GROWTH OF *ENDOMYCOPSIS LIPOLYTICA* ON HYDROCARBONS

J.N. NIGAM, B.K. LONSANE, K. VADALKAR, H.D. SINGH,
J.N. BARUAH AND M.S. IYENGAR

*Biochemistry Division
Regional Research Laboratory, Jorhat-6, Assam, India*

(Received March 4, 1971)

Ten strains of yeast isolated from the oil fields of Assam in India and identified as *Endomycopsis lipolytica* were studied. The characteristics of the growth of these ascosporogenous yeast strains on various petroleum fractions and *n*-alkanes are reported for the first time.

Ascosporogenous yeasts belonging to genus *Endomycopsis* were extensively studied by various workers, for their ability to assimilate hydrocarbons. *E. fibuliger* IFO 0103 was tested by TANAKA and FUKUI (1) for growth on decane, octadecane, and mixture of alkanes but with negative results. IIZUKA *et al.* (2) studied strains from culture collection belonging to *E. capsularis* and *E. fibuliger* for assimilation of kerosine. They observed that *E. capsularis* was not able to utilize kerosine even after 6 days of incubation while *E. fibuliger* gave good growth after 6 days of incubation though the growth was negative after 3 days of incubation. KOMAGATA *et al.* (3) reported the inability of the strains of *E. fibuliger* and *E. capsularis* from culture collection to assimilate kerosine. The inability of the strains of *E. fibuliger* and *E. javanensis* to utilize alkanes and alkenes was observed by MARKOVETZ and KALLIO (4).

In the beginning of 1970, WICKERHAM *et al.* (5) isolated from nature an ascosporogenous diploid culture of *Candida lipolytica* which was identified as *E. lipolytica*. Recently, from the oil fields of Assam, India, we have isolated 10 strains of yeast which were identified as *E. lipolytica*. This paper describes, for the first time, the ability of this species of yeast to utilize hydrocarbons.

MATERIALS AND METHODS

A large number of soil samples collected from the oil fields of Digboi,

Duliajan, and Sibsagar in Assam were screened by enrichment culture technique for the isolation of hydrocarbon-utilizing yeasts. Composition of the screening and growth media, enrichment culture technique, and methods of isolation, purification, and preservation of yeasts have been reported elsewhere (6). Determination of the morphological and biochemical characteristics of the yeast strains and studies of identification were made according to LODDER and KREGER-VAN RIJ (7).

Hydrocarbon utilization: Biomass yield from gas-oil, diesel oil, and white kerosine by all the strains were studied in the shake flask under condition of culture described in a previous paper (8). The boiling ranges of these petroleum fractions are 250–350° for gas-oil, 130–334° for diesel oil, and 150–280° for white kerosine. Specificity of the strains, Y-15 and Y-16, on different n-alkanes (decane to eicosane) was also determined (8).

Fermentation studies: Cultivation of the strains Y-15 and Y-16 on gas-oil was done in a 3-litre fermentor at 10% gas-oil concentration under condition of culture described before (6). Growth of the organisms was followed by the amount of ammonium hydroxide added to maintain the operational pH.

Crude protein content in the dry cell was estimated by the conventional method of Kjeldahl nitrogen estimation. The procedure used for the preparation of dry cells was described in a previous paper (6).

RESULTS

Ten yeast cultures capable of utilizing hydrocarbons were isolated from soil and silt samples collected from Assam oil fields and identified as strains of *Endomycopsis lipolytica*. All these strains produced ascospores within 10 days on gypsum blocks, potato, carrot, and Gorodkowa agar slants. In most cases the fat-splitting test was positive within 24 hr of incubation while the rest gave positive test after 48 hr. Some of the differentiating characteristics of the strains are depicted in Table 1.

The data on the comparative utilization of gas-oil, diesel oil, and white kerosine by all the strains for biomass formation are shown in Table 2. In general, the degree of utilization of these substrates for biomass formation is different for all the strains. The strains Y-15 and Y-16 produce the highest biomass yield on all substrates. Comparative utilization of the three petroleum fractions, gas-oil, diesel oil, and white kerosine, by the strains shows a general pattern. Gas-oil promotes highest biomass formation in all the strains except Y-14, followed by diesel oil and white kerosine in that order.

The crude protein content of the dry cells ranges from 38.9% in Y-9 to the highest value of 61% in Y-12 (Table 2). Strains Y-15 and Y-16 show clear substrate specificity to members of n-alkane series (Table 3). Strain Y-15 shows profuse growth on hexadecane, octadecane, and decane. Strain Y-16 shows highest biomass formation on decane and, in general, it utilizes

Table 1. Certain morphological and biochemical characteristics of the strains.

Strain	Cell size (μ)		Pellicle formation, surface growth		Splitting of arbutin	Reduction in litmus milk
	Length	Width	3 days	1 month		
Y-1	10.5-21	3-4.5	Ring	Thin pellicle	+	—
Y-2	9-60	3-4.5	Ring	Thin pellicle	—	—
Y-9	7.5-10.5	3-4.5	Thin pellicle	Thin pellicle	—	—
Y-12	4.5-21.0	3-3.5	Ring	Ring	—	—
Y-14	4.5-19.5	3.0-6.0	Thin pellicle	Pellicle	+	—
Y-15	6.0-31.5	3.0-4.5	Thin pellicle	Pellicle	—	—
Y-16	6.0-60.0	3.0-4.5	Loose islet of cells	Thin pellicle	—	+
Y-20	6.0-24.0	3.0-3.75	Pellicle	Pellicle	+	—
Y-24	6.0-13.5	3.0-4.5	Ring	Thin pellicle	—	—
Y-31	6.0-22.5	3.0-4.5	Pellicle	Ring	—	—

Table 2. Comparative biomass yield on petroleum fractions.

Strain	Protein N $\times$ 6.25 (%)	Yield from 100 ml medium (mg dry cell)		
		Gas-oil	Diesel oil	White kerosine
Y-1	49.05	182.5	92.5	77.5
Y-2	40.37	117.5	75.0	82.5
Y-9	38.92	177.5	117.5	87.5
Y-12	61.06	107.5	107.5	87.5
Y-14	36.38	62.5	107.5	95.0
Y-15	39.13	457.5	125.0	135.0
Y-16	45.1	235.0	132.5	122.5
Y-20	36.73	97.5	70.0	70.0
Y-24	39.20	100.0	67.5	50.0
Y-31	42.5	95.0	75.0	60.0

shorter-chain n-alkanes (decane to dodecane) better than longer ones with the exception of hexadecane and nonadecane.

Cultivation of strains Y-15 and Y-16 on gas-oil in a 3-litre fermentor shows a specific growth rates of 0.16 hr^{-1} and 0.14 hr^{-1} for Y-15 and Y-16, respectively. After 22 hr of cultivation in the fermentor, a total biomass yield of 4.0 g·litre for Y-15 and 3.1 g·litre for Y-16 was obtained.

DISCUSSION

The yeast species *E. lipolytica* is ascosporeogenous diploid culture of *C. lipolytica* and was isolated and reported for the first time by WICKERHAM *et*

Table 3. Substrate specificity of strains Y-15 and Y-16 for members of *n*-alkane series.

Substrate	Yield from 100 ml medium (mg dry cell)	
	Strain Y-15	Strain Y-16
n-C ₁₀	496.25	313.25
n-C ₁₁	322.5	292.65
n-C ₁₂	231.5	196.50
n-C ₁₃	241.75	139.75
n-C ₁₄	260.50	113.5
n-C ₁₅	371.75	144.00
n-C ₁₆	822.00	195.75
n-C ₁₇	291.25	36.25
n-C ₁₈	508.25	77.75
n-C ₁₉	183.00	278.75
n-C ₂₀	225.5	85.50

al. (5) in the beginning of 1970. The strains of *C. lipolytica* are considered of great potential in hydrocarbon fermentation for the production of single cell proteins, lipids, and other products of economic and industrial importance. Strains of hydrocarbon-utilizing *E. lipolytica* may offer advantages over strains of *C. lipolytica* in hydrocarbon fermentation due to the possibilities of hybridization on selected lines. As WICKERHAM *et al.* (5) suggested, diploids with higher specific growth rate may improve the economics of the process as the large-celled diploid will offer greater ease in harvesting from fermentation broth. However, surprisingly enough, *E. lipolytica* has not so far been studied for its hydrocarbon assimilation characteristics.

Out of the 10 strains of *E. lipolytica* isolated from oil fields of Assam, strains Y-15 and Y-16 are promising for the production of single-cell protein from gas-oil and some of the *n*-alkanes because of their profuse growth on these substrates.

The ability of these strains to grow moderately well on nonadecane and eicosane which are solid at the temperature of incubation (30°) is interesting. Generally, yeast species show very poor growth on solid alkanes unless a liquid carrier or emulsifier is used (8-10). Exceptionally low affinity of various species of yeast to certain members of *n*-alkane having odd number of carbons is well-documented (8). This inexplicable phenomenon is also shown by strain Y-16 which utilizes heptadecane very poorly.

REFERENCES

- 1) A. TANAKA and S. FUKUI, *J. Ferm. Technol.*, 48, 137 (1970).

- 2) H. IIZUKA, A. HIRANO, T. SHIO, Y. YAMAMOTO and S. TSURU, Vorträge des Internationalen Symposiums Erdölmikrobiologie, Brno CSSR (1964), p. 163.
- 3) K. KOMAGATA, T. NAKASE and N. KATSUYA, *J. Gen. Appl. Microbiol.*, **10**, 313 (1964).
- 4) A.J. MARKOVETZ and R.E. KALLIO, *J. Bacteriol.*, **87**, 968 (1964).
- 5) L.J. WICKERHAM, C.P. KURTZMAN and A.I. HERMAN, *Science*, **167**, 1141 (1970).
- 6) K. VADALKAR, H.D. SINGH, J.N. BARUAH and M.S. IYENGAR, *J. Gen. Appl. Microbiol.*, **15**, 375 (1969).
- 7) J. LODDER and N.J.W. KREGER-VAN RIJ, *The Yeasts-A Taxonomic Study*, 2nd Printing, North Holland Publishing Co., Amsterdam (1957).
- 8) P.K. BARUA, S.D. BHAGAT, K.R. PILLAI, H.D. SINGH, J.N. BARUAH and M.S. IYENGAR, *Appl. Microbiol.*, **20**, 657 (1970).
- 9) T.L. MILLER and M.J. JOHNSON, *Biotechnol. Bioeng.*, **8**, 549 (1966).
- 10) K. YAMADA and M. YOGO, *Agr. Biol. Chem. (Tokyo)*, **34**, 296 (1970).