Simple and Rapid Method for Screening of Heavy Oil-degrading Bacteria from Marine Environment*1

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Petroleum-degrading bacteria occur extensively in the aquatic environment. Several methods were used to enumerate and isolate the petroleum-degrading bacteria, including MPN methods^{1,2)} and membrane filter methods.^{3,4)} Each of these technique is based on the growth of bacteria using petroleum as a sole carbon source. They have a common limitation. Some bacteria will always grow on the low concentration of organic nutrients found as impurities in the media. Therefore, in order to confirm the counts or isolate oil-degrading bacteria, it is necessary to test the individual isolates for the degrading ability of petroleum, which is time consuming process (as for individual isolates, preparation of liquid medium with petroleum, incubation, and determination by either visual inspection, gravimetric, or gaschromatographic analysis are necessary).

In the course of a program for selection of bacterial isolates able to degrade heavy oil (desulfurized fuel oil), we developed a rapid method for screening heavy oil-degrading bacteria. A nitrocellulose membrane filter (pore size 0.45 μm , Toyo Roshi Co. LTD) was dipped in a hexane solution containing 2% (w/vol) desulfurized fuel oil for 10 seconds. After hexane was evaporated from the filter at room temperature (it takes less than 15 seconds), the filter was placed on the top of $\frac{1}{8}$ ZoBell 2216E agar plate on which bacterial colonies were present. After incubation at 25°C for 2 days, the filter was taken out from the agar plate and washed with hot water (about 50°C). Colonies with the ability of oil-degradation make hydrophilic spots (due to hydrophilicity of oxidation products of hydrocarbons)

on the filter which could be easily recognized by visual inspection (absorbing water, the color of the hydrophilic spot turns into darker than the hydrophobic background). Microscopic observation could be used to confirm the results, when necessary. Eight or more isolates can be tested at one time on a single agar plate by this membrane filter (MF) colony assay method.

Table 1 shows the results of screening tests using this method. Among 23 bacterial strains which were isolated from Bisan Seto and Hibiki Nada following a procedure previously described,⁴⁾ 7 isolates demonstrated positive results with this method. When these bacterial strains were tested for oil degradation determined by gravimetric analysis,⁵⁾ all of the positive isolates probed to degrade desulfurized fuel oil, whereas none of negative isolates demonstrated oil degradation.

One unique feature of the method is that, since the process in this method is rapid and simple, it greatly facilitates screening oil-degrading bacteria. Another feature of the method is that, since this method is based on sensitive and direct enzymatic reaction on the filter, it allows us to isolate oil-degrading bacteria even in the presence of organic nutrients other than hydrocarbons, such as peptone or yeast extract, which have been regarded as undesirable additives for isolation of oil-degrading bacteria.⁶⁾

References

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Table 1. Correlation of the determination by MF colony assay and the degree of oil degradation

Source of strain	Bacterial strain	MF colony assay*1	Oil degradation*2 (%)
Bisan Seto sea water	WB5521	+	25.3
	WB5522	—	0.0
	WB5523	—	0.0
	WB5561	+	15.7
	WB5562	—	0.0
	WB5563	—	0.0
	WB5564	—	0.0
	WB5211	+	20.5
	WB5212	+	24.8
	WB5213	—	0.0
Bisan Seto sediment	MB5211	—	0.1
	MB5212	+	35.5
	MB5213	+	26.8
	MB5214	—	1.3
	MB5215	—	0.0
	MB5216	—	0.0
	MB5217	—	0.6
	WB56C1	—	0.0
Hibiki Nada sea water	WB56C2	—	2.0
	WB56C3	+	18.3
Hibiki Nada sediment	MB56A1	+	28.3
	MB56A2	—	0.0
	MB56A3	—	0.0

*1 +, hydrophilic spot positive; —, hydrophilic spot negative.

*2 Determined by gravimetric analysis.⁵⁾

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