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Characterization and Biomass Production Potential of Yeast Flora of Some Natural Sources of Kalyani

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Yeast flora of different natural sources such as palm syrup, molasses, toddy, canejuice and grape were isolated and characterized. Canejuice and Palmjuice yielded *Candida* sp. and *Saccharomyces cerevisiae* respectively. Other species isolated were *Schizosaccharomyces pombe*, *S. kluyveri* and *Rhodotorula* sps. All test isolates showed good growth on media containing sucrose or glucose as carbon source and ammonium nitrogen production of biomass by the test isolates in complete, malt extract or molasses medium was comparable. Protein content of cells grown on 2% molasses medium was comparable to that of complete medium. The importance of exploitation of yeasts as single cell protein source has been discussed.

Key words : Yeast flora, Single cell protein, Biomass

INTRODUCTION

In recent years, increasing interest has been focused on yeast as source of single cell protein (SCP) both for man and animals (Altschul, 1974 ; Kharatyan, 1978). It is now well established that yeasts grow rapidly and can produce a biomass which is 50% protein in dry weight by utilizing cheap nutrient sources (Peppler, 1970 ; Kharatyan, 1978). Moreover yeast SCP has been found to be adequate in lysine and other amino acids essential for human diets (Peppler, 1970). In several advanced countries, natural yeast flora occurring in soil, on plant surfaces and fruits have been investigated and a few selected ones are exploited industrially for the production of dry yeast powder as SCP source (Kharatyan, 1978)

Although yeasts occur on many natural sources, most abudant source, however, has been found to be tree-exudates such as mapple exudates (Carmo-Sousa, 1969). The yeasts present in exudates of different Indian plants, leaf surfaces, soils and other natural sources have not been properly investigated.

The objective of this work was to isolate and identify yeasts commonly occurring on natural sources such as plant juices and fruits commonly available in an around Kalyani. An attempt was also made to determine the growth characteristics and biamass producing ability of different isolates using different nutrient media.

MATERIALS AND METHODS

Presence of yeast in natural sources such as palm juice, toddy, cane juice, molasses and market grapes was examined. Yeast cells from these natural sources were isolated follewing the method of Beech and Davenport (1971) using Malt Extract Agar medium (MA)

The liquid sources were serially diluted and plated on MA. Known weight of grape or molasses was washed with known volume of sterilized distilled water by shaking on a rotary shaker. The washings were serially diluted and plated on MA. Single cell culture of each type of yeast was isolated and maintained on Wickerham's medium (1951) at 28°C with monthly sub-culturing. Morphological characters of vegetative cells and buds of the individual isolate were determined following the method of Barnett *et al.* (1983). Induction of ascospores in different isolates was done by the method of Smith (1960). Diazonium blue—B test (DB-B) of different isolates was done following the method of Vander Walt and Hopsu-Havu (1976).

Production of biomass in different media was determined by growing the isolates in respective nutrient broths at 28°C on a metabolic shaker. After 3 days. the cells were harvested by centrifugation using pre-tared centrifuge tubes. The cell pellets were dried at 80°C till constant weight. The biomass was expressed as dry weight of cells/unit volume of culture medium.

For protein extraction, samples (300 mg) of cells were homogenized with 0.2 M sodium phosphate buffer (pH, 6). The homogenate was centrifuged at 1000 g for 10 minutes in a cold centrifuge. The supernatant was treated with equal volume of cold 40% Trichloro-acitic acid (TCA) in a refrigerator for 48 hours. The precipitate was washed three times with cold ether, dissolved in phosphate buffer (ph, 6) and washed with cold ethanol. The pellet was finally dissolved in 1 ml of 1 N NaOH and incubated at 30°C for 1 hour. Protein centent of aliquots (0.5 ml) of this solution was determined following the methods of Lowry *et al.* (1951).

RESULTS

Isolation and identification

Yeast cells naturally occurring on palm syrup, cane juice, molasses, toddy and grapes were isolated in pure culture. The descriptive features of different isolates

along with the source materials are presented in Table 1. It was noted that the three isolates from palm juice, toddy and molasses were spore formers, whereas the isolates from grapes and cane juice were non-spore formers. Except the grape isolate which was red, other isolates were white or creamy in colour.

Table 1. Descriptive features of different isolates of yeasts.

Source	Isolate No.	Colour of Colony	Shape & Size	Nature of Budding	Ascospores
Palm juice	1	White or Cream	Round or ovate and 4-14 x 3-7 μ	Multilateral, no filament	1—12
Toddy	2	Cream	Ovate or cylindrical and 6-16 x 3-6μ	Polar, no filament	1—4
Molasses	3	White	Round or ovate and 3-15 x 3-6μ	Multilateral, No filament	1—4
Grape	4	Red	Ovate or cylindrical 5-15 x 3-7μ	Polar, no filament	No ascospore
Can juice	5	White	Round or ovate and 4-15 x 2-8μ	Multilateral, pseudo filament	No ascospore

For identification of different isolates, besides the morphological characters described in Table 1, standard biochemical tests concerned with utilisation of different carbohydrate and nitrate or nitrite nitrogen, and diazonium blue—B test were performed. The isolates were grown on basal medium containing the test substances separately and the results are shown in Table 2. Although several other tests were necessary for confirmation of the identification, the isolates examined in this study were identified as shown in Table 2. It is apparent that palm juice yielded *Saccharomyces cerevisiae* (isolate 1), toddy gave *Schizosaccharomyces pombe* (isolate 2), molasses yielded *Saccharomyces kluyveri* (isolate 3), grape gave *Rhodotorula* sp. (isolate 4) and cane juice yielded *Candida* sp. (isolate 5).

Effects of carbon sources on growth

Yeasts can utilized diverse carbon sources for growth and biomass production. Effect of different carbon sources when added to the basal medium were examined in this experiment. The isolates were grown on basal medium supplemented with test carbohydrates and the growth, as measured by increasing in O.D. at 550 nm,

Table 2. Biochemical characteristics of different isolates

Isolate No.	D-Glucose	Galactose	Maltose	Sucrose	Lactose	Starch	Nitrate	Nitrite	DBB	Tentative identification
1	+	V	V	V	—	V	—	—	—	*Saccharomyces cerevisiae*
2	+	—	+	+	—	V	—	—	—	*Schizosaccharomyces pombe*
3	+	+	—	+	—	—	—	—	—	*Saccharomyces kluyveri*
4	V	+	+	+	—	—	+	+	+	*Rhodotorula* spp.
5	—	+	+	+	—	—	—	—	—	*Candida* spp.

Codes in table : + positive ; — negative ; V variable.

Table 3. Effect of different carbon sources on growth of yeast isolates

Isolates No.	Days	D-Glucose	D-Galactose	Maltose	Sucrose	Lactose	Starch
1	1	0.52	0.93	0.10	0.08	0.01	0.02
	2	0.61	0.90	0.19	0.27	0.03	0.02
2	1	0.48	0.02	0.75	0.42	0.03	0.04
	2	0.52	0.04	0.79	0.51	0.05	0.34
3	1	0.52	0.87	0.05	1.00	0.02	0.03
	2	0.43	1.00	0.15	1 40	0.12	0.05
4	1	0.07	0.14	0.17	0.12	0.05	0 09
	2	0.17	0.17	0.20	0.22	0.25	0.43
5	1	0.02	0 50	0.13	0.41	0.01	0 02
	2	0.17	1.00	0.19	1.10	0.04	0.03

was determined. The results are shown in Table 3. It is evident from the data that different isolates utilised different carbohydrates differentially, The best growth of isolate 1 was in galactose, but that of isolate 5 in sucrose medium. In general, the sucrose and glucose media either supported the best of the next best growth for majority of the isolates. It was of interest to note that the best growth of isolate 4 was in lactose medium.

Effects of different nitrogen sources on growth

Effects of inorganic and organic nitrogen sources including amino acids on the growth of different yeast isolates were examined. The isolates were inoculated to basal medium supplemented with the test compounds and growth, as measured by increase in O. D. at 550 nm, was determined. The results are presented in

Table 4. Effect of different N-sources on growth of yeast isolates

Isolates No.	Days	NH_4NO_3	$NaNO_3$	NH_4Cl	Asparagine	Ammonium tartrate
1	1	1.00	0.50	0.45	0.68	0.27
	2	1.20	0.95	0.48	0.61	0.92
2	1	0.17	0.51	0.10	0 27	1.10
	2	0.19	0.57	0.13	0 26	1.55
3	1	1.00	0.88	0.90	0.26	1.10
	2	1.32	1.20	1.50	0.38	1.00
4	1	—	—	—	0.13	0.005
	2	0.16	0.16	0.36	0.17	0.16
5	1	0.82	1.00	1.10	0.27	0.80
	2	1.10	1.10	1.51	0.17	1.10

Table 4. It is apparent from the results (Table 4) that ammonium nitrogen supported best growth for isolate 3, 4 and 5 as well as isolate 1. Good growth of isolate 1 and 2 in presence of ammonium nitrate also indicated its preference for amonium nitrogen.

Comparison of biomass production by different isolates

Biomass production as measured by dry weight yield from unit volume of culture medium of different test isolates was determined. The isolates were grown on unit volume of basal or different concentrations of malt extract or molasses media and the dry weight was determined. The results are shown in Table 5. It may be noted from the data that both malt extract and molasses media at 2% concentration produced biomass equivalent to or better than basal medium. At 5% concentration the dry weight yield per unit volume was significantly higher in molasses and malt extract media.

Table 5. Comparison of biomass production by the isolates of yeasts

Isoiate No.	Dry wt/unit volume of culture mass (mg/10 ml) obtained from media						
	Basal	Malt Extract			Molasses		
		1%	2%	5%	1%	2%	5%
1	35.0	22.0	33.0	50.0	20.0	29 0	45.5
2	32.0	20.0	31.0	47.0	19.0	29.5	46.5
3	29.0	25.0	30.0	39 0	22.0	28.7	38.0
4	10.0	02.0	04.0	08.0	02.0	03.0	07.0
5	12.0	07.0	10.0	20.0	05.0	12.0	25.0

Comparison of protein content of different isolates

Protein contents of the test isolates grown on basal or molasses media were compared. The results are shown in Table 6. It is evident from the result that calls grown on 2% molasses medium had protein contents comparable to or more than cells grown on basal media.

Table 6. Comparison of TCA insoluble protein content of yeast isolates

Isolate No.	Protein content μg/g fresh wt. when grown on media	
	Basal	Molasses (2%)
1	933.0	906.0
2	840.0	880.0
3	826.0	840.0
4	666.0	640.0
5	676.0	733.0

DISCUSSION

It is evident from this study that the different yeast species are naturally occurring on diverse source materials examined. Cane juice yielded *Candida* species. The identification procedures of Barnett *et al.* (1983) suggested that it is *Candida utilis*, which is commonly used for industrial food yeast production in America (Peppler, 1970), England (Barnett *et. al.*, 1963) and USSR (Kharatyan, 1978).
The result concerned with growth studies of different isolates of yeasts clearly indicated that the isolates differed in their ability to utilise different carbon and nitrogen sources. In general glucose and sucrose as carbon sources and amonium nitrogen as nitrogen source were preferred by most isolates.

It was of interest to note that all the test isolates produced significant amount of biomass utilsing cheap nutrient source such as 2% molasses. No protein source was added to this medium of Barnett *et al.* (1983). Furthermore the protein contents of cells of the test isolates grown in molasses medium were comparable to those grown in complete medium. Apparently, the test isolates possess good potential for industrial exploitation. The characterisation of natural yeast flora of this country warrants more attention before industrial exploitation of them can be initiated.

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