Emulsifying Factor of Hydrocarbon Produced by a Hydrocarbon-Assimilating Yeast

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Hydrocarbon fermentation has been widely investigated by many researchers. Especially the production of single cell protein from hydrocarbons has become a matter of great interest in the world. The first step, in which microorganisms utilize the liquid hydrocarbons as carbon sources, will be emulsifying phenomena of hydrocarbon and water. The hydrocarbons, emulsified by microorganisms, may be absorbed easily into the cells.

We have recently found the hydrocarbon-emulsifying factors produced into the cultural fluid by a hydrocarbon-utilizing yeast, *Candida*

petrophilum.¹⁾ One of these factors was isolated as a crude compound by ion exchange resin from the cultural fluid and showed the strong activity for emulsifying a mixture of hydrocarbon and water. This compound was named as "Emulsifying factor". This factor was composed of peptide and unidentified fatty acid. Glutamic acid, aspartic acid, alanine and leucine were identified after the hydrolysis of this factor with concentrated hydrochloride. Such a emulsifying factor seems to play an important role in the assimilation of hydrocarbons by microorganisms.

TABLE I. INFLUENCES OF THE SUPERNATANT OF BROTH ON THE EMULSIFICATION OF HYDROCARBON AND WATER

	Carbon sources of culture medium			
	<i>n</i> -Hexadecane		Glucose	
	Growth	Emulsification (10 min)	Growth	Emulsification (10 min)
<i>Pseudomonas aeruginosa</i> IAM 1057	+	—	+++	—
<i>Brevibacterium lactofermentus</i> ATCC 13869	+	—	++	—
<i>Bacillus subtilis</i> IAM 1069	±	—	++	±
<i>Corynebacterium petrophilum</i> nov. sp.	++	±	++	+
<i>Candida fimetaria</i> NI 7631	±	—	+	—
<i>Mycotorula japonica</i> IAM 4225	++	+	++	+
<i>Candida tropicalis</i> IAM 4076	+++	++	+++	±
<i>Candida petrophilum</i> nov. sp.	+++	++	+	—

Methods: 1 ml of each supernatant of broth cultured in the medium A for 24 hr with shaking was added in the following reaction mixtures: *n*-hexadecane 0.5 ml and water 1 ml (in a test tube of 18 mm in diameter). Emulsification was carried out with shaking at 30°C for 2 min and estimated after 10 min.

Culture medium A: (NH₄)₂SO₄ 0.1%, (NH₂)₂CO 0.1%, KH₂PO₄ 0.15%, MgSO₄·7 H₂O 0.05%, MnSO₄·7H₂O 0.002%, FeSO₄·7H₂O 0.002%, NaCl 0.1%, cane molasses 0.05%, glucose 4% or *n*-hexadecane 2% (vol).

Estimation of emulsification: — none, ± a little bit, + good, ++ very good.

1) I. Takeda, T. Iguchi, T. Kawamura, S. Hayakawa and S. Senoh, *Agr. Biol. Chem.*, **29**, 796 (1965).

Such an emulsifying factor is produced in a considerable amount in cultural broth. Most of hydrocarbon utilizing microorganisms tested are able to produce such a factor which emulsify a mixture of hydrocarbons and cultural fluid as shown in Table I.

Candida petrophilum was cultured in the hydrocarbon—medium described above and the supernatant after 2 days' culture was added to the new medium in the same concentration as described above. The growth rate of yeast was increased in proportion to the concentration of the cultural fluid. (Fig. 1)

FIG. 1. Influence of the Supernatant of Broth on the Growth Rate of Yeast.

Basal medium: *n*-hexadecane 2% (vol), $(\text{NH}_2)_2\text{CO}$ 0.3% $(\text{NH}_4)_2\text{SO}_4$ 0.1%, KH_2PO_4 0.15%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.05%, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.004%, $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.004%, CaCl_2 0.01%, C. S. L. 0.1%. The supernatant of 2 day old culture in the basal described above was added to the same medium as follows:

○—○ cultural fluid 50%, ×—× 25%,
△—△ 10%, □—□ 5%, ●—● 0%

This phenomenon indicates the presence of materials which stimulate the growth rate of yeast in the supernatant of 2 day old culture.

Candida petrophilum was aerobically grown in synthetic hydrocarbon—medium at 30°C for 0 hr on a reciprocal shaker. After the

centrifugation of the cells, the supernatant was passed through a column of Amberlite IRC 50 (H^+ Type). The fractions having emulsifying activity were eluted with 50% solution of ethylalcohol from the above column and decolorized by passing an active charcoal column pretreated with ethylalcohol. An active component "Emulsifying factor" was obtained as a crude material by concentrating this eluate. The properties of an isolated compound which shows emulsifying activity were as follows. The ultraviolet absorption spectrum did not show any peaks (in alkaline solution) in the range of 230 $\text{m}\mu$ to 400 $\text{m}\mu$. On the other hand, the presences of peptide bonds were demonstrated in infra-red spectrum (Fig. 2). Ninhydrin reaction of this

FIG. 2. Infrared Spectrum of Isolate Emulsifying Factor.

compound is negative. When emulsifying factor was subjected to acid hydrolysis in 6N HCl in a sealed tube at 110°C for 20 hr, L-aspartic acid, L-glutamic acid, L-alanine and L-leucine were detected by an amino acid analyzer and microbioassay. By the acid hydrolysis in 6N HCl, a brown-colored, hydrochloric acid insoluble and oily material was liberated. This oily material was extracted with ether and showed the typical features of fatty acids in infra-red spectrum.

From these data, the emulsifying factor is confirmed to be composed of peptide and fatty acids. The crystallization of this crude compound and the identification of fatty acids are now in progress.