppm) of H₂S.
2. It does not require pre-feeding of or pre-exposing to a particular sulfur compound.
3. It retains its activity for a long period.

Therefore, strain DO-1 is shown to be useful as a biological deodorant for the malodorous gases surrounding us in daily life.

Further studies to discover the mechanisms of H₂S removal by strain DO-1 are in progress.

References