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Growth of an Aquatic-derived Bacterium in the Presence of Long-chained Alkanes[1]

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In a study of microbial utilization of paired hydrocarbon and nonhydrocarbon substrates, an isolate derived from the Mary's River, Benton County, Oregon, was found to have limited growth on glucose alone in a mineral salts medium at 30 C (L. D. Bushnell and H. F. Haas, J. Bacteriol. **41**:653, 1944). However, in the presence of 0.5% *n*-hexadecane (99% purity; Humphrey Chemical Co., North Haven, Conn.), vigorous growth ensued under the same conditions.

In Nutrient Broth (Difco), this organism was found to be pleomorphic, growing from a gram-variable coccoid stage through a rod stage where gram-positive granules were present (36 to 48 hr). After this period, the organism fragmented to gram-variable cocci. The isolate, growing in a thin surface pellicle, was aerobic, catalase-positive, unable to reduce nitrates, indole- and hydrogen sulfide-negative, urease-positive, and gelatin-negative. Starch was hydrolyzed, but the organism produced no acid or gas with glucose, lactose, sucrose, mannitol, and galactose. These tests were performed by standard methods (Manual of Microbiol. Methods, Soc. Am. Bacteriologists, Geneva, N.Y., 1957). Colonies of the organism, after growth on nutrient agar at 30 C for 24 hr, were circular, entire, creamy white, raised, glistening, translucent by transmitted light, and viscous. Based on these data, this organism, isolate 1-80-3, was tentatively considered to be a member of the genus *Arthrobacter*.

Growth of the organism with and without 0.5% *n*-hexadecane, in the presence of other carbon sources at 0.5% (w/v), was carried out in 250-ml Erlenmeyer flasks containing 50 ml of mineral salts medium incubated at 30 C with reciprocal agitation. The inoculum was grown in nutrient broth for 24 hr at 30 C, washed three times in 0.1 M phosphate buffer, *p*H 7.2, and added to the flasks to give an initial population of 5×10^4 organisms per ml. The cells were in a gram-variable coccoid state at this culture age. All studies were carried out by use of filter-sterilized carbon sources, added aseptically with autoclave-sterilized hexadecane after sterilization of the medium. Results of the growth of the organism at 30 C are given in Fig. 1. In all cases, growth was stimulated in the presence of the hydrocarbon. In controls containing only the organism and hydrocarbon, no visible turbidity or emulsification of the medium was observed in a 2-week period. Parallel experiments carried out with *n*-decane and *n*-dodecane (99% purity; Humphrey Chemical Co., North Haven, Conn.) indicated a similar stimulation of growth.

To determine the effects of temperature on nutrient utilization in the presence of hexadecane, cultures were incubated with reciprocal agitation at 42, 35, 30, 25, 18, and 5 C. Growth was negligible at 42 and 5 C, and the optimal growth was found at 25 and 30 C. When growth was observed, it was stimulated in the presence of the hydrocarbon. Controls containing only hydrocarbon were negative at all temperatures.

Microbial oxidation of normal alkanes occurs primarily through the formation of homologous *n*-alcohol, aldehyde, and acid (E. J. McKenna and R. E. Kallio, Ann. Rev. Microbiol. **19**:183, 1965). If microbial oxidation of the hydrocarbon was occurring concomitantly with growth on glucose, *n*-hexadecanol (if it was being formed) could stimulate growth owing to its known surfactant action (L. I. Osipow, Surface Chemistry, Am. Chem. Soc. Monograph 153, p. 314, 1962). Such stimulation was observed with this isolate when hexadecane containing 1% (v/v) *n*-hexadecanol was used in the presence of glucose. No stimulation was observed when the homologous fatty acid was added.

To check for the formation of *n*-hexadecanol from hexadecane during growth on glucose, portions of 3- and 6-day cultures, grown on glucose in the presence of *n*-hexadecane, were extracted three times with equivalent volumes of hexane and benzene, respectively. The extracts were dried under a flow of nitrogen gas and were subjected to gas-liquid chromatography. A model 402 chromatograph (F & M Scientific Corp., Avondale, Pa.) having flame ionization was used.

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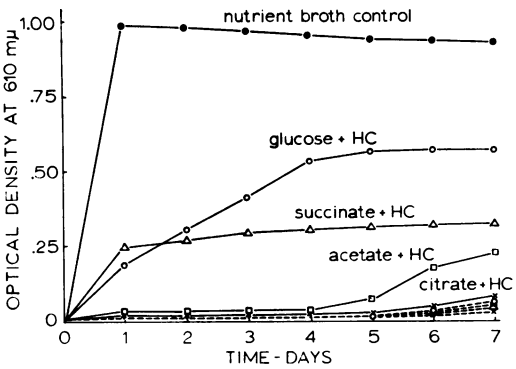

FIG. 1. *Growth of isolate 1-80-3 on four carbon sources with and without the presence of n-hexadecane (HC), as compared with growth in nutrient broth. The dotted lines indicate growth without hydrocarbon present on the same carbon sources. Absorbance (610 mμ) of whole broths was measured with a model B spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) using 13 × 100 mm matched circular cuvettes.*

The 0.61 × 194 cm stainless steel column contained 20% free fatty acid polar liquid phase (Varian Aerograph, Walnut Creek, Calif.,) on 60 to 80 mesh Chrom-sorb P (Johns-Manville Products, New York, N.Y.). The column was held at 215 C, and the helium flow used was 40 ml per min. Samples (1 μliter) with an attenuation of 8 and range setting of 10 were used. Calibration curves and internal extraction standards indicated that this procedure would allow detection of 0.001% *n*-hexadecanol (w/v) in extracts. Under these conditions, it was not possible to detect peaks having retention times similar to *n*-hexadecanol. These results would indicate that, within the sensitivity limit of the detection procedure, stimulation of growth in the presence of the hydrocarbon may not be due to free alcohol formation.

Hydrocarbons can be present in aquatic environments as products of biological decomposition, and, in addition, increasing levels of these materials are present owing to industrial pollution (C. E. ZoBell, Intern. Conf. on Water Pollution Control, Pergamon Press, London, p. 85, 1964). Nevertheless, the interactions of aquatic microorganisms with hydrocarbons which may be present in these environments have been considered primarily from the standpoint of microbial utilization of these materials, as well as from the standpoint of relevant oxidation mechanisms (E. J. McKenna and R. E. Kallio, *in* H. Heukalekian and N. C. Dondero [ed.], Principles and Application of Aquatic Microbiology, John Wiley and Sons, New York, p. 1–14, 1964).

The present data indicate, however, that long-chained aliphatic alkanes may influence growth rates of an organism unable to use these alkanes as a sole source of carbon and energy. This would suggest that, in studies of microorganisms from environments where hydrocarbons may be present, even if the hydrocarbon cannot be utilized for growth, appropriate experiments should be conducted to determine possible influences of these compounds on physiological responses.