HALOPHILIC n-ALKANE UTILIZING YEAST FROM THE ARABIAN GULF

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SUMMARY

A facultative halophilic yeast Torulopsis candida (Saito) Lodder, isolated from the Arabian Gulf, was found to grow on n-alkanes (C_{13}-C_{19}) at 37°C and pH 3-7 in media containing 1.4 - 3M NaCl or 30-90% seawater. The influence of pH, temperature, growth factors, salt and pure n-alkanes on its growth were studied. Its amino acid profile and crude protein were compatible with those of commercial n-alkane yeast products.

INTRODUCTION

Production of protein-rich feed supplements, derived from microbial biomass and grown on n-alkanes, is of considerable interest to oil-producing industries. Halophilic microorganisms have been isolated from natural salt lakes, seawater, and from industrial wastes of canneries and hide, skin and refrigeration industries utilizing substantial quantities of salt. The importance of halophilic cultures in the manufacture of single cell protein (SCP) has never been emphasized. In countries where the availability of ground or river water is extremely limited and seawater abundant, the halophilic property of industrial micro-organisms can possibly significantly reduce production costs through the use of seawater as process water and fermentation hardware compatible with seawater.

Unlike the primitive marine fungi Phycomycetes and Labyrinthulales, which grow within a narrow range of salinity, the higher fungi (including marine yeasts) are reported to grow over a wide salinity range as well as in media prepared with distilled water (Macleod, 1976). The ability of yeasts to withstand low pH values is a definite industrial advantage in maintaining sterility during the production phase. This communication briefly describes our preliminary studies on a facultative halophilic n-alkane-utilizing yeast isolated from the Arabian Gulf.

MATERIALS AND METHODS

Procedure: The yeast was isolated by enrichment technique in shake flasks, using the medium of Pal and Hamdan (1979). Unless otherwise indicated, the physiological studies were undertaken with Medium A prepared in one litre

distilled water containing: KH_2PO_4, 9.5 mg; $(NH_4)_2SO_4$, 5 g; $FeSO_4 \cdot 7H_2O$, 10 mg; $ZnSO_4 \cdot 7H_2O$, 5 mg; $MnSO_4 \cdot 7H_2O$, 1.0 mg; $Co(NO_3)_2 \cdot 6H_2O$, 0.25 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.25 mg; $CuSO_4 \cdot 5H_2O$, 0.25 mg; the pH was 4 - 4.5. The media were either filtered through 0.22/μ millipore membrane or steam sterilized at 121°C for 30 minutes. Membrane sterilized yeast extract and n-alkanes (C_{13}-C_{19} mixed alkanes of British Petroleum) were added to the medium to final concentrations of 200 mg/l and 0.5% v/v, respectively. Batch cultures were grown in 100 ml media in 250 ml Erlenmeyer flasks using a New Brunswick G-20 Gyrotary shaker or a 14 l fermenter (MF-14), equipped to maintain the pH, temperature and dissolved oxygen levels. Cell biomass was determined by measuring the optical density of cultures at 620 nm in a Spectronic 20.

Amino Acid Analysis: 100 mg of yeast was refluxed for 24 h in 6N HCl; a portion of this was evaporated to dryness and taken up in pH 2.2 sodium citrate buffer (Davies and Thomas, 1973). Analysis was carried out on the Technicon NC-2 amino acid analyzer using a 75 cm column, packed with type A resin. Norleucine was used as an internal standard. Two standard runs were averaged and used in the calculation.

RESULTS AND DISCUSSIONS

Seawater in proximity to an oil-contaminated area was employed to isolate the yeast. While growing on n-alkanes in oxygen limited shake flask cultures, the yeast exhibited pleomorphy within and around the hydrocarbon oil globules. It was identified by CBS, Delft, The Netherlands, as Torulopsis candida (Saito) Lodder* The yeast was grown for 24 h in Medium A containing 1-6M NaCl (Fig. 1). Although the biomass (and growth rate) at 2M NaCl was higher than the corresponding values at 1M (5.85%) or 3M (17.5%) NaCl or Medium A (1.45% salts), there was a total cessation of growth at 4, 5 and 6M NaCl. The yeast may therefore be classified as a facultative halophile (Flannery, 1956). Incorporation of Arabian Gulf water in Medium A caused increase in the biomass up to 75% strength of seawater.

Seawater contains growth-enhancing as well as inhibitory heavy metals (Ca, K, Mg, Co, Mn and Fe), each having a threshold level for its activity on the physiology of yeasts (Lotan et al., 1976; Okorokov et al., 1979). The total dissolved solids in the seawater employed were approximately 46 000 mg/l, with 12 000 mg/l Na and 22 800 mg/l Cl. Despite the reported debilitation of micro-organisms in seawater (Dawe and Penrose, 1978), some extreme halophiles can survive in the marine environment (Rodriguez-Valera et al., 1979). Our observed enhanced yeast biomass formation in media

*For a previous characterisation of n-alkane-utilizing *T. candida* from seawater, cf P. G. Gimenez and E. Stahl (1972), *Mycopathologia et Mycologia Applicata*, 47, 161-165.

containing 30-75% seawater could possibly be ascribed to the presence of growth-enhancing salts (and organic matter) present in the seawater. Meyers and Ahearn (1971) and Walker et al. (1975) have likewise reported a better or comparable utilisation of petroleum fractions by yeasts in seawater media than in media prepared in distilled water.

The effect of initial pH of the medium on the growth of T. candida shown in Fig. 2 indicates that the optimum pH was 4.0 - 4.2. However, the actual pH of the 42- to 48-hour-old cultures was 4.0 - 3.7. It is interesting to note that although in the natural marine environment of the Arabian Gulf the pH values exceed 8, our yeast isolate showed insignificant growth at pH above 8. The presence of acid-tolerant yeasts in the sea explains hydrocarbon biodegradation within the micro-environment of the oil globule, where the metabolically-produced organic acids cause a substantial decline in the pH.

The amount of biomass formed by T. candida at 32°C was not significantly different from that at 37°C. However, at 40°C and above yeast growth was drastically impaired. In the absence of growth factors there was an increase in the lag phase, and the rate of biomass formation was less than half of that observed in the presence of 400 mg of yeast extract or corn steep liquor per litre of medium. Biomass contributed by the above growth factors was negligible.

The mixed n-alkanes (C_{13}-C_{19}) produced considerably higher amount of biomass than that obtained with pure C_{10} or C_9. Lower alkanes, gasoline and kerosene were poor carbon sources.

The yeast was grown in a 14 l fermenter in batch culture, using a medium compounded with 75% seawater, 5 ml/l of BP's n-alkanes (C_{13}-C_{19}) and yeast extract 500 mg/l. The course of a typical batch fermentation is shown in Fig. 3. The measured values of μ, T_d and Y_S were 0.198 h^{-1}, 3.5 h and 0.8, respectively. The observed depletion of dissolved oxygen and generation of metabolic heat during the exponential phase in our trial experiment indicate the importance of adequate oxygen supply, foam suppression and cooling during n-alkane fermentations. Some preliminary data on a continuous culture of the yeast using a 4 l LKB fermenter are recorded in Table 1, where the presence of n-alkanes in the effluent was not determined.

Analysis of the lyophilised T. candida, grown in a batch culture (Table 2), shows the amino acid content of our yeast (47.05% protein) in comparison with Brewer's yeast (54% protein), gas-oil yeast (69.70% protein) and soyabean meal (48% protein). Amino acid requirements for poultry feeds include lysine and sulphur containing amino acids (methionine

and cystine). To be accepted as a protein supplement for feeds these amino acids must not only be in adequate amounts but in the proper ratios with other amino acids. T. candida is relatively abundant in lysine compared to soyabean meal. However, as in Brewer's yeast and gas-oil yeast, the lysine-arginine ratio is high, making arginine a limiting amino acid requiring supplementation. T. candida is superior to Brewer's yeast in methionine and cystine content but slightly inferior to gas-oil yeast and soyabean meal in methionine alone; cystine content was found to be higher in soyabean meal. Our yeast could be a good source of proline, an amino acid essential to the diet of the growing chick.

TABLE 1

Continuous Culture Studies with T. candida

INCUBATION TEMPERATURE (^{o}C)	CULTURE VOLUME (ml)	MEDIUM FEED RATE (h^{-1})	DILUTION RATE (h^{-1})	SUBSTRATE FEED RATE ($g\ h^{-1}$)	BIOMASS (DRY WT) IN HARVESTED CULTURE ($g\ h^{-1}$)
37	3000	390	0.13	1.33	0.514
37	3000	456	0.152	1.4	0.716
37	3000	480	0.16	1.33	0.7397

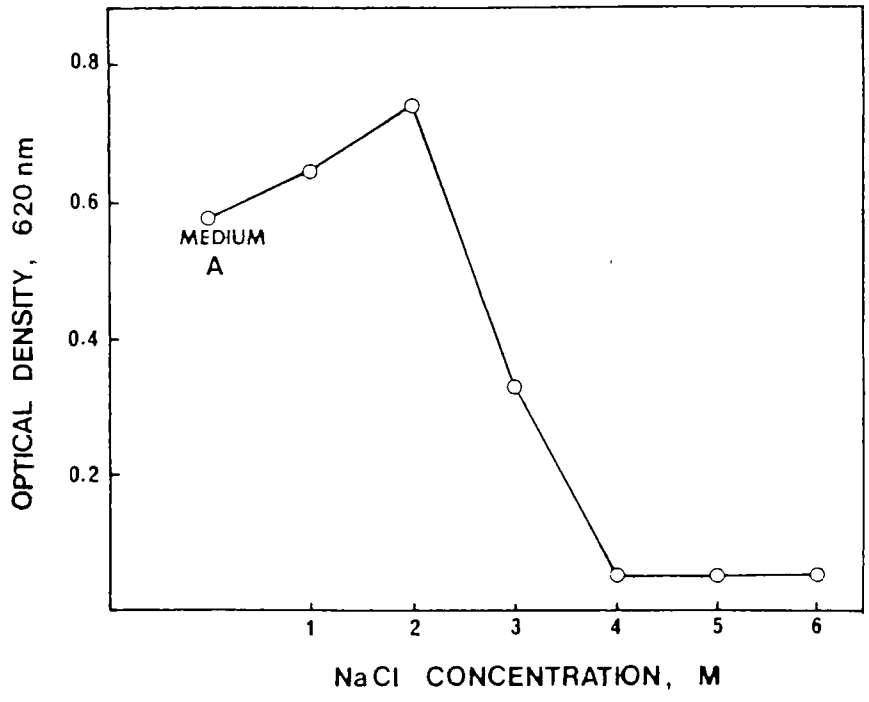

Fig. 1. Effect of NaCl on the growth of T. candida.

TABLE 2

Amino Acid Profile of T. candida Yeast, Brewer's Yeast,* Gas-Oil Yeast,* and Soyabean Meal* (g / 16gN)

SCP / Amino Acid	T. CANDIDA	BREWER'S YEAST	GAS-OIL YEAST	SOYABEAN MEAL
Asp.	8.10	9.2	-	-
Threo.	4.00	3.2	5.4	4.0
Ser.	3.28	3.0	-	-
Glu.	9.86	6.2	-	-
Prol.	4.07	1.6	-	-
Gly.	3.73	3.1	-	4.2
Ala.	4.73	5.1	-	-
Val.	4.81	4.3	5.8	5.0
Cys.	1.3	1.0	0.9	1.5
Meth.	1.23	0.8	1.6	1.4
Isol.	5.10	2.8	5.3	4.8
Leu.	3.9	5.3	7.8	7.5
Phenyl.	4.28	2.9	4.8	4.9
Tyro.	3.32	1.7	4.0	3.5
Lys.	7.35	9.0	7.8	6.1
Hist.	2.19	7.1	2.1	2.5
Arg.	3.54	4.0	5.0	7.2

* P. Vananuvat (Nov. 1977), Value of yeast protein for poultry feeds, CRC Critical Reviews in Food Science and Nutrition, p. 328.

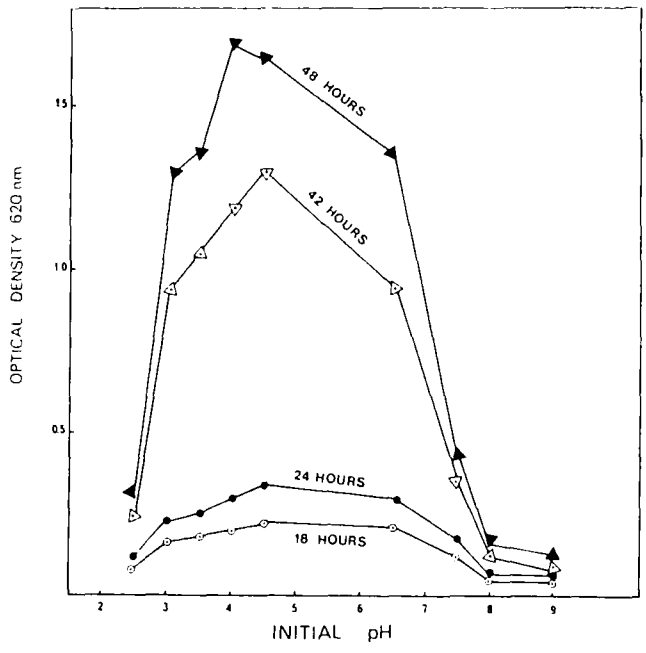

Fig. 2. Effect of initial pH on the growth of T. candida yeast.

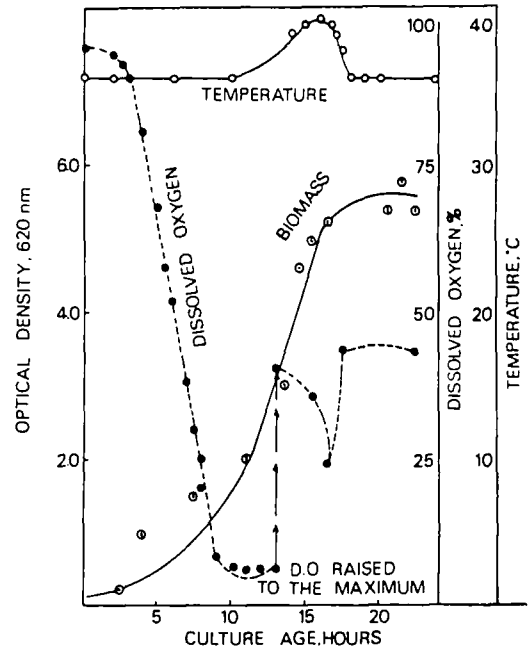

Fig. 3. Growth of T. candida in a 14 l fermenter.

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