海洋微生物による脂肪族炭化水素の分解に及ぼす塩分と温度の影響

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Influence of Salinity and Temperature on the Aliphatic Hydrocarbon Degradation by Marine Bacteria

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Degradation rates of *n*-alkane substrate were obtained as functions of salinity (0~5%) and temperature (10°C~50°C) for marine petroleum-degrading bacteria, *Corynebacterium* sp. and *Flavobacterium* sp. For both strains, only a slight dependency on salinity was found in the range of 0~5% NaCl. Temperature dependency, however, was quite large, i.e., while the most appropriate temperature for *n*-hexadecane degradation was at ca. 30°C for both isolates, the activity was lost completely at 10°C and at 50°C.

In studying the physiology and ecology of marine organisms, the characteristic features of marine environment—salinity, low temperature, hydrostatic pressure, etc.—should be taken into consideration. While the important role of marine bacteria in the environmental preservation against pollution has been well documented from various viewpoints, there exists, in fact, the intimate relationships in the field between their activities and environmental factors mentioned above.[1] That is to say, the activity of marine petrolcum-degrading bacteria, which possess an indispensable function for oil contamination in the ocean,[2,3] will also be affected by these factors as well as the amount of energy source from organic matters and nutrients.

For marine microbes, the physiological processes requiring specific ions[4] and ability to grow and/or survive under various ranges of temperatures or extreme pressure have been extensively investigated in the laboratory scale,[5] in order to clarify their essential or inherent characters. In the present work, the effect presented by the salinity and temperature in the microbial degradation of hydrocarbon was discussed in relation to their activities for two species of marine petroleum-degrading bacteria, *Corynebacterium* sp. and *Flavobacterium* sp. strains.[6]

Materials and Methods

The isolation and identification methods for the marine petroleum-degrading bacteria used in the present study, i.e., *Corynebacterium* sp. and *Flavobacterium* sp., were described previously.[6] Also, degradation experiments with substrate (*n*-hexadecane (*n*-C_{16})), extraction, and pH or gas-liquid chromatographic measurements were carried out by the same methods and conditions[6,7] except for the following points, i.e., the composition of salt solution in the degradation experiments studied here was as follows: $CaCl_2 \cdot 2H_2O$, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.7 g; KCl, 0.3 g; NH_4Cl, 1.0 g; K_2HPO_4, 0.7 g; KH_2PO_4, 0.3 g; per 1000 m*l* of distilled water, pH 7.6±0.1. Incubation of mixtures (*n*-C_{16} concentration: 2.5 m*l*/*l*) was carried out in the flasks using a reciprocal shaker (88, 6.2 cm strokes/min) for 7 days. All treatment and analyses were performed in triplicate.

Growth data of these two isolates during *n*-C_{16} degradation was not shown in this paper, because their results obtained were found out to be almost parallel to the degradation rates in the ranges of NaCl concentration and temperature examined. The experimental results of *n*-C_{16} degradation at 50°C for both strains were also not referred, as no degradation took place for both isolates regardless of the NaCl concentration. In addition, no breakdown products through *n*-C_{16} degradation were evident in any of the GLC profiles under the present conditions.[8]

Results and Discussion

Changes in pH accompanied with *n*-C_{16} degradation for *Corynebacterium* sp. and *Flavobacterium* sp. strains were shown in Figs. 1 and 2, respectively. As a whole, at a fixed pH value temperature dependency rather than salinity ap-

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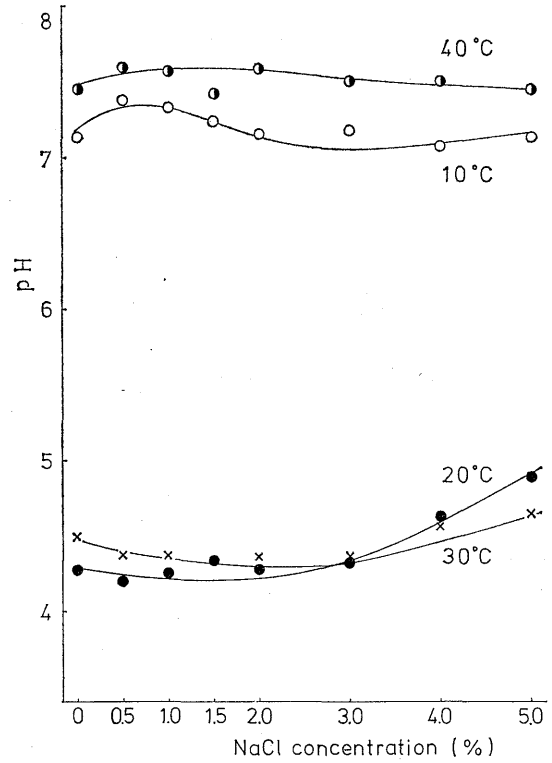

Fig. 1. Changes in pH accompanied with degradation of n-C_{16} (2.5 m*l*/*l*) by *Corynebacterium* sp. at different concentrations of NaCl (Incubation time: 7 days); 10°C (○), 20°C (●), 30°C (×), 40°C (◑).

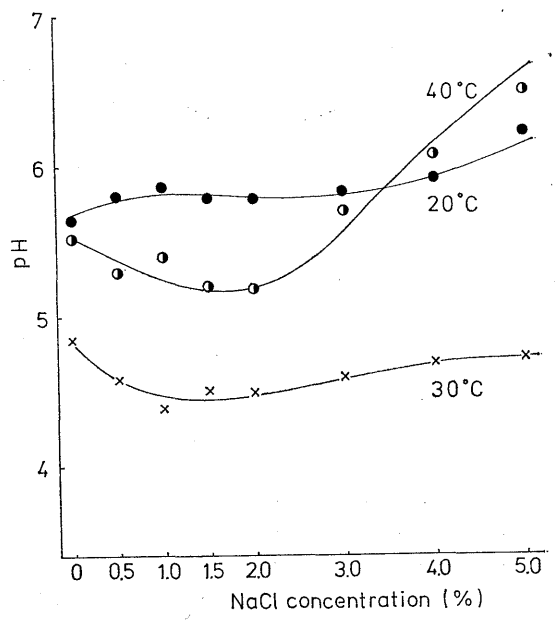

Fig. 2. Changes in pH accompanied with degradation of n-C_{16} (2.5 m*l*/*l*) by *Flavobacterium* sp. (Incubation time: 7 days); 20°C (●), 30°C (×), 40°C (◑).

pears to be much more evident for both strains. For instance, two different types of pH data for *Corynebacterium* sp. strain (7.1～7.6 for 10°C and 40°C; 4.2～4.6 for 20°C and 30°C) appeared throughout the variation range of NaCl concentration, as shown in Fig. 1. Salinity dependency of pH in this strain was comparatively samll in the ranges of temperature examined, although the decrease in pH was a little smaller at NaCl concentration of more than 4.0% at 20°C and 30°C. For *Flavobacterium* sp., on the other hand, pH changes are somewhat complicated except for the case of 10°C (Not shown in this figure. Initial value of pH (7.6) was completely maintained, because the degradation for hydrocarbon substrate was not proceeded at all like the case of 50°C), i.e., there existed no remarkable difference among these temperatures. As a whole, salinity dependency in this strain was also small, and its variation seems to be similar to the case of *Corynebacterium* sp. At 40°C, however, a pH curve, which had minimum (5.16) at about 1.7% NaCl concentration and almost linear relationship above 2% NaCl, was observed.

Degradation rates of n-C_{16} for two strains were shown in Figs. 3 and 4. The results are roughly in accordance with the pH changes discussed above. In the case of *Corynebacterium* sp., about fifty to sixty percent of degradation was observed at 20°C or 30°C except for the cases of higher NaCl concentrations. The degradation of substrate at 10°C was at most 18% (2.5% NaCl concentration). Moreover, at 40°C n-C_{16} substrate was scarcely degraded in consistent with the anticipation from pH data. For *Flavobacterium* sp. the degradation rate of n-C_{16} was largest at 30°C and the same degree of n-C_{16} degradation was observed at 20°C and 40°C. These results were also consistent with the pH data. Although an incubation temperature of 30°C was most appropriate for both isolates, temperature dependency for n-alkane degradation of these two strains seems to be a little different each other. *Corynebacterium* sp. might be designated as obligate mesophile, having larger activity at narrow ranges of temperature, 20°C～30°C, while *Flavobacterium* sp. might be called facultative mesophile with ability of degradation at somewhat broad ranges of temperature, 20°C～40°C.

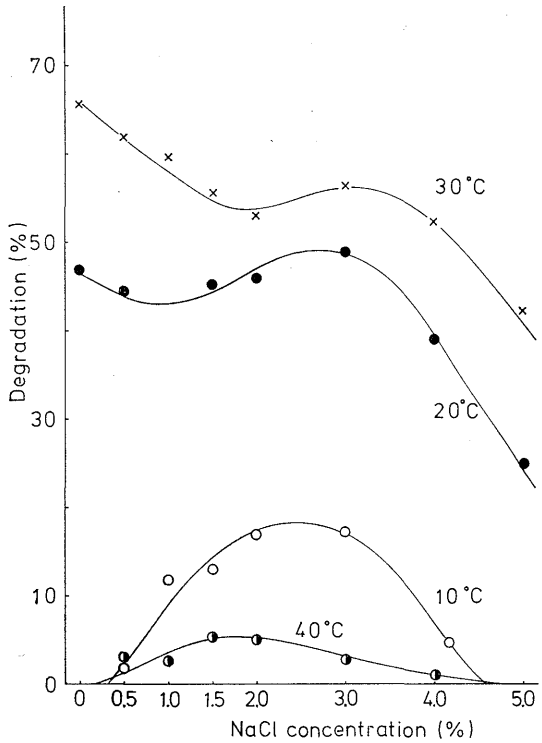

Fig. 3. Biodegradation of n-C_{16} (2.5 ml/l) by *Corynebacterium* sp. at different concentrations of NaCl (Incubation days: 7 days); 10°C (○), 20°C (●), 30°C (×), 40°C (◑).

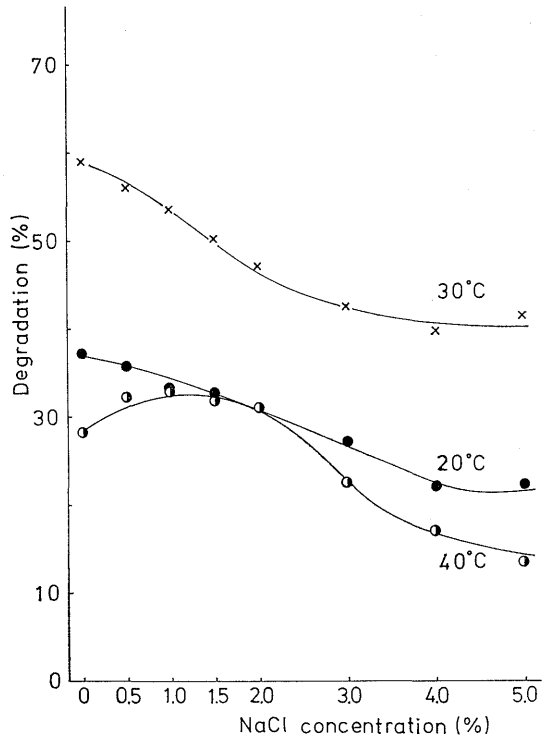

Fig. 4. Biodegradation of n-C_{16} (2.5 ml/l) by *Flavobacterium* sp. at different concentrations of NaCl (Incubation days: 7 days); 20°C (●), 30°C (×), 40°C (◑).

Compared to the pH data, which showed little dependency on salt concentration, salinity dependency of degradation rates seems to have rather peculiar feature. At first, degradation rates for *Flavobaterium* sp. become gradually lower as the concentration of NaCl increases regardless of temperature (see Fig. 4). Accordingly, this strain may be reasonably designated to non-halophilic bacteria with little dependency of NaCl concentration, at least in the present study of n-alkane degradation. On the contrary, maximal degradation rates for *Corynebacterium* sp. were found out at ca. 2.5~3% NaCl concentration at 10°C and 20°C, although at 30°C the degradation rate was largest at 0% NaCl.

From these results, it can be concluded that both bacteria have, in general, flexible characters for salinity and temperature. Further studies should be carried out on the interaction between membrane function of cell itself or enzyme activity and salinity or temperature, in order to explain these phenomena reasonably.

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References

1) R. R. COLWELL and R. Y. MORITA: Effect of the Ocean Environment on Microbial Activities, University Park Press, Baltimore, Md., 1974. pp. 587.
2) R. M. ATLAS and R. BARTHA: *Environ. Pollut.*, **12**, 186–191 (1973).
3) D. G. AHEARN and S. P. MEYERS: The microbial degradation of oil pollutants, Louisiana State University, Baton Rouge, 1973, pp. 1–322.
4) R. A. MACLEOD: *Bacteriol. Rev.*, **29**, 9–23 (1965).
5) R. Y. MORITA: *Bacteriol. Rev.*, **39**, 144–167 (1975).
6) S. NAGATA: *Bull. Japan. Soc. Sci. Fish.*, **48**, 781–786 (1982).
7) S. NAGATA, G. KONDO, and T. ASANO: *J. Oceanogr. Soc. Japan*, **34**, 105–107 (1978).
8) J.R. SCHWARZ, J.D. WALKER, and R.R. COLWELL: *Can. J. Microbiol.*, **21**, 682–687 (1975).