Isolation of Dimethyl Sulfone-Degrading Microorganisms and Application to Odorless Degradation of Dimethyl Sulfoxide

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With the objective of developing an odorless biodegradation process for dimethyl sulfoxide (DMSO), Hyphomicrobium sp. WU-OM3 was isolated. During the cultivation of strain WU-OM3 cells with 20 mM dimethyl sulfone (DMSO2) as the sole carbon source, DMSO was completely consumed within 48 h and sulfate ion accumulated in the culture broth. Methanesulfonate was also detected as an intermediate of DMSO degradation. By combining the DMSO-oxidizing microorganism and strain WU-OM3 cells, 0.64 mM (50 mg/l) DMSO was degraded to sulfate ion with 80% molar conversion ratio.

[Key words: dimethyl sulfoxide, dimethyl sulfone, methanesulfonate, wastewater, Hyphomicrobium]

Dimethyl sulfoxide (DMSO) is widely used in various industries, since it dissolves many organic and inorganic substances (1). In the manufacture of semiconductors or liquid crystal displays, DMSO is used as a detergent or a photoresist stripping agent, and a large amount of wastewater containing DMSO is discarded from the washing or rinsing process (2). It has been reported that DMSO is easily reduced to dimethyl sulfide (DMS) which is a malodorous toxic compound by microorganisms in an activated sludge process (3). With the objective of safely and efficiently degrading DMSO, we screened for microorganisms, which can utilize DMSO as the nutrition.

Previously, we reported on Hyphomicrobium denitrificans WU-K217 which utilizes DMSO as the sole carbon source (4). Similar to most DMSO-degrading microorganisms (5, 6), strain WU-K217 seemed to reduce DMSO to DMS as the initial step of DMSO degradation.

On the other hand, we also isolated Cryptococcus humincola WU-2 which utilizes DMSO as the sole sulfur source and reported on its taxonomical characteristics and DMSO-degrading properties (7). Both growing and resting cells of strain WU-2 oxidized DMSO to dimethyl sulfone (DMSO2), which is an odorless compound with a high molar conversion ratio based on the DMSO consumed. However, under any reaction condition for the DMSO degradation by strain WU-2, sulfite ion was not detected as a metabolite of DMSO (7).

Considering the DMSO-degrading properties of microorganisms such as strains WU-K217 and WU-2 (4, 7), with the objective of degrading DMSO without the release of DMS, we screened for microorganisms which utilize DMSO2 as the sole carbon source. We describe here the isolation of Hyphomicrobium sp. WU-OM3 and its DMSO2 degradation pathway with quantitative analysis of the metabolites. Although it has been suggested that some microorganisms degrade DMSO or DMS to sulfate ion via methanesulfonate (MSA) (8–11), the detection of MSA as a metabolite of these organosulfur compounds has not been performed and a clear evidence of this suggestion has not been shown. In this report, the degradation of DMSO to sulfate ion by combining strains WU-2 and WU-OM3 cells is also described.

RT medium was used for the screening and cultivation of DMSO2-degrading microorganisms (4). Unless otherwise indicated, 20 mM DMSO2 was added to the RT medium as the sole carbon source. All reagents were obtained from Kanto Chemicals (Tokyo). For the screening of DMSO2-degrading microorganisms, each sample was suspended in physiological saline solution, and a 0.2-ml aliquot of each suspension was inoculated into an 18-mm-diameter test tube containing 5 ml of RT medium. After cultivating for 7–14 d at 30°C with reciprocal shaking at 240 rpm, a 0.2-ml aliquot of the broth was subcultivated five times into 5 ml of fresh RT medium. As a parameter of microbial growth, the optical density (OD660) of the culture broth was measured at 660 nm using a Shimadzu UV-1200 spectrophotometer (Shimadzu, Kyoto). The broth showing growth after the subcultivations was subjected to the analysis of residual DMSO2 concentration. DMSO2 was measured by gas chromatography–mass spectrometry (GC–MS) as described previously (7). DMSO and sulfate ion were measured by high-performance liquid chromatography (HPLC) as described previously (4). MSA was measured by the same method as that used for sulfate ion.

Approximately 200 samples of soil, wastewater and activated sludge were subjected to screening, and strain WU-OM3 was isolated on KM agar medium which contained methylamine as the carbon source (4). During the cultivation of strain WU-OM3 cells, 20 mM DMSO2 was completely consumed within 48 h and both MSA and sulfate ion
were detected in the culture broth (Fig. 1). After 108-h cultivation of strain WU-OM3 cells, sulfate ion accumulated with almost 100% molar conversion ratio on 20 mM DMSO. DMSO was not detected throughout the cultivation period. Strain WU-TDL1 cells which were obtained by this screening method accumulated MSA in the culture broth. In the case of strain WU-TDL1 cells, it required 84 h for the complete consumption of 20 mM DMSO. In the case of strain WU-OM3 cells, it required 84 h for the complete consumption of 20 mM DMSO and 10.6 mM MSA accumulated after the 108-h cultivation period. Neither DMSO nor sulfate ion was detected throughout the cultivation of strain WU-TDL1 cells. Considering the growth level and the metabolites of DMSO, it seemed likely that strain WU-TDL1 cells utilized only half of the moiety of carbon in DMSO. Therefore, we considered that strain WU-OM3 might be more suitable for DMSO degradation than strain WU-TDL1 and used the former in further experiments. Strain WU-OM3 was identified as *Hyphomicrobium* sp. by the partial sequence of 16S rRNA. There have been few detailed studies on microorganisms which utilize DMSO as the sole carbon source. Only Borodina et al. reported that *Arthrobacter methylotrophus* TGA and *Hyphomicrobium sulfonivorans* S1 degraded DMSO to sulfate ion via DMSO and DMS (12, 13). In contrast, by analyzing the metabolites of DMSO, it seemed likely that strain WU-OM3 cells degraded DMSO to sulfite ion via DMSO and DMS (12, 13). As a result, the degradation properties of strain WU-OM3 cells toward DMSO, and related compounds were evaluated using resting cells. Strain WU-OM3 cells were cultivated for 3 d in a 500-ml Erlenmeyer flask containing 200 ml of RT medium for the preparation of resting cells. The cells were harvested by centrifugation at 10,000 × g for 5 min at 4°C to separate the cells and/or cell debris as precipitate. During DMSO degradation by the resting cells of strain WU-OM3, MSA accumulated as an intermediate. DMSO (2.56 mM) was degraded within 45 min to sulfate ion with almost 100% molar conversion ratio without the release of odor (Fig. 3). DMS was not detected by GC–MS (4) throughout the DMSO degradation by the resting cells of strain WU-OM3. The resting cells of strain WU-OM3 also degraded 2.56 mM MSA to sulfate ion with almost 100% molar conversion ratio within 90 min (Fig. 3). We considered that the lower degradation rate for MSA than for DMSO might be caused by the toxic effect of MSA. The resting cells of strain WU-OM3 could not degrade DMSO or DMS.

Previously, we reported on the isolation of strain WU-2 and on its DMSO-degradation properties (7). Strain WU-2 cells utilize DMSO as the sole sulfur source and oxidize it to DMSO with a high molar conversion ratio. Therefore, we examined the degradation of DMSO to sulfate ion by combining strains WU-2 and WU-OM3 cells. First, strain WU-2 cells were cultivated on TA-1 medium which contained DMSO as the sole sulfur source. The initial DMSO concentration in the TA-1 medium was 0.64 mM (50 mg/l), which is the concentration in wastewater. After the 48-h cultivation period, DMSO was completely consumed and DMSO accumulated with 92% molar conversion ratio. Then, strain WU-OM3 cells were inoculated and the cultivation was continued. With further cultivation for 72 h, DMSO was completely consumed and sulfate ion was accumulated with 91% molar conversion ratio based on 0.64 mM DMSO.

DMSO degradation by combining strains WU-2 and WU-OM3 was also performed using resting cells. First, 0.64 mM DMSO was added to the resting cells of strain WU-2 with a cell density of 10 at OD_{660}. After the 24-h resting cell reaction, the cells and/or cell debris of strain WU-2 were separated by centrifugation. Then the supernatant was mixed with the same volume of resting cells of strain WU-OM3 to give a final cell density of 10 at OD_{660}. Following the resting cell reaction of strain WU-OM3, sulfate ion accumulated with 80% molar conversion ratio based on 0.64 mM DMSO within 15 min. Neither DMSO nor DMSO was detected in the reaction mixture.

In this report, we described the isolation of *Hyphomicrobium* sp. WU-OM3 and its application to DMSO degrada-

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**FIG. 1.** Degradation of DMSO by growing cells of strain WU-OM3. Strain WU-OM3 cells were cultivated at 30°C in RT medium with 20 mM DMSO as the sole carbon source. Symbols: circles, growth (OD_{660}); diamonds, DMSO; closed triangles, MSA; open triangles, sulfate ion.

**FIG. 2.** Proposed pathway for DMSO degradation by *Hyphomicrobium* sp. WU-OM3 (A) and those by *Arthrobacter methylotrophus* TGA and *Hyphomicrobium sulfonivorans* S1 (B) (12, 13). Abbreviations: DMSO₂, dimethyl sulfone; MSA, methanesulfonate; DMSO, dimethyl sulfoxide; DMS, dimethyl sulfide.
We considered that the biodegradation of DMSO by combining a DMSO-oxidizing microorganism and a DMSO-degrading microorganism might be applicable to the odorless degradation of DMSO.

REFERENCES

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