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Isolation of a Bacterium from Mangrove Soil for Degradation of Sea Sludge

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Abstract

Sea sludge, which is sediment of fish excrement and sewage on the sea bottom, continues to be a serious environmental problem. It has the potential to cause eutrophication and red tide, resulting in the death of shellfish and leading to an offensive odor. Soil taken from a mangrove swamp was added to sea sludge, which promoted an initial fermentation of the sludge components. This article reports on the isolation of a bacterium from mangrove soil that is involved in that fermentation. Three bacteria were isolated on a marine agar plate after incubating for 12 h at 60°C. One of these bacteria fermented sea sludge. 16S rDNA of this bacterium was sequenced, and it had a high homology with that of *Bacillus fumarioli* LMG17489^T (AJ250056).

Index Entries: Sea sludge; mangrove; fermentation; isolation; bacterium.

Introduction

Cultivation of fish and shellfish in a cove has a great potential to cause red tide, which results in offensive odors and fish kills *(1,2)*. Sea-life excrement accumulates as sea sludge and remains for a long time because there is little exchange between seawater in a cove and seawater in the open ocean. In addition, high concentrations of nitrogen, phosphorus, and organic compounds from an influx of domestic wastewater remain at the bottom of a cove as sea sludge. Therefore, the treatment of sea sludge has the potential to make a significant contribution to environmental protection.

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Composting sea sludge may solve these problems. It is simple and cost-effective to use microorganisms for decomposition of organic materials. To date, municipal waste *(3,4)* and sewage sludge *(5,6)* have been composted, but there are no reports of sea sludge being used as a substrate for composting.

Adding soil taken from a mangrove swamp to sea sludge promoted an initial fermentation of sludge components (first stage in a composting reaction; personal communication). The results suggested that microorganisms, which can degrade organic compounds in sea sludge, exist in mangrove soil.

In this article, we report on the isolation of bacteria from mangrove soil and the evaluation of one bacterium involved in the fermentation of sea sludge. A phylogenetic tree for this bacterium was prepared based on its 16S rDNA sequence.

Materials and Methods

Sea Sludge

Sea sludge was taken from the bottom of a cove in Ago Bay, Mie Prefecture, Japan. The bottom was about 8 m under the sea, and the sea sludge was scooped at a depth of 0–20 cm from the surface using a bottom sampler. Pearl cultivation in this bay is considered a main cause of eutrophication. The sludge was stored at 4°C until use.

Other Materials

Rice chaff from a farm was used as a bulking agent for fermentation. All the chemicals used in this study were analytical grade and obtained from Wako (Osaka, Japan) and Tokyo Chemical (Tokyo, Japan). Artificial seawater was purchased from Yashima (Osaka, Japan), and marine broth was purchased from Difco (Detroit, MI).

Isolation of Bacteria from Mangrove Soil

Mangrove swamp soil was collected at Iriomote Island (Taketomi-cho, Yaeyama County, Okinawa Prefecture, Japan). The collection was completed at low tide at a depth of 10–20 cm in a tidal estuary.

For the extraction of bacteria, 15 mL of artificial seawater was added to 5 g of soil, and the mixture was centrifuged at 2000*g* for 15 min. The supernatant was diluted at various concentrations with artificial seawater and applied onto marine agar plates. The plates were incubated at 60°C for 12 h.

Fermentation Procedure

Three hundred grams of sea sludge and chaff at a ratio of 4:1 (dry wt) was prepared with the moisture content adjusted to 60%. Separate fermentation experiments were conducted with three isolated bacteria, and the inoculum was 10 mg (dry wt) of cells. Sea sludge and chaff with no addition

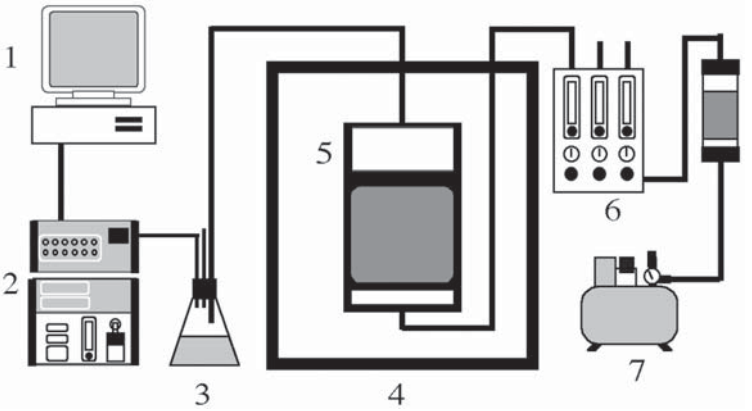

Fig. 1. Schematic diagram of fermentation system. 1, Personal computer; 2, CO_2 analyzer; 3 and 8, silica gel column; 4, thermostated chamber; 5, compost reactor; 6, reducing valve and flowmeter; 7, air compressor.

was used as a control. Another experiment used wet mangrove soil that was equivalent to 5 g of dry soil as inoculum for the sea sludge and chaff.

A schematic diagram of the fermentation system is shown in Fig. 1. Experiments with the three bacterial isolates were conducted simultaneously using three reactors connected to the chamber in parallel. The chamber, controlled by a thermostat, was maintained at 60°C. The airflow was kept at 150 mL/min. The CO_2 concentration from the reactor was monitored using a CO_2 analyzer (Exhaust CO_2 meter; ABLE, Japan).

Phylogenetic Tree

The V3 region of 16S rDNA (*Escherichia coli* position 341–534) of the isolated bacterium, which effectively degraded sea sludge, was sequenced with a DNA sequencer (PRISM Model 377; ABI). The sequence was subjected to a homology search by BLAST *(7)* from the DNA Data Bank of Japan (DDBJ), and the phylogenetic tree of the sequences was made using CLUSTAL W *(8)* from the DDBJ.

Calculation of Amount of Organic Compounds (Carbohydrate, Protein, Lipid) in Sea Sludge After Fermentation

The amounts of organic compounds (carbohydrate, protein, lipid) in three samples—sea sludge before fermentation (*a*) and the mixture of sea sludge with chaff before (*b*) and after (*c*) fermentation—were measured as described in ref. *9*. Amounts of protein and lipid were measured by Kjeldahl's method and Soxhlet method, respectively. Regarding carbohydrate, the total amount of organic compounds was first measured using the ashing method, and then the amount of carbohydrate was calculated by subtracting those of protein and lipid from that of organic compounds.

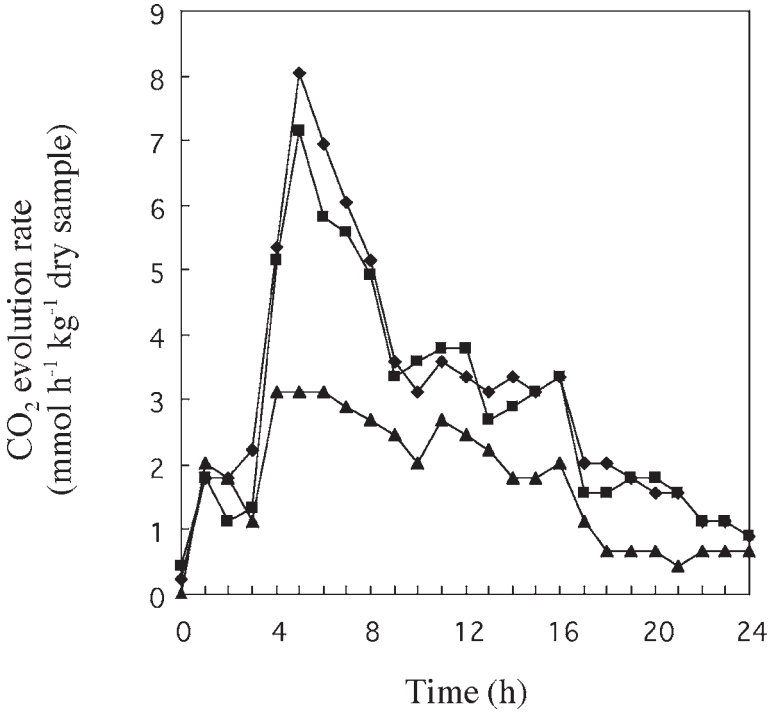

Fig. 2. Time course of CO_2 evolution during fermentation of sea sludge with rice chaff as bulking agent. Inoculum: WC bacterial isolate (■); mangrove soil (◆); and control, which is sea sludge and rice chaff without added bacteria (▲).

The amount of organic compounds in sea sludge after fermentation (x) was calculated using the following equation:

$$x = a - 1.25 \times (b - c)$$

in which a = organics in sea sludge before fermentation (g/100 g of dried sea sludge), b = organics in sea sludge + chaff mixture before fermentation (g/100 g of dried mixture), and c = organics in sea sludge + chaff mixture after fermentation (g/100 g of dried mixture). A correction factor of 1.25 was used because 80 g of sea sludge (dry wt) was contained in 100 g of the mixture (dry wt).

Results and Discussion

Isolation of Bacteria from Mangrove Soil

Three bacteria (WC, WD, and WE) were isolated on marine agar plates incubated at 60°C. The morphologic shapes of the colonies of WC and WD were similar with an ivory color, while WE exhibited a white colony with spores observed by microscopy.

Three isolates were cultured at 60°C for 12 h in the marine broth to obtain enough biomass for the fermentation experiment. The cells were

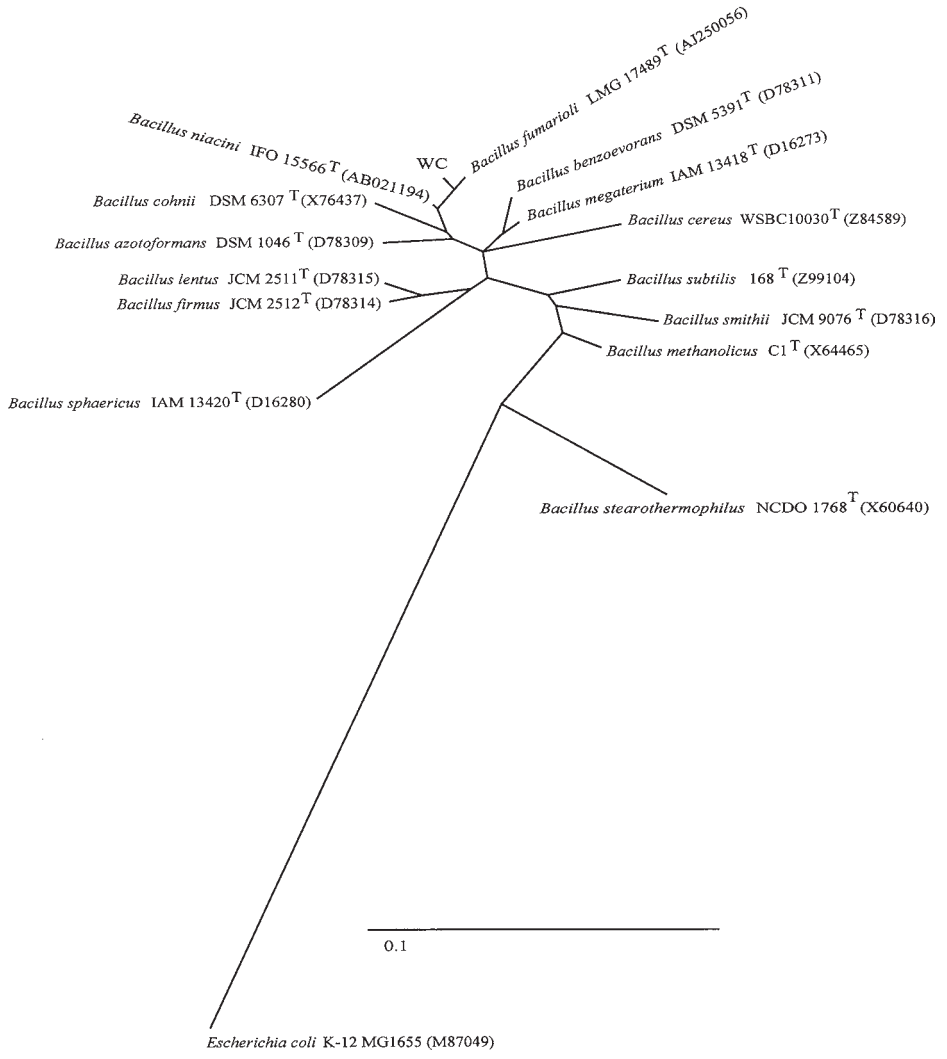

Fig. 3. Phylogenetic tree based on 16S rDNA sequences of WC and some related bacteria.

collected by centrifugation, and the pellets were washed with artificial seawater to remove the marine broth.

CO_2 Monitoring During Fermentation

During the fermentation process, the control batch containing only sea sludge and rice chaff showed a slow rate of CO_2 evolution without any clear peak observed (Fig. 2). On the other hand, the time course of CO_2 evolution during the fermentation using WC bacteria showed a rapid CO_2 evolution rate resulting in a clear peak in the time course profile. Interestingly, the profile of the mangrove soil inoculum was identical to the profile of the WC bacteria. The same profiles were obtained in three replicate experiments.

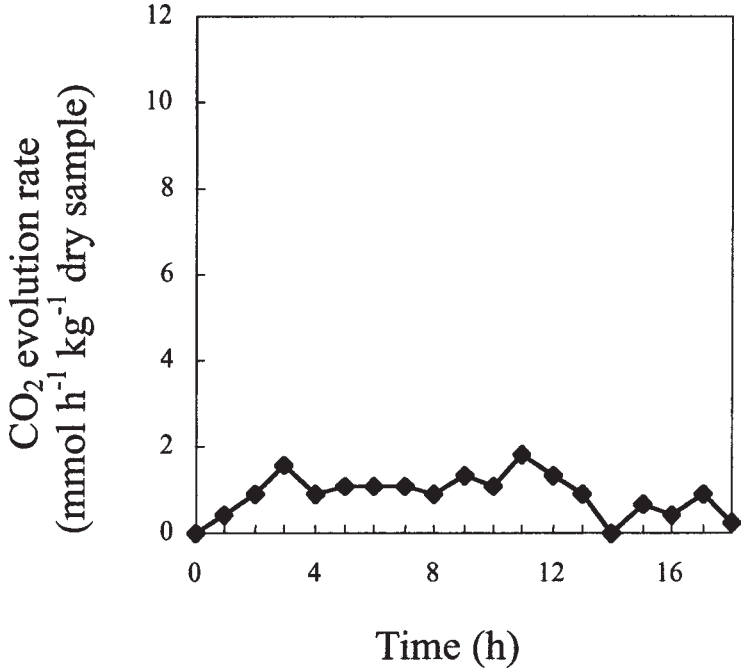

Fig. 4. Time course of CO_2 evolution using WC, rice chaff, and sea sand.

These results suggest that the WC isolate was a main constituent in the mangrove soil for the degradation of sea sludge and that fermentation of sea sludge could be conducted using only the WC isolate.

Phylogenetic Tree

The V3 region of the 16S rDNA of WC was sequenced, analyzed by BLAST, and subjected to phylogenetic analysis using CLUSTAL W. Figure 3 shows the phylogenetic relationship. It was found from the tree that WC had the highest homology with the *Bacillus fumarioli* LMG17489^T (AJ250056). This bacterium was first isolated from geothermal environments in Antarctica and shown to grow at 50°C *(10)*. Further characterization of WC is under investigation.

Degradation of Organic Compounds in Rice Chaff in Fermentation

In this study, rice chaff was used as a bulking agent. However, since it consists of large amounts of organic compounds, it might be dominantly degraded in the fermentation process. Therefore, another fermentation test was conducted using sea sand, rice chaff, and WC without sea sludge. No significant change in the CO_2 evolution rate was observed during the time course of the fermentation (Fig. 4). Therefore, the organic compounds in the rice chaff were not significantly degraded in the fermentation studies.

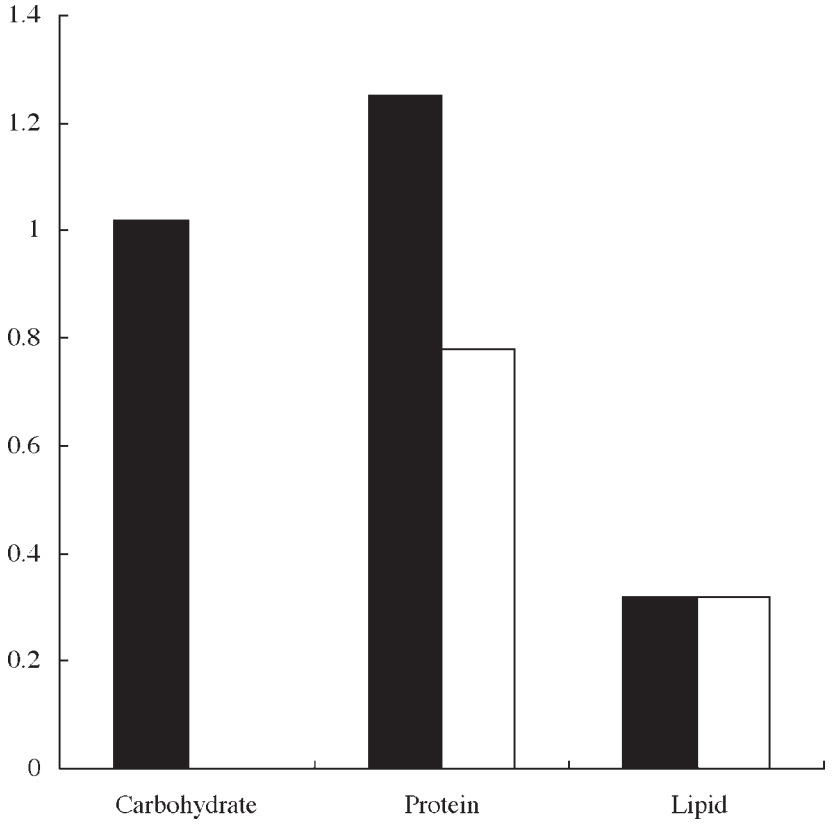

Fig. 5. Quantitative change in sea sludge of organic compounds (carbohydrate, protein, and lipid) by fermentation with WC bacteria. (■), Before fermentation; (□), after fermentation.

Quantitative Change in Organic Compounds in Sea Sludge by Fermentation

The amount of organic compounds (carbohydrate, protein, and lipid) in sea sludge before and after fermentation (*a* and *x*, respectively) is shown in Fig. 5. The efficient degradation activity of WC was observed: almost all carbohydrate and 40% of protein were degraded. By contrast, the amount of lipid did not change. This is more than likely owing to the fact that the bacterium could barely degrade lipid during this time course.

Conclusion

This was the first attempt to use a bacterial isolate from a mangrove soil to degrade sea sludge. It was found that the isolate could degrade sea sludge effectively and was closely related to the *Bacillus* group. This bacterium may have potential in solving environmental problems related to sea sludge.

Acknowledgments

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