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# PERMEABILIZATION OF YEAST CELLS (*Kluyveromyces fragilis*) TO LACTOSE BY CETYLTRIMETHYLAMMONIUM BROMIDE

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## SUMMARY

Whole cells of lactose fermenting *Kluyveromyces fragilis* had very low β-galactosidase activity. Treating the yeast cells with a cationic detergent cetyltrimethylammonium bromide (0.1%) at 4°C for 5 mins increased the enzyme activity 480 fold. Detergent treated cells readily hydrolysed lactose present in milk and sweet whey and glucose produced was not further metabolized. These detergent permeabilized cells could be used to produce low lactose milk, in the utilization of whey and saccharification of lactose or whey for the production of alcohol.

## INTRODUCTION

The enzyme β-galactosidase (lactase) which hydrolyses lactose into glucose and galactose has several biotechnological applications in food industries and nutrition. It is widely used to reduce the lactose content of certain dairy products and whey utilization (Mahoney, 1985). A number of enzyme preparations from various microbial sources have been developed, of these the enzyme from a yeast *Kluyveromyces fragilis* has an optimum pH suitable for hydrolysis of lactose in milk and use of this organism is permitted in food industries (Mahoney *et al.*, 1975). Currently, efforts are mainly focussed on the use of purified enzyme in either a soluble or immobilized form. The use of whole cells containing β-galactosidase as a source of enzyme is an interesting alternative which has not yet been fully explored. However, a major drawback in the use of whole yeast cell is the poor permeability of the cell membrane to lactose. Here we report that treating the yeast cells with cetyltrimethylammonium bromide (CTAB) a cationic detergent rendered the cells freely permeable to lactose and the catalytic potential of the cells was greatly increased.

## MATERIALS AND METHODS

o-Nitrophenyl β -D-galactopyranoside (ONPG) was from Sigma Chemical Co., USA. Cetyltrimethylammonium bromide is a product of Fluka, Switzerland. Triton X-100 was purchased from V.P. Chest Institute, Delhi, India. All other chemicals used were of reagent grade.

Micro organisms and culture conditions. The yeast Kluyveromyces fragilis (NRRL-Y-1196) was obtained from Northern Regional Research Centre (NRRL), USDA, Agricultural Research Service Peoria, IL, USA. Maintenance and growth condition of organism in medium containing 10% lactose was essentially followed as reported earlier (Mahoney et al., 1975). The cells were harvested at the beginning of the stationary phase by centrifugation and washed twice with cold water. Cell free extract was prepared by toluene autolysis according to the method described earlier (Mahoney et al., 1975).

Cell permeabilization. Harvested cells (1 g wet wt) were suspended in 10 mL of cold 0.1M potassium phosphate buffer pH 7.0 containing 0.1% cetyltrimethylammonium bromide and held at 4°C for 5 mins. Cells were separated from detergent solution by centrifugation at 6,000 x g for 10 mins and washed twice with ice cold 0.1M potassium phosphate buffer, pH 7.0 and finally suspended (1 g wet wt/5 ml) in the same buffer.

Enzyme assay. β -Galactosidase activity was assayed using O-nitrophenyl-β -D-galactopyranoside following the method described earlier (Mahoney et al., 1975). One unit of enzyme activity is defined as the amount of enzyme that liberates 1 μ mole of o-nitrophenol per min at 37°C.

Lactose hydrolysis in milk by yeast cells. 1 g of yeast cells (CTAB treated or untreated) were added to 50 mL of pasteurized skim milk and incubated at 37°C with gentle shaking in a Dubnoff metabolic shaking incubator. Samples were removed at various time intervals, the reaction was terminated by placing the sample in a boiling waterbath for 10 mins. The glucose produced by the reaction was determined by glucose oxidase/peroxidase coupled assay system (Dahlqvist, 1968). Lactose hydrolysis was also followed by HPLC analysis of lactose in a Waters Associate HPLC system (column μ Bondapak CN packing, 3.9 mm x 30 cm, acetonitrile/water 85:15, flow rate 2 mL/min) equipped with a RI detector. Alcohol produced was measured enzymatically using yeast alcohol dehydrogenase as described earlier (Bernt and Gutmann, 1974).

## RESULTS AND DISCUSSION

Intact whole cells of the yeast, K. fragilis has very low β -galactosidase activity as compared to the isolated enzyme. As shown in Table 1 the enzyme activity of whole cells is less than one unit/g whereas the cell free extract prepared by toluene autolysis shows an activity of 175 units/g. The low enzyme activity exhibited by whole cells is probably due to the poor permeability of the substrate across the cell membrane. Therefore attempts were made to permeabilize the cells to lactose. When cells were treated with 0.1% CTAB at 4°C for 5 mins,

Table 1. β-Galactosidase activity of K. fragilis

| Cell treatment | Enzyme activity units/g cells |
|---|---|
| None | 0.46 |
| CTAB[a] | 220.90 |
| Triton-X-100[b] | 50.70 |
| Cell free extract[c] (Toluene autolysed) | 175.2 |

(a) 1 g cells treated with 10 mL 0.1% CTAB in 0.1M potassium phosphate buffer pH 7.0 at 4°C for 5 mins.
(b) 1 g cells treated with 10 mL 1% Triton X-100 in 0.1M potassium phosphate buffer pH 7.0 at 40°C for 1 hr.
(c) 10 g cells suspended in 50 mL 0.1M potassium phosphate buffer pH 7.0 containing 0.1 mM $MnCl_2$, 0.5 mM $MgSO_4$ and 2% toluene and stirred at 30°C for 21 hrs.

the cellular β-galactosidase activity measured was 480 fold greater than that of the control cells. In fact the β-galactosidase activity of CTAB treated cells is 20-30% more than that of the cell free extract. Recently Vlach and Prenosil (1984) reported that K. lactis another strain of yeast was permeabilized to lactose by treating the cells with 1% Triton X-100 for 1 h at 40°C. However the extent of increase in enzyme activity compared to the isolated enzyme was not reported. Therefore, K. fragilis cells were permeabilized with Triton X-100 under the conditions reported (Vlach and Prenosil, 1984). Results show that Triton X-100 is only 20% as effective when compared to CTAB (Table 1).

Results of optimization of conditions are shown in Fig.1. Permeabilization is dependent on the concentration of detergent used and reaches a maximum at 0.1 - 0.2% CTAB (Fig. 1a). At higher concentrations of the detergent, cellular β-galactosidase activity decreased with concomitant increase in enzyme activity in the supernatant detergent solution (results not shown), indicating that CTAB at higher concentrations caused either leaking of enzyme from cells or cell lysis. Maximum enzyme activity was observed when the cells were treated with CTAB at 4°C (Fig. 1b). At higher temperatures cellular enzyme activity slightly decreased and

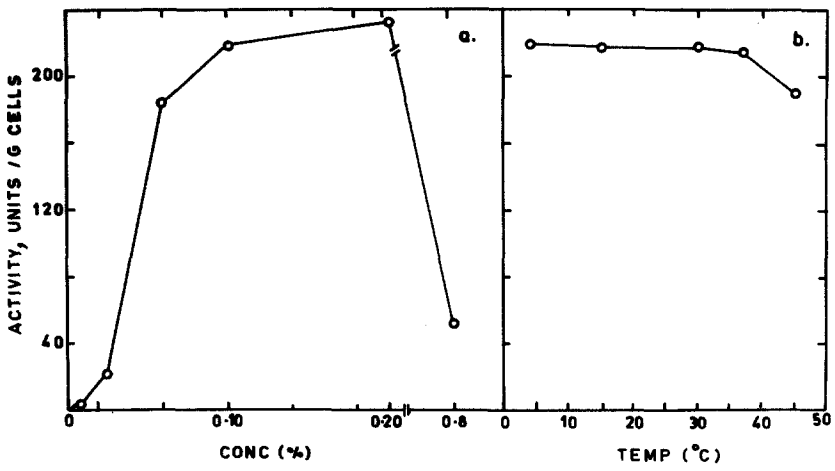


Fig. 1. Optimization of conditions for CTAB treatment of yeast cells.

a) Effect of varying concentrations of CTAB at 4°C for 5 mins.
b) Effect of temperature on CTAB (0.1%) treatment for 5 mins.

the enzyme activity appeared in the supernatant. Five minutes exposure of cells to detergent yielded the maximum cellular enzyme activity. Prolonged treatment even upto 2 hrs at 4°C did not appreciably decrease enzyme activity. Thus maximum β-galactosidase activity was observed in the cells treated with 0.1% CTAB in 0.1M potassium phosphate buffer pH 7.0 for 5 mins at 4°C.

Ability of CTAB treated cells to hydrolyse lactose present in milk was studied and compared to that of untreated cells. Cells suspended in 0.1M potassium phosphate buffer pH 7.0 were added to pasteurized skim milk and lactose hydrolysis was followed by estimating glucose formed or lactose hydrolysed as described under Materials and Methods. About 90% lactose was hydrolysed in 1 hr milk treated with CTAB permeabilized cells. The extent of lactose hydrolysed calculated based on either the glucose formed or lactose disappeared correlated well indicating that the glucose produced was not further metabolized by the permeabilized cells. No detectable amount of alcohol was formed in the milk

sample. In addition neither was there any curdling of the milk nor were there any off flavours generated during the hydrolysis process.

In contrast milk treated with non-permeabilized (Control) cells, lactose hydrolysis calculated by the glucose estimation method was much lower than that by lactose disappearance (Fig. 2). Significant quantity of alcohol was present in the milk sample (Fig. 2, inset), thus suggesting that part of the glucose formed by the normal cells was further

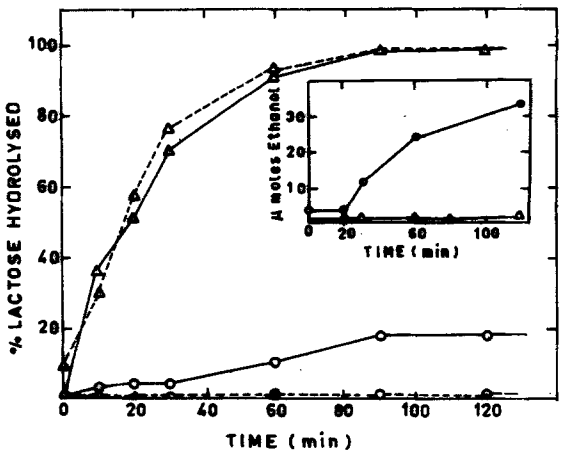


Fig. 2. Lactose hydrolysis in skim milk by yeast cells.
△ CTAB treated cells, O control cells, Lactose hydrolysis followed by HPLC analysis ————, glucose estimation ·····.
Inset: Alcohol produced during lactose hydrolysis in skim milk.

converted to other metabolites including alcohol. Even Triton X-100 permeabilized yeast cells were reported to produce alcohol (Vlach and Prenosil, 1984). CTAB permeabilized cells also readily hydrolysed lactose present in sweet whey (results not presented).

K. fragilis cells are known to possess the lactose carrier protein (lactose permease) on their membrane that mediates the transport of lactose across the cell membrane (Dickson and Barr, 1983). Yet availability of substrate seems to be the limiting factor in expressing the full enzymatic activity of whole cells. Here in we have demonstrated that CTAB (cationic detergent) treatment renders the cells freely permeable to lactose and the full biocatalytic potential of the cell is available for catalysis. Thus CTAB permeabilized cells could be readily used

to hydrolyse lactose instead of isolated pure enzyme. These permeabilized whole yeast cells may have wide application in the food industry for producing low lactose milk, in the utilization of whey and saccharification of lactose or whey for the production of alcohol.

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