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ORIGINAL PAPER

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Immobilization of recombinant strains of *Saccharomyces cerevisiae* for the hydrolysis of lactose in salted Domiati cheese whey

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Abstract Intergeneric protoplast fusants SK-26 and SK-35 between *Saccharomyces cerevisiae* ATCC 4126 and *Kluyveromyces lactis* CBS 683 produced 2.70 ml dl^{-1} and 1.52 ml dl^{-1} (v/v) of ethanol during fermentation of lactose at 25 °C in salted Domiati cheese whey containing 6.1 g dl^{-1} (w/v) NaCl when entrapped in alginate spheres, whereas the free recombinant cells produced 2.36 ml dl^{-1} and 1.09 ml dl^{-1} (v/v) of ethanol. Yeast hybrids spheres can be used nine times with accumulated increases of ethanol production of 9.1 ml dl^{-1} and 8.98 ml dl^{-1} (v/v), respectively.

Keywords Immobilization · Entrappment · *Saccharomyces cerevisiae* · Ethanol production · Salted cheese whey · Recombinant yeast strains

Introduction

World output of whey has increased as a result of the development of the cheese industry. Whey and whey protein hydrolysates have many commercial uses as food additives and in the preparation of special diets for the enteric feeding of babies and sick adults [1]. Although whey has nutritional and economical values, most whey production is not economical and represents a potential pollutant in many countries. Utilization of unsalted whey as a fermentation substrate to produce biomass and organic acid has been described [2]. In Egypt, salted whey [up to 6 g dl^{-1} (w/v) NaCl] is an abundant by-product of Domiati cheese manufacture. This waste whey represents a serious environmental problem and economic losses. Yeasts have been shown to be the most promising organism for the conversion of lactose to ethanol [3, 4]. *Saccharomyces cerevisiae* and *Kluyveromyces lactis* have been intensively used for this purpose. *S. cerevisiae* is unable to ferment lactose because it lacks the β-galactosidase system but it can produce ethanol. On the other hand, *K. lactis* utilizes lactose but cannot carry out alcoholic fermentation [5] and is inhibited by high concentrations of salt [6]. To overcome these problems, two hybrids of yeast containing the characteristics of the parent yeast strains [7] were immobilized to maximize ethanol production. The previous hybrids from the intergeneric protoplast fusion between *S. cerevisiae* ATCC 4126 and *K. lactis* CBS 683 showed a high level of ethanol yield, and immobilization of microbial cells in alginate systems provides an ideal means of biocatalyst recycling [8–10], prevents alcohol accumulation and accelerates lactose fermentation [11]. The present study was carried out in a simple batch reactor configuration to evaluate ethanol production by immobilized and free cells of fusant yeast strains from salted Domiati cheese whey. The stability of immobilized cells during fermentation eas also studied.

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Materials and methods

Microorganisms

Yeast strains used in the present investigation were recombinant hybrids (SK-26 and SK-35) obtained from intergeneric protoplast fusion of industrial strains of *S. cerevisiae* ATCC4126 (Cycloheximide$^-$, Imazilil$^+$), fermenting sucrose, glucose, fructose, *K. lactis* CBS 683 (Imazilil$^-$, Cycloheximide$^+$), fermenting lactose, glucose and galactose [7]. Recombinant strains were maintained on yeast extract peptone dextrose (YEPD) medium [12] and stored at 4 °C.

Media

Yeast extract peptone dextrose. The medium was prepared as previously described [12], and slants from it were sterilized at 110 °C for 30 min.

Cheese whey medium. The salted whey was produced from Domiati cheese manufactured in the Dairy Science and Technology

Department factory and prepared [13] by adjusting the whey to pH 4.6 with concentrated HCl, heating at 90 °C for 15 min, cooling and centrifugation at 800 *g* for 10 min to remove coagulated proteins. Whey medium containing 6.1 g dl^{-1} (w/v) NaCl and 4.6 g dl^{-1} (w/v) lactose was supplemented with 0.3 g K_2HPO_4, 0.5 g yeast extract and 0.3 g dl^{-1} (w/v) of 5 mol l^{-1} NH_4OH. The medium was adjusted to pH 4.5 with H_2SO_4, autoclaved for 15 min at 121 °C, cooled to 30 °C and inoculated.

Immobilization of yeast hybrids in calcium alginate

Ten millilitre cultures of fusants were grown overnight at 25 °C in YEPD broth medium. Cells were harvested by centrifugation at 1500 g for 5 min and subsequently washed in YEPD broth. A sample of 10^6 cells was then suspended in 4 g dl^{-1} (w/v) sodium alginate (Medium Viscosity, Sigma) and the suspension was added dropwise to a 50 m/mole l^{-1} $CaCl_2$ solution. The resulting spheres (average diameter 4 mm) were washed in 50 ml YEPD broth [14].

Fermentation process

A volume of 50 ml of the cheese whey medium used for ethanol production was transferred to 100 ml fermentation bottles with rubber stoppers equipped with hypodermic syringes. The bottles were inoculated with 10^6 ml^{-1} of free cells or entrapped cells and incubated with mild shaking at 25 °C for 96 h.

Determination of ethanol concentration

Ethanol concentration was determined using a gas chromatograph (PYE UNICAM, PYe series 104) equipped with a flame ionization detector. A column (2 mm × 2 m) packed with 80/120 carbopack B AW/15 g kg^{-1} PEG 20 M was used. The temperatures of the injector and detector were maintained at 200 °C and the column oven was operated isothermally at 130 °C. The carrier gas was nitrogen and the chart speed was 2.5 mm per min.

Sugar measurement

The sugar content was determined using the phenol/sulphuric acid reagent method [15].

Salt determination

The salt content in whey was determined using silver nitrate reagent according to the method of Ling [16].

Results and discussion

It has been demonstrated that the immobilization of yeast strains in calcium alginate enhances ethanol production during fermentation [17]. In order to determine whether or not immobilization of the yeast hybrids has a similar effect during growth on salted Domiati cheese whey medium containing 6.1 g dl^{-1} (w/v) NaCl, ethanol production by the immobilized microorganisms (10^6 cells) was compared with that by the free microorganisms in a batch-fed system. The results are shown in Fig. 1 and demonstrate that while the immobilized system produced 2.70 ml dl^{-1} and 1.52 ml dl^{-1} (v/v) of ethanol for the fusants SK-26 and SK-35, respectively, the free cells produced 2.36 ml dl^{-1} and 1.09 ml dl^{-1} (v/v) of ethanol for the other hybrids.

The decrease in ethanol production by the free cells compared with immobilized cells in a batch-fed system has been observed previously [17, 18]. The percentage of increases in ethanol yield for the fusants SK-26 and SK-35 by immobilized cells were 14.4% and 39.4%, respectively. Such values are greater than the values reported previously [14], indicating that the percentage increase in ethanol production by immobilized *Kluyveromyces marxianus* IMB3 reaches 54.5% rather than free cells. The increases in ethanol yield in the present study were higher than that reported previously [18]. Similarly, Brady et al. [19] reported that ethanol production by the immobilized system of *K. marxianus* IMB3 in the absence of Mn^{+2} increased from 0.8 ml dl^{-1} (v/v) to 1.3 ml dl^{-1} (v/v) in the fermentation of 4 g dl^{-1} (w/v) of lactose.

The results obtained suggest that immobilization of the yeast hybrids in alginate provides a suitable system for the development of continuous ethanol processes in salted cheese whey.

The stability of reusing yeast immobilized spheres, or the ability of a known amount of yeast hybrid cells to complete the same fermentation several times was studied. The results are presented in Table 1 and indicate that SK-26 and SK-35 spheres could be used for the completion of the same reaction nine times with an

Table 1 Ethanol production by immobilized yeast hybrids in salted Domiati cheese whey medium containing 6.1 g dl^{-1} (w/v) NaCl at 25 °C (Average of three replicates)

Number of reactions	SK-26		SK-35	
	Ethanol yield (ml dl^{-1}, v/v)	Accumulated ethanol yield (ml dl^{-1}, v/v)	Ethanol yield (ml dl^{-1}, v/v)	Accumulated ethanol yield (ml dl^{-1}, v/v)
1	2.70	2.70	1.52	1.50
2	1.14	3.84	1.09	2.61
3	1.14	4.98	1.09	3.71
4	0.79	5.77	1.05	4.75
5	0.74	6.51	0.95	5.70
6	0.74	7.25	0.95	6.65
7	0.74	7.99	0.90	7.55
8	0.57	8.56	0.84	8.39
9	0.54	9.10	0.59	8.98

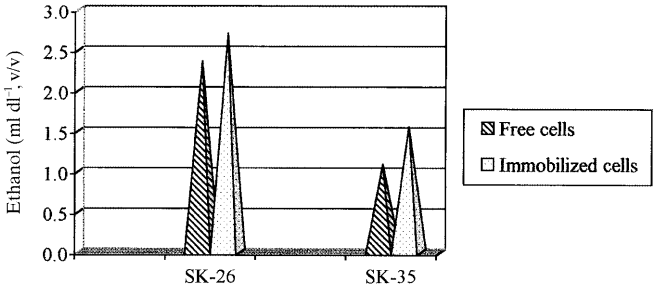

Fig. 1 Ethanol production by free and immobilized yeast hybrids in salted Domiati cheese whey medium containing 6.1 g dl^{-1} (w/v) NaCl at 25 °C for 96 h (Average of three replicates)

accumulated yield of ethanol production reaching 9.1 ml dl^{-1} and 8.98 ml dl^{-1} (v/v), respectively. This means that the immobilization of these recombinant strains of yeast into the alginate system gives a good recovery of ethanol yield. From these results it can be concluded that the immobilization process into alginate spheres enhances the production of ethanol and shows accumulated increases of ethanol from salted Domiati cheese whey.

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