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Microbial Degradation of Alkane Components in Crude Oil

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미생물에 의한 원유중 Alkane 성분의 분해

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ABSTRACT: The isolates biodegrading crude oil were examined to characterize their properties. Isolates which were identified as *Acinetobacter lwoffii* G1, *Klebsiella pneumoniae* L25, *Pseudomonas maltophilia* N246, *Xanthomonas campestris* M12, and *Xanthomonas* sp. M28. The optimum concentration of crude oil was 0.15% for the bacterial growth. *X. campestris* M12, *Xanthomonas* sp. M28, and *K. penumoniae* L25 showed the maximal growth at the concentration of 3.5% sodium chloride, indicating that they were derived from sea water. Among the isolates, *X. campestris* M12, *Xanthomonas* sp. M28 specially utilized hexadecane and octane, and *P. maltophilia* N246 utilized octane with optimum concentration of 0.2-0.3% as sole carbon source. The utilization of components of saturate fraction by *K. pneumoniae* L25 was examined by gas-liquid chromatography. The short-chain saturates are used before the long chain ones although they almost disappear within 8 days of incubation at 30°C.

KEY WORDS Bacterial degradation, alkane

Pollution of the oceans by oil constitutes a serious environmental problem. The possibility of employing bacteria for the degradation of petroleum and its derivatives in minimizing contamination due to oil leaks and spills has prompted a number of investigators to study the process in laboratories (Jobson *et al.*, 1972; Atlas and Bartha, 1972; Reisfeld *et al.*, 1972; Westlake *et al.*, 1974; Atlas, 1975). Reisfeld *et al.* (1972) and Miget *et al.* (1969) described the isolation of active oil-degrading cultures from sea water and sediments containing crude oil. In the most active preparations, 40 to 55% of the oxidizable crude oil was degraded in enriched sea water in 60 hr. The degradation by bacteria was accompanied by an emulsification, resulting in a greater oil-water interface, which is significant to improve oil decomposition by microorganisms (Reisfeld *et al.*, 1972; Jobson *et al.*, 1972; Atlas and Bartha, 1972). The present investigation was undertaken in order to study the isolation of microorganisms utilizing crude oil, characterization of the isolates, the biodegradability of crude oil by them.

MATERIALS AND METHODS

Sampling and identification of isolates

Sea water samples were collected from the coasts of South Korea as shown in Fig. 1. Methods for sampling water and sediment and procedures for identifying the pure cultures isolated have been described by Walker and Colwell (1976).

Isolation of bacteria

For the isolation of bacteria utilizing crude oil as a carbon source, enrichment cultures were carried out in 250 ml Erlenmeyer flasks containing 50 ml of basal salt medium as the described publication (Hong *et al.*, 1986), to which was contained with 3.5% of NaCl and 1 ml per liter of crude oil. Each culture medium was incubated at 30°C on a rotary shaker (240 rev/min). Growth or isolation of bacteria was monitored on the plate of basal medium contained crude oil and sodium chloride. The hydrocarbon sub-

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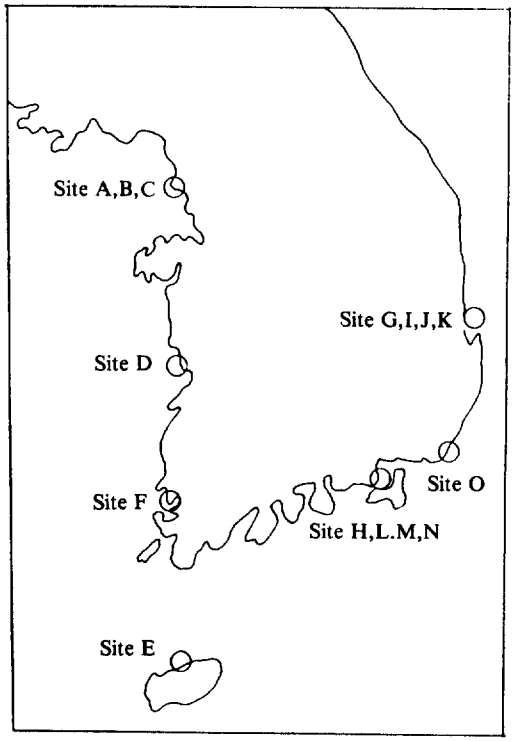

Fig. 1. *Map of sampling sites.*

strate, hexadecane or octane, was added by placing of few drops on a piece of filter paper in the lid of petri dish, and plates were incubated for one week in sealed tins at 30°C (Nieder and Shapiro, 1975).

Biodegradation experiments

The isolate was transferred into the basal medium for enrichment culture and incubation was carried out with the above condition. The crude oil used in this biodegradation experiments was obtained from Kuwait.

Cell growth

Unless otherwise stated, incubation was at 30°C with reciprocal shaking. Flasks of 250 m*l* were used for 50 m*l* of culture medium for enrichment. Dilutions for viable cell number were performed with sterile filtered sea water. The viable counts were determined by use of plate counting on nutrient agar media.

Gas-liquid chromatographic analysis of saturated hydrocarbons

Residual crude oils were extracted from cultures by *n*-hexane as a solvent and subjected to chemical analysis by gas-liquid chromatographic technique (Lee *et al.*, 1987).

Chemicals

The solvents used in the fractionation of the crude oil were of special quality and obtained from Sigma Chemical Company.

RESULTS

Enrichment cultures used to isolate crude oil-degrading bacteria yield different strains even after several transfer. The isolates utilizing crude oil were examined to degrade hexadecane or octane as shown in Table 1. The G1 strain utilized crude oil, while L25 and N246 did not degrade hexadecane. However, N246 shows excellently biodegradation of octane as a carbon source. Specially M12 and M28 were high ability to degrade crude oil, hexadecane and octane. Identification of isolates was carried out by using standard techniques with reference to Bergey's Manual of Determinative Bacteriology. Isolates which were identified as *Acinetobacter lwoffii* G1, *Klebsiella pneumoniae* L25, *Pseudomonas maltophilia* N246, *Xanthomonas campestris* M12 and *Xanthomanas* sp. M28. (Table 1) *A. lwoffii* G1, *X. compestris* M12 and *Xanthomonas* species M28 utilized crude oil, hexadecane and octane but *m*-toluate and salicylate as a sole carbon source. This result showed that these strains biodegraded specially hexadecane

Table 1. *Identification and cultural properties of isolates biodegrading crude oil*

Strain	species name	Substrate				
		crude oil	hexadecane	octane	*m*-toluate	salicylate
G1	*Acinetobacter lwoffii*	+	+	+	–	–
L25	*Klebsiella pneumoniae*	+	–	–	–	–
N246	*Pseudomonas maltophilia*	+	–	+	–	–
M12	*Xanthomonas campestris*	+	+	+	–	–
M28	*Xanthomonas* sp.	+	+	+	–	–

**m*-Toluate and salicylate (10 mM) were added to the plate of basal media instead of crude oil.
+ ; growth, – ; no growth, ND; no identification.

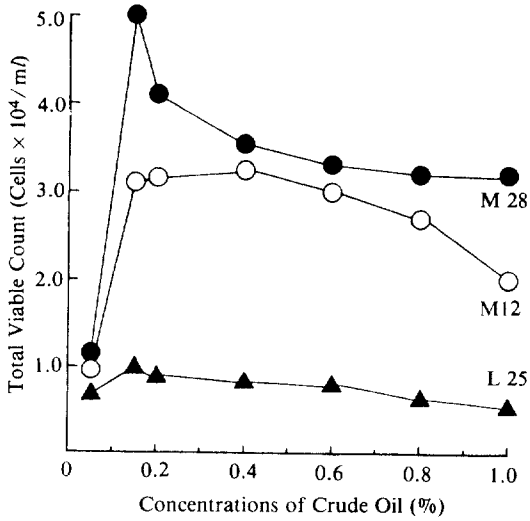

Fig. 2. *Effect of the concentration of crude oil on the growth of the isolates.*
The culture was carried out for 48 hr at 30°C and total viable counts were estimated.

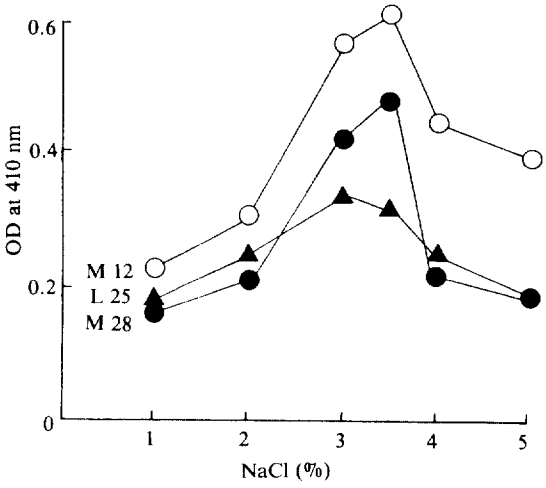

Fig. 3. *Effect of salinity on the growth of the isolates using crude oil as carbon source.*
The culture was incubated for 48 hr at rotary shaking incubator (30°C).

and octane components in crude oil.

Growth of isolates on crude oil

The final yield as a function of the concentration of crude oil in the growth medium for each of the newly isolated strains was shown in Fig. 2. The growth of strains, *X. campestris* M12, *Xanthomonas* sp. M28 and *K. pneumoniae* L25, was directly proportional to oil concentrations up to 0.15%

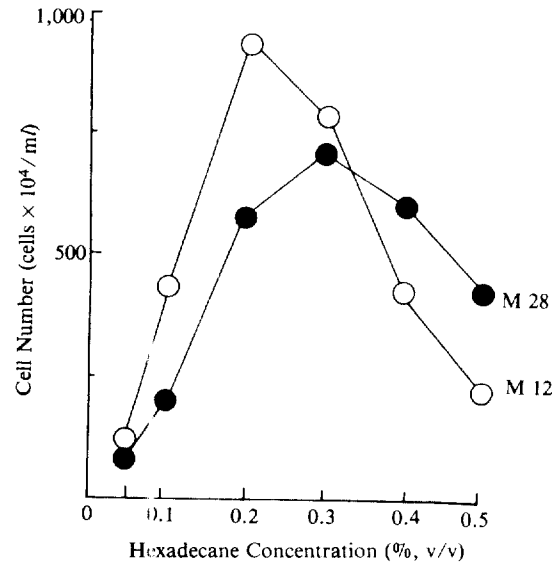

Fig. 4. *Effect of the concentration of hexadecane on the growth of the isolates.*
The culture was carried out for 48 hr at 30°C and total viable counts were estimated.

(v/v), yielding approximately 5.0×10^7 cells per ml, respectively. At higher oil concentrations, growth of these strains was greatly inhibited.

Salinity concentration on the growth of the isolates

In order to study the effect of sodium chloride on the growth of the isolates, various concentrations of sodium chloride were added to the enrichment culture media. Three isolates, *X. campestris* M12, *Xanthomonas* sp. M28 and *K. pneumoniae* L25, showed the maximal growth at the concentration of 3.5% NaCl as shown in Fig. 3. It means that these isolates derived from sea water will be able to grow well in marine environment.

Effect of alkane concentration on the growth isolates

Among the isolates, *X. campestris* M12 and *Xanthomonas* sp. M28 strains specially utilized hexadecane as a carbon source on the basal medium. Growth of isolates on *n*-alkane concentration was examined as shown in Fig. 4 and 5, resulting the optimum concentration of substrate such as hexadecane and octane was approximately from 0.2% to 0.3%. This results showed that optimum concentration of hexadecane or octane was higher than that of crude oil (0.15%). It was assumed that hexadecane or octane was good substrate for bacterial growth because they were more easily degradable component than crude oil.

Microbial degradation of crude oil

The utilization of components of saturated fraction

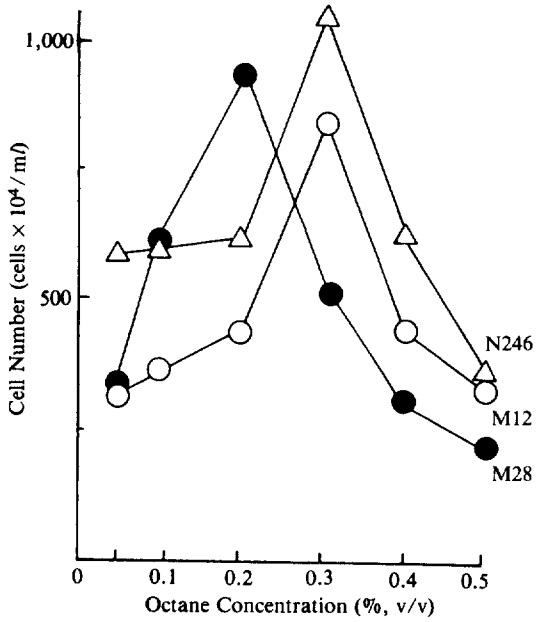

Fig. 5. *Effect of the concentration of octane on the growth of the isolates.*
The culture was carried out for 48 hr at 30 °C and total viable counts were estimated.

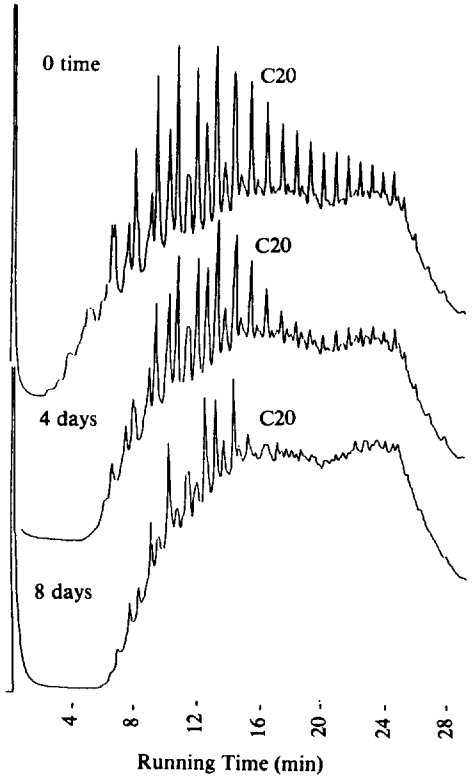

Fig. 6. *Gas chromatographic tracing of crude oil biodegradation after 8 days incubation by L25.*

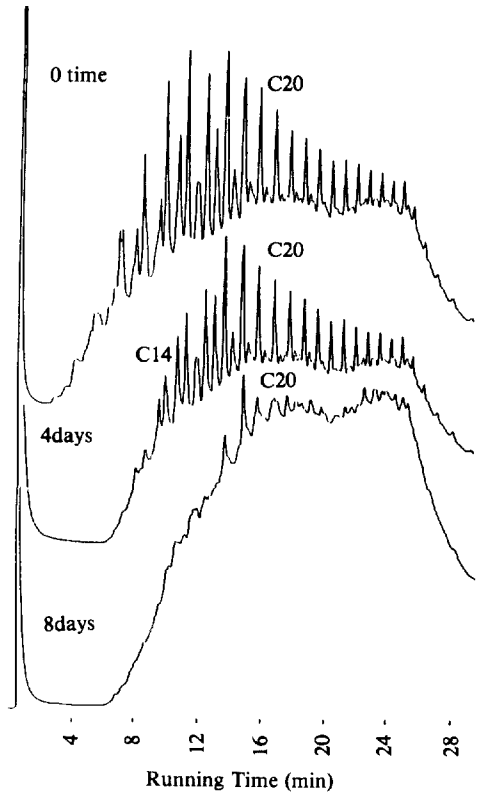

Fig. 7. *Gas chromatographic tracings of crude oil biodegradation after 8 days incubation by M12.*

by L25, tentatively identified as *Klebsiella penumoniae,* was followed by gas-liquid chromatography and the results are presented in Fig. 6. It was assumed that the short-chain saturates are used before the long chain ones although they almost disapper within 8 days of incubation at 30 °C. Similar results were obtained at 30 °C with pure culture, *X. campestris* M12, that there are preferential earlier digestion of the shorter chain saturates (Fig. 7). These results are good agreement with the result of sequential utilization of crude oil by mixed population at 30 °C (Jobson *et al.*, 1972). However, it was found that branched paraffins appeared to be somewhat more slowly degraded than straight-chain paraffin (Atlas, 1975).

DISCUSSION

Walker and Colwell (1974) described that all of the strains utilizing crude oil had been isolated from Chesapeake Bay and were identified as *Acinetobacter lwoffii, Pseudomonas* sp., and *Nocardia* sp. Traxler and Bernard (1969) observed that *Pseudomonas* sp. grow very poorly on *n*-alkanes if provided forced

aeration by vigorous shaking. *P. putida* strains can grow on straight-chain primary aliphatic alcohols from propanol to dodecanol as sole carbon and energy source (Nieder and Shapiro, 1975).

Our result showed that *Acinetobacter lwoffii* and *Pseudomonas* sp. grow considerably on crude oil. Specially it was found that we had isolated *Xanthomonas campestris,* which strongly degraded crude oil.

The biodegradability of a variety of crude oils was found to be highly dependent on crude oil composition and temperature. Of special importance was the relative amounts of high and low molecular-weight compounds in the various crude oils. Heavy oils contain a greater percentage of high molecular-weight components, whereas light oils contain a greater percentage of low molecular-weight components. Unlike some predictions based on findings with pure hydrocarbons, hexadecane and octane, all hydrocarbon classes were found to be subject to biodegradation within the context of the whole crude oil. Some preference was shown for degradation of paraffins.

Several reports have appeared in the literature regarding the effect of temperature on oil biodegradation. Under our experimental condition at 30 °C, it has been impossible to degrade a crude oil completely under laboratory conditions. But these results seem to be noteworthy and of possible practical significance for the treatment of spilled oil.

적 요

한국 해안으로부터 우수 원유분해능 균주를 분리동정하여 그 특성을 고찰하였다. 이들 분리균주 중 우수 균주로는 *Acinetobacter lwoffii* G1, *Klebsiella pneumoniae* L25, *Pseudomonas maltophilia* N246이 동정되었다. 이들 균주의 생장은 0.15%의 원유농도와, 3.5% sodium chloride에서 최대의 생장을 보여주었다. 이들 균주 중 *X. campestris* M12와 *Xanthomonas* sp. M28은 특별히 hexadecane과 octane을 그리고 *P. maltophilia* N246은 octane을 탄소원으로 0.2-0.3%의 농도에서 최대로 분해하였다. *K. pneumoniae* L25에 의한 crude oil의 경시적인 분해과정을 gas-liquid chromatography로 분석하였으며, 그 결과 긴 사슬의 alkane 보다 C 14 이하의 짧은 사슬의 alkane을 보다 쉽게 이용하였으며 30℃에서 8일간 배양시에 거의 모두 이용되었다.

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