ETHANOL PRODUCTION BY FREE AND IMMOBILIZED "YEAST PHASE" OF *COKEROMYCES*

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ABSTRACT

Cokeromyces (NCL 83-2-5) showed yeast-mycelial dimorphism and ethanol production in the "yeast phase" from glucose. The yeast phase was induced readily in shake cultures and also aerobically by high sugar concentrations and/or high incubation temperature (37°C) in agar cultures. Fermentation studies with free as well as immobilized yeast phase cells showed quantitative ethanol production in 4% glucose media. The flocculent nature of the yeast phase and ethanol production at 37°C are features of special interest in this mucoraceous fungus.

INTRODUCTION

COKEROMYCES is a rare genus of thamnidiaceous mucors characterized by homothallic zygospores, and uni- or multispored sporangioles borne on strongly recurved pedicels[1,2]. A saprophytic strain of *Cokeromyces* isolated in our laboratory showed interesting colonial morphogenesis and yeast-mycelial dimorphism depending on the cultural conditions, and in the "yeast phase" fermented glucose to ethanol in the free as well as in immobilized conditions. Some observations on the morphogenetic behaviour as well as ethanol production from glucose are presented in this paper.

MATERIALS AND METHODS

Culture

The fungus was isolated on malt extract glucose yeast extract peptone (MGYP) agar plates from a soil sample collected at Doddabetta, Udhagamandalam (altitude 2461.5 m), Tamil Nadu, India, and grown by periodic subculture on the same medium. Morphogenetic studies were carried out in MGYP liquid as well as agar medium, modified to contain glucose concentrations ranging from 2–25%. The strain has been designated *Cokeromyces* (NCL-83-2-5).

Induction of yeast phase, immobilization and ethanol fermentation.

When MGYP (2% glucose) liquid medium was inoculated with spores of *Cokeromyces* and incubated on rotatory shaker (220 rpm) at 28°C for 24 hr, yeast phase was induced. Alternately, when inoculated into large tubes (25 × 150 mm) in which the medium was filled to 2/3 capacity and incubated at 37°C, yeast phase cells were readily formed and deposited at the base as a whitish flocculent mass.

20 g (wet weight) of yeast phase cells were thoroughly mixed with 100 ml of 4% (w/v) of sodium alginate solution and added dropwise through a microsyringe into 6% calcium chloride solution. After 2 hr the calcium alginate beads with the entrapped cells were removed, washed and used for fermentation studies. The immobilization was carried out under aseptic conditions.

5 g (wet weight) of free cells or calcium alginate beads containing the equivalent of 5 g (wet weight) of cells were added to MGYP liquid medium (4% glucose) contained in 150 ml Erlenmeyer flasks filled to 2/3 capacity and incubated at 30°C, 37°C and 50°C to evaluate ethanol production at different temperatures.

Ethanol was estimated at different intervals by gas chromatography, using a Poropak-Q (80–100 mesh) and FID detector. Samples were prepared as follows: 5 ml of fermentation broth was diluted to 7.5 ml with distilled water and exactly 5 ml was distilled; 1 μl of the distillate was used for estimating ethanol by gas chromatography. Ethanol concentration was expressed in per cent. (w/v).

RESULTS AND DISCUSSION

When inoculated on MGYP agar (5% glucose) and incubated for 8 hr at 30°C or 37°C, the spores of *Cokeromyces* enlarged , became spherical and yeast-like. Several multipolar daughter cells were budded off resulting in the formation of micro-colonies composed of many rounded yeast phase cells. At 30°C tubular mycelial growth took place from the yeast-like cells, and after 48–72 hr a well established mycelial colony forming the asexual and sexual spores developed. At

37 C however, growth was essentially yeast-like with a suppression of mycelial growth and sporulation. Figure 1 shows the comparative appearance of the colonies at 30 C and 37 C. Figure 2 is a photomicrograph of the budding yeast phase cells under ethanol fermentation conditions. Besides temperature, sugar concentration had a distinct effect on morphogenesis. Cultures raised on MGYP (25 % glucose) were only yeast-like with total suppression of mycelial growth and sporulation when incubated at either 30°C or 37 C. Figure 3 shows an enlarged view of the yeast-like colonies on 25 % glucose medium.

Table 1 presents comparative data on ethanol production from 4 % glucose by free and calcium alginate immobilized yeast phase cells (10^6–10^8 per head of 2–3 mm diameter). Complete conversion of glucose to ethanol occurred in either case in 48 hr. The ethanol conversion was similar over a wide pH range (4–7) and at 30°C or 37°C. When pregrown cells were incubated at 50°C in 4% glucose medium, ethanol production was very poor.

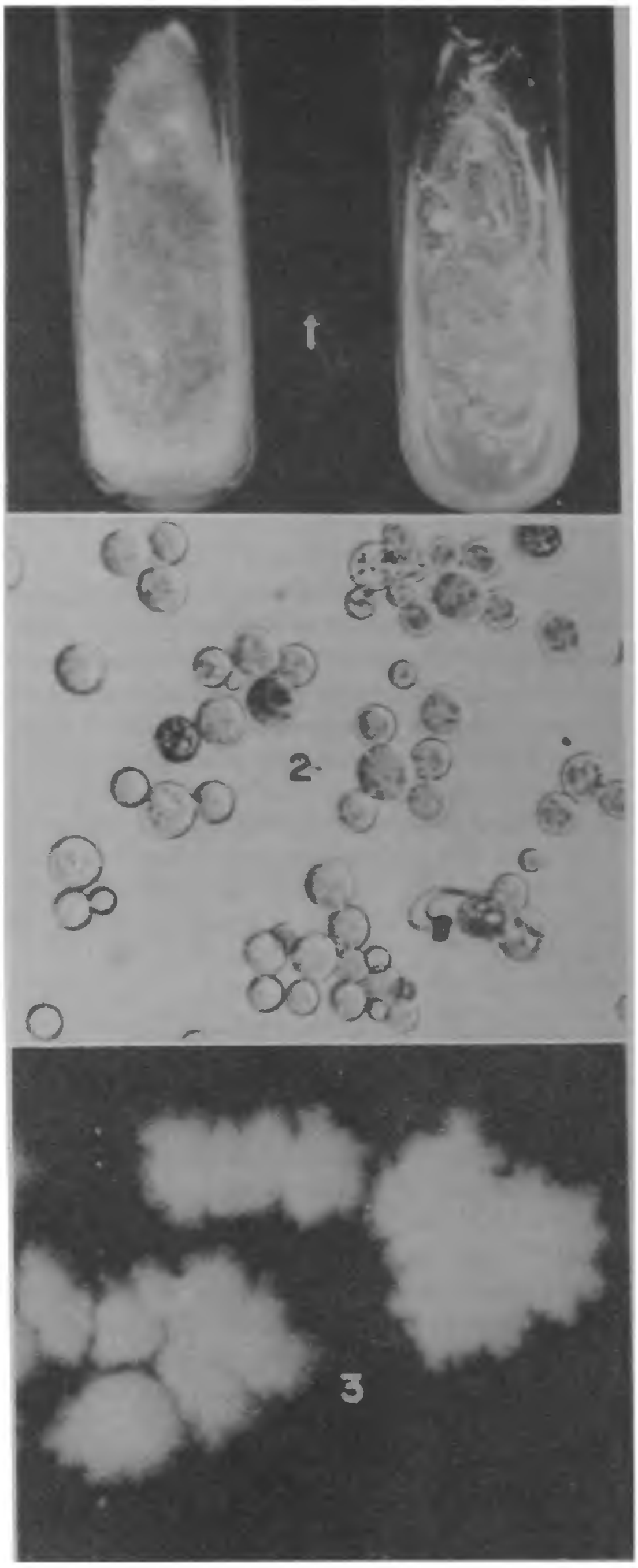
The present study on the morphogenetic variations exhibited by *Cokeromyces* (NCL 83-2-5) in relation to nutritional and cultural conditions and the fermentation of glucose to ethanol by the yeast phase cells present several interesting aspects. The suppression of mycelial phase as well as sporulation in cultures incubated either at 37°C or even at 30°C, when high sugar concentrations are used, is significant from the point of view of regulatory controls related to morphogenesis and phenotypic expression. Formation of yeast phase in submerged culture has been readily observed in MGYP medium at all the glucose concentrations tested.

Price *et al*[3] reported yeast phase formation in *C. poitrasii* only in shake cultures containing more than 3 % glucose and 0.1–0.2 % (v/v) phenethyl alcohol.

Table 1 *Comparative ethanol production by free and immobilized yeast phase cells from 4 % glucose at 37°C*

	Period of fermentation (hr)	Ethanol concentration (w/v)
Free cells*	5	0.8 %
	22	1.1 %
	48	2.0 %
Immobilized cells*	5	0.8 %
	22	1.04 %
	48	1.9 %

*See Materials and Methods



Figures 1–3. 1. *Cokeromyces* (NCL 83-2-5) on MGYP (5 % glucose) agar at 30°C and 37°C. **2.** Budding yeast phase cells. **3.** Yeast-like colony on MGYP (25 % glucose) agar.

The flocculent nature of the yeast phase during alcohol production at 37°C is an advantageous feature, since screening for such flocculent yeast strains is being extensively made for ethanol biotechnology. We are currently in the process of selecting mutants and variants totally suppressed for mycelial growth under all cultivation conditions and for ethanol tolerant and thermotolerant strains of the culture.

10 February 1986

1. Shanor, L., Poitras, A. W. and Benjamin, R. K., *Mycologia*, 1950, **42**, 271.
2. Benjamin, R. K., *Aliso*, 1960, **4**, 523.
3. Price, J. S., Storck, R. and Gleason, F. H., *Mycologia*, 1973, **65**, 1274.

ANNOUNCEMENTS

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The Centre for Advanced Study in Botany, University of Madras is organizing a National Seminar on Marine Microbiology in the second week of October 1986. The main objective of the seminar is to bring together all the Marine Microbiologists of the country and take stock of the research work done in India and identify problems for future research. For additional information regarding the seminar, please contact: Prof. A. Mahadevan, Director, C.A.S. in Botany, University of Madras, Guindy Campus, Madras 600 025.