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Biodegradation of Formaldehyde by a Formaldehyde-Resistant Bacterium Isolated from Seawater

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Abstract

A formaldehyde-tolerant bacterium designated as a DM-2 strain was used to biodegrade formaldehyde. The cells, precultivated in the presence of 400 ppm of formaldehyde, were able to degrade formaldehyde in a minimal medium supplemented with up to 400 ppm of formaldehyde in the presence of 3% NaCl. The rate of formaldehyde degradation achieved in this study was 45 ppm/h when the DM-2 culture's optical density at 660 nm was 1.2.

Index Entries: Biodegradation; formaldehyde; marine bacterium; screening.

Introduction

Formaldehyde, a volatile organic compound, is widely used in medicine, agriculture, and industrial processes as a disinfectant for killing bacteria and fungi. Moreover, formaldehyde is contained in materials such as pesticides, plastics, and adhesives. However, considering its high cytotoxicity toward human health and the environment, the removal of formaldehyde from soil, water, and air has become a necessity.

Regarding the biodegradation of formaldehyde from a polluted environment, a number of studies have been conducted on the isolation of bacteria resistant to high concentrations of formaldehyde. *Pseudomonas* sp. *(1,2)*, *Escherichia coli (3)* and *Halomonas* sp. *(4)*, and *Trichosporon* sp. *(5)* have been isolated and characterized. These bacteria were isolated from soil or river water near a chemical plant that used formaldehyde. The biodegradation of formaldehyde under anaerobic conditions was also reported *(6,7)*.

In the marine environment, wastewaters containing formaldehyde are being discharged from rivers. In addition, formaldehyde has also been used as a fungicide for fishing implements such as nets. Formaldehyde has

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been detected in seawater, and the removal of formaldehyde has also been required. However, the isolation of formaldehyde-degrading bacterium and the biodegradation of formaldehyde in seawater have not been reported.

Recently, we succeeded in isolating a formaldehyde-tolerant bacterium from a marine environment. Since this bacterium can use formaldehyde as a carbon source, its application for the bioremediation of formaldehyde in a marine environment is expected.

In this article, we describe the application of the formaldehyde-tolerant bacterium for the degradation of formaldehyde.

Materials and Methods

Chemical

All chemicals used in this study were reagent grade. Formaldehyde (36% solution) was purchased from Kanto (Tokyo, Japan). Iron (III) chloride and 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) were obtained from Wako Pure Chemical (Osaka, Japan). Yeast extract and trypton were from Difco (Detroit, MI).

Bacteria and Culture Conditions

A formaldehyde-resistant bacterium, DM-2 strain, used in this study was isolated from coastal seawater in Japan. It was cultured aerobically at 28°C with shaking in a modified DM-2 medium (30 g of NaCl, 5 g of trypton, 5 g of yeast extract, 6 g of Na_2HPO_4, 3 g of KH_2PO_4, 1 g of NH_4Cl, 1.2 g of $MgSO_4$, and 111 mg of $CaCl_2$ in 1 L, pH 6.8). Formaldehyde was added to the medium as indicated in each experiment. Cell density was monitored by measuring optical density at 660 nm (OD_{660}). Cells cultivated in the medium in either the presence or the absence of formaldehyde were collected by centrifugation at 4000*g* for 10 min at 4°C. The pellet was washed two times with 10 m*M* potassium phosphate buffer (pH 7.0) containing 3% NaCl and used for biodegradation experiments.

Biodegradation of Formaldehyde

Washed cells of the DM-2 strain that had been grown until the late log growth phase with or without 200 or 400 ppm of formaldehyde were resuspended in 50 mL of M9S medium (30 g of NaCl, 6 g of Na_2HPO_4, 3 g of KH_2PO_4, 1 g of NH_4Cl, 1.2 g of $MgSO_4$, and 111 mg of $CaCl_2$ in 1 L) in 100-mL Erlenmeyer flasks. Formaldehyde was added to the medium at the concentration indicated in each experiment. Cell density in the medium was adjusted by measuring OD_{660}. The medium containing cells was incubated at 28°C with shaking (130 strokes/min). One milliliter of sample was removed from the medium, and the sample was centrifuged at 4000*g* for 3 min to remove cells. Supernatant of the medium was collected, and the formaldehyde concentration was analyzed.

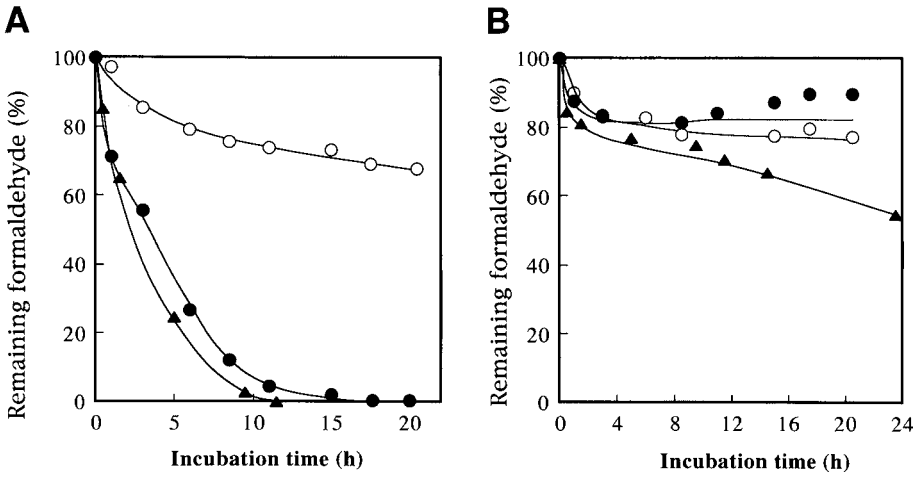

Fig. 1. Time course of formaldehyde degradation by a formaldehyde-tolerant bacterium, DM-2. Formaldehyde concentration in the minimal medium was 200 **(A)** and 400 ppm **(B)**, respectively. DM-2 strains were precultivated in modified DM-2 medium in the absence of formaldehyde (○), in the presence of 200 ppm of formaldehyde (●), or in the presence of 400 ppm of formaldehyde (▲). Cell density in the medium was adjusted to 0.6 OD_{660}.

Analysis of Formaldehyde Concentration

Formaldehyde concentration in the culture supernatant was determined using a colorimetric reaction with $FeCl_3$ and MBTH as reported previously *(8)*. To 200 µL of sample containing up to 10 ppm of formaldehyde, 40 µL of 0.4% MBTH in 2 *N* HCl was added. After 20 min of incubation at room temperature, 10 µL of 1% (w/v) $FeCl_3$ was added. After 10 min of incubation at room temperature, 250 µL of acetone was added. The final solution was incubated for 20 min at room temperature for the color to stabilize. The absorbance at 670 nm was measured using a spectrophotometer (UV1200, Shimazu, Kyoto, Japan).

Results and Discussion

Figure 1 shows the time course of formaldehyde degradation in minimal medium containing 3% NaCl. DM-2 strain cells were precultivated until the late logarithmic growth phase in either the presence or the absence of 200 or 400 ppm of formaldehyde and were used for the biodegradation experiments. Cells were added to M9S minimal medium containing either 200 ppm (Fig. 1A) or 400 ppm (Fig. 1B) of formaldehyde as the sole carbon source.

DM-2 was able to degrade formaldehyde in M9S medium containing 200 ppm of formaldehyde, as shown Fig. 1A, and formaldehyde was completely degraded within 15 h. DM-2, which was precultivated in the absence of formaldehyde, also showed the degradation of formaldehyde; however, >70% of formaldehyde remained even after 20 h.

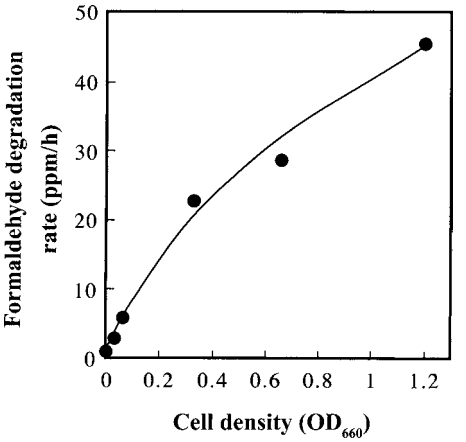

Fig. 2. Effect of cell density on formaldehyde degradation rate. Cell density was indicated as the absorbance at 660 nm (OD_{660}). The formaldehyde concentration in the M9S minimum medium was 200 ppm.

The biodegradation of 400 ppm of formaldehyde was also attempted. DM-2 cells were able to degrade formaldehyde; therefore, the cells were tolerant of 400 ppm of formaldehyde. However, the ability of formaldehyde degradation was dependent on the precultivation conditions. The cells precultivated in the presence or absence of 200 ppm of formaldehyde could degrade formaldehyde for 4 h (Fig. 1B). After 4 h, these cells did not show formaldehyde degradation. On the other hand, the cell precultivated in the presence of 400 ppm of formaldehyde degraded formaldehyde continuously and 45% of formaldehyde in the medium was degraded within 24 h. Therefore, the DM-2 strain is classified as a high formaldehyde-tolerant microorganism. It can degrade and tolerate up to 400 ppm of formaldehyde, a concentration at which most, if not all, formaldehyde-tolerant bacteria, such as *E. coli (3)* and *Halomonas* sp. *(4)*, are unable to survive. The DM-2 strain can be used for the construction of a formaldehyde degradation system that may be adapted to a higher formaldehyde concentration environment.

It has been reported that formaldehyde is metabolized in a reaction catalyzed by the combination of formaldehyde dehydrogenase and formate dehydrogenase in *Pseudomonas* sp. *(1,2)*, *E. coli (3)*, and *Halomonas* sp. *(4)*. Induction of glutathione-dependent formaldehyde dehydrogenase activity in *E. coli* and *Haemophilus influenza* was reported *(3)*. Production of formaldehyde dehydrogenase may be induced by formaldehyde during the growth phase; consequently, precultivated DM-2 strain in the presence of formaldehyde showed high formaldehyde degradation activity.

Figure 2 shows the effect of cell concentration in the medium on the formaldehyde degradation rate. In minimal medium, 200 ppm of formaldehyde was added. As seen from Fig. 2, increased cell concentrations resulted in increased rates of formaldehyde degradation. The formalde-

hyde degradation rate was dependent on the cell density. The highest formaldehyde degradation rate achieved was 45 ppm/h (45 mg of formaldehyde/(L·h)) at a DM-2 strain concentration corresponding with an OD_{660} of 1.2. Lu and Hegemann *(6)* reported that under 200 or 400 ppm of formaldehyde concentration, >90% of formaldehyde was degraded by incubation with anaerobic sludge for 20 d *(6)*. This study demonstrated, for the first time, the potential application of marine bacteria for the biodegradation of formaldehyde.

Conclusion

We demonstrated formaldehyde degradation by using a novel formaldehyde-tolerant bacterium isolated from a marine environment, the DM-2 strain. The bacterium could degrade high concentrations of formaldehyde up to 400 ppm in M9 minimal medium containing 3% NaCl. This bacterium can be used as the basis of a bioremediation system that can remove formaldehyde from marine environments.

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References

1. Kato, N., Kobayashi, H., Shimao, M., and Sakazawa, C. (1984), *Agric. Biol. Chem.* **48,** 2017–2023.
2. Yanase, H., Noda, H., Kiyotaka, A., Kita, K., and Kato, N. (1995), *Biosci. Biotechnol. Biochem.* **59,** 197–202.
3. Gutheil, W. G., Kasimoglu, E., and Nicholson, P. C. (1997), *Biochem. Biophys. Res. Commun.* **238,** 693–696.
4. Azachi, M., Henis, Y., Oren, A., Gurevich, P., and Sarig, S. (1995), *Can. J. Microbiol.* **41,** 548–553.
5. Kaneko, Y., Ito, M., Ogura, Y., and Ishikawa, J. (1985), US patent no. 4,505,821.
6. Lu, Z. and Hegemann, W. (1998), *Water Res.* **32,** 209–215.
7. Omil, F., Méndez, D., Vidal, G., Méndez, R., and Lema, J. M. (1999), *Enzyme Microb. Technol.* **24,** 255–262.
8. Eberhardt, M. A. and Sieburth, J. M. (1985), *Mar. Chem.* **17,** 199–212.