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Screening of beta-galactosidase producing yeasts

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SUMMARY

Various strains of yeasts were tested to determine the β-galactosidase yield. *Cryptococcus* were the best producers. Enzyme activities of some strains of *Cryptococcus laurentii*, *Cr. albidus* and *Cr. luteolus* were superior to those of yeasts like *Kluyveromyces fragilis* and *K. lactis* which have been reported as the best enzyme source for use in the dairy industry.

RESUMEN

Se evaluaron varias cepas de levaduras para determinar los rendimientos en β galactosidasa. La mejor cepa productora resultó *Criptococcus*. La actividad enzimática de algunas cepas de *Cr. laurentii, Cr. albidus y Cr. luteolus* fueron superiores a las cepas de levadura *Kluyveromyces fragilis y K. lactis*, que han sido reportadas en la literatura como las mejores fuentes de la enzima para su utilización en la industria alimenticia.

INTRODUCTION

Lactose is a disaccharide found in mammalian milk. Since cow's milk and various products made from it are major items for human beings, lactose constitutes a substantial portion of the daily carbohydrate intake (Shula, 1975). Furthermore, the presence of lactose in some dairy products discourages consumption of milk and some milk products by lactose intolerant individuals.

There is widespread interest, from both nutritionists and commercial producers in reducing the lactose content of some dairy products. One of the most promising methods utilizes hydrolysis of lactose to glucose and galactose by lactate (β-D-galactosidase, EC 3.2.2.23), (Mahoney *et al.*, 1974; Barbosa *et al.*, 1985).

Considerable effort has been expended in assessing and developing β-galactosidase preparations from various microbial sources of many demonstrated microorganisms; however, only a few species of yeast and molds are currently used as sources for commercially available enzyme preparations for industrial use (Itoh *et al.*, 1982). Yeasts have been considered as the predominant microbial enzyme source for food applications and both *Kluyveromyces fragilis* and *K. lactis* have been most investigated (Van Dam *et al.*, 1950, Shula, 1975; Guy and Bingham, 1978) for this purpose. They are now used as preferential sources for β-galactosidase production. The aim of the present study was the screening of several yeasts to determine the β-galactosidase producing strains as possible sources of this enzyme for industrial use.

MATERIALS AND METHODS

Microbial sources

Two yeasts strains of *Kluyveromyces fragilis* (145 and 276) and one of *K. lactis* from Food Sciences Department, University of California, Davis, were kindly supplied by Dr. D.O. Silva from Universidade Federal de Vicosa, Brasil; 330 yeasts isolated from a small urban lake were obtained from Mycology Laboratory, ICB UFMG, Belo Horizonte, Brasil.

The selection of β-galactosidase producing yeasts was made through assimilation tests by auxonographic method in basal medium I (Lodder and Kreger-van Rij, 1952) with lactose as the carbon source.

The microorganisms were maintained by monthly transfer in malt agar and kept at 4°C. Identification of yeasts was carried out as described by Freger-van Rij,1984.

Culture and growth conditions

The medium for enzyme production consisted of 2% (w/v) lactose and 0.5% (w/v) yeast extract, adjusted to pH 7.0 and sterilized at 120°C for 15 min. The microorganisms were cultivated aerobically in 250 ml shaking flasks containing 30 ml of medium. The inoculum was prepared by the addition of sterile water to slant cultures of yeasts growing in malt agar to give 10^8 cells/ml. One ml of cell suspension of each strain was inoculated in 100 ml shaking flasks containing 10 ml of medium for enzyme production and incubated at 30°C. Three ml of 24 h inoculum were used. Incubation was carried out by rotatory shaker at 150 rpm, 30°C for 24 h. The cells were harvested by centrifugation at 5000 xg for 10 min at 4°C and washed twice with 0.1 M sodium phosphate buffer, pH 7.0.

Permeabilization

The pellets were suspended in 10 ml of 70% (v/v), ethanol for 15 min. with stirring in an ice-bath. The cells were them harvested by centrifugation and washed twice with 0.1 M phosphate buffer, pH 7.0 according to the method described by Decleire *et al.*, 1985.

Enzyme assay

After cells permeabilization, the β-galactosidase activity was assayed by measurement of glucose released from lactose by glucose oxidase-peroxidase-chromogen method (Decleire *et al.*, 1985). It is a fast method; therefore, it is useful for enzyme screening of a great number of microorganisms. The permeabilized cells were incubated in 10 ml of 2% (w/v) lactose at 40°C. The reaction was interrupted after 30 min by cooling in an ice-bath. The cells were harvested by centrifugation. Glucose was determined in the supernatant by oxidase-peroxidase- chromogen method using Glucose ENZ-COLOR kit (Bio-Diagnóstica S P). The pellets were utilized for dry-weight determination. β-galactosidase activity was measured by the glucose production/mg of dry cells, and one unit of enzyme activity was defined as the amount of enzyme that liberates 1 μmole of glucose/min. mg^{-1} dry cells under the conditions specified above.

O-nitrophenyl-β-galactopyranoside (ONPG) assay

The best producers of lactase obtained as described above were compared with strains of *K. fragilis* and *K. lactis* used as control. The b-galactosidase activity of these yeasts was determined by ONPG assay described by Park et al., 1979. This method is more specific for lactase assay. The enzymatic activity was measured by adding 4 ml of the substrate (8.2 mM ONPG in 0.1 M sodium acetate buffer, pH 5.0) to 1 ml of permeabilized cell suspension (25 mg of dry cells/ml). The mixture was incubated at 40°C. The reaction was interrupted after 30 min by adding 2.5 ml of 1 M sodium carbonate. The cells were harvested by centrifugation and the supernatants absorbance was measured at 420 nm. One unit of ß-galactosidase is defined as the amount of enzyme that liberates 1 μmole of 0-nitrophenol (ONP)/min · mg^{-1} dry cells under the conditions described above.

RESULTS AND DISCUSSION

A total of 330 yeast strains were tested, of which 72 strains were able to grow on medium with lactose as the carbon source. These strains were tested for β-galactosidase production by measurement of glucose released from lactose, and 60 of them were found to produce the enzyme (table 1). The *Cryptococcus* species were the best producers, followed by *Candida* and *Trichosporon*. Yeast of genera *Aureobasidium*, *Debaryomyces*, *Hansenula* and *Rhodotorula* showed no lactose activity. The possibility

of the presence of intracellular enzyme was not investigated. The enzyme may be present in concentrations too small to be perceptible with the enzymatic assay used in this experiment. As we only tested two species of *Candida*, and as this genus accommodates a heterogeneous and unnatural group of yeasts (Meyer *et al.*, 1984), it is not possible to affirm whether *Candida* is a good lactase producer.

As shown in table 2, the dry weight average of the *Trichosporon* species was larger than those of *Candida* and *Cryptococcus* but that species showed a lower production of lactase. It is possible that these variations are related to permeabilization treatments which are more efficient for one species than for another due to differences in cell wall structure, presence of pseudomycelium and

Table 1
β-GALACTOSIDASE ACTIVITY OF LACTOSE ASSIMILATING YEASTS

Yeasts		Dry weight (mg)[1]			Lactase activity[2]		
		Min	Max	$\bar{x}$	Min	Max	$\bar{x}$
Aureobasidium pullulans	(1)*		7.2			0	
Candida famata	(1)		48.1			3.1	
C. parapsilopsis	(1)		61.7			1.2	
Cryptococcus sp	(8)	29.5	196.4	107.3	2.3	6.5	3.8
Cr. albidus	(10)	34.4	245.3	113.7	2.1	6.2	3.7
Cr. laurentii	(9)	101.1	181.8	134.6	2.4	5.0	3.5
Cr. luteolus	(3)	60.1	165.5	122.7	2.5	4.6	3.4
Debaryomyces sp	(1)		11.5			0	
Hansenula fabianni	(1)		12.8			0	
Rhodotorula sp	(3)	6.7	20.2	14.1		0	
Rh. acheniorum	(1)		11.5			0	
Rh. minuta	(4)	6.6	22.6	15.8		0	
Rh. rubra	(1)		25.5			0	
Trichosporon sp	(7)	63.2	187.2	150.9	0.1	2.4	0.5
Tr. cutaneum	(20)	77.6	243.8	148.6	0.1	1.2	0.5
Tr. pullulans	(1)		122.6			0.3	

* In parenthesis the number of strains tested.
[1] *Min.* Minimum; *Max.* Maximum; $\bar{x}$ Average.
[2] Lactase activity is defined as the amount of enzyme that liberates 1 μmole of glucose/min•mg^{-1} dry cells under the assay conditions specified.

true mycelium common in the *Trichosporon* species resulting in greater resistance to ethanol treatment. The strains that showed lactase activity higher than 3.0 unit by measurement of glucose release (table 1), were tested using ONPG as substrate (table 3). The results were compared with those of *K. fragilis* 145.276 and *K. lactis*. In table 3, only the strains which showed activities higher than 0.100 units are shown. Five strains of *Cr. laurentii* and three strains of *Cr. albidus* showed a range of activities between 0.132 and 0.496, 0.332 and 0.464 units respectively.

The results show that the enzyme production, like many other physiological activities of microorganisms, is more a strain than a species-bound characteristic. The strains of *Cryptococcus laurentii* 02, 03, 04, *Cr. albidus* 01, 02, 03 and *Cr. luteolus* produced more enzymes than the strains of *K. fragilis* and *K. lactis*.

Table 2
AVERAGE OF DRY WEIGHT AND ENZYMATIC ACTIVITIES OF β-GALACTOSIDASE GENERA YEASTS

Yeasts	Number of strains	Dry weight (mg)[1] $\bar{x}$	Lactase activity[2] $\bar{x}$
Candida	2	55	2.1
Cryptococcus	30	120	3.6
Trichosporon	28	141	0.4

[1] $\bar{x}$. Average
[2] Lactase activity is defined as the amount of enzyme that liberates 1 μmole of glucose/min.mg^{-1} dry cells under the assay conditions specified.

The *Cr. laurentii* 04 was the best producer and showed 1.9 fold greater β-galactosidase activity than *K. fragilis* 276.

Table 3
COMPARATIVE LACTASE ACTIVITY FOR THE BEST β-GALACTOSIDASE PRODUCERS WITH *Kluyveromyces lactis* AND *K. fragilis* USED AS CONTROL, USING O-NITROPHENYL-β-GALACTOPRANOSIDE (ONPG) AS SUBSTRATE

Strains	Lactase activity[1]
Cryptococcus laurentii-01	0.157
Cr. laurentii-02	0.413
Cr. laurentii-03	0.413
Cr. laurentii-04	0.496
Cr. laurentii-05	0.132
Cr. albidus-01	0.464
Cr. albidus-02	0.438
Cr. albidus-03	0.332
Cr. luteolus	0.339
Kluyveromyces lactis	0.223
K. fragilis-145	0.187
K. fragilis-276	0.259

[1] One unit of lactase is defined as the amount of enzyme that liberates 1 μmole of o-nitrophenol/min · mg^{-1} dry cells under the assay conditions specified.

Cr. laurentii, *Cr. albidus* and *Cr. luteolus* are saprophytic species.

In the genus *Cryptococcus*, only the species *Cr. neoformans* is pathogenic in man (Davis *et al.*, 1973). Therefore, strains of *Cr. laurentii* 02, 03, 04 and *Cr. albidus* 01, 02 could be used as β-galactosidase source in the dairy industry. Besides, they could be used in genetic improvement of industrial yeasts strains through experiments of protoplast fusion or DNA-transformation. Biochemical characterization studies of β-galactosidase produced by these yeasts are in course in our laboratory.

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