

INFLUENCE OF BILE ON β -GALACTOSIDASE ACTIVITY OF *LACTOBACILLUS BULGARICUS*

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Story in Brief

In the presence of 0.15% oxgall, the β -galactosidase activity of whole cells of *Lactobacillus bulgaricus* was increased. However, higher concentrations of oxgall decreased the enzyme activity. In the absence of oxgall, little or no β -galactosidase was exhibited by the whole cells. When the cells were lysed to obtain cell-free extracts prior to assay, there was some decrease in activity of the enzyme in the presence of 0.15% oxgall. Increasing concentrations of oxgall did not further reduce the activity. Apparently, the oxgall increases the permeability of the cells upto a certain concentration (0.15%) to allow more substrate to enter, resulting in more hydrolysis of the substrates. However, at concentrations higher than that, the enzyme activity was inhibited by the oxgall.

(Key Words: *Lactobacillus bulgaricus*, β -galactosidase, Bile.)

Introduction

There have been reports that the cells of *Lactobacillus acidophilus* showed an increased β -galactosidase activity in the presence of bile (Fisher, et al., 1985 and Kim, 1981). This is an important factor enabling this organism to improve lactose utilization in persons classified as lactose malabsorbers (Gilliland, 1979).

Lactobacillus bulgaricus, an organism used to manufacture yogurt, also contains this intracellular enzyme. There are, however, no reports concerning the effect of bile on its enzyme activity. The present experiments measured the β -galactosidase activities of *L. bulgaricus* under different

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Table 1. Effect of oxgall on β -galactosidase activities of *Lactobacillus bulgaricus*.

Oxgall Content	Enzyme Activity ^a	
	Lu 42	Alta Dena
0%	0.32 ^b	6.05 ^d
0.15%	68.46 ^c	81.04 ^e
0.3%	0.73 ^b	3.75 ^d
0.45%	1.45 ^b	4.31 ^d
0.6%	1.25 ^b	4.59 ^d

^a μ g ONP produced/10 min. incubation at 37⁰C/ml of cell suspension. Each value is an average of 3 trials.

^{b,c,d,e} Those in the same column with different superscripts differ significantly ($P < .05$).

concentrations of bile to ascertain whether or not bile had an effect similar to that reported for *L. acidophilus*.

Materials and Methods

Bacterial Strains and Growth Conditions

Two strains of *L. bulgaricus* (Lu42 and Alta Dena), isolated from commercial yogurt, were used to check the effect of bile. Oxgall was used as the bile source. The cultures were grown in Peptonized Milk Nutrient (PMN) broth at 37⁰C for 18 hr. The cells were subcultured at least 3 times prior to the experiment. The cultures were stored at 5⁰C between uses.

Preparation of cells for β -galactosidase Activity

Cells from cultures which had been grown in 10 ml PMN broth for 18 hrs at 37⁰C were harvested by centrifugation, washed with cold 0.05 M

sodium phosphate buffer (pH 7.0) and resuspended with 10ml of same buffer. The washed cell suspension was held in an ice water bath until assay (not more than 30 min.).

Measurement of β -galactosidase Activity in presence and absence of oxgall

Washed cell suspensions were added to four different concentrations of oxgall (0.15, 0.3, 0.45 and 0.6% as a final concentration) to measure the influence of bile on enzyme activity (oxgall is a dried bile). O-nitrophenyl- β -D-galactopyranoside (ONPG) was used as a substrate for enzyme activity according to Noh (1991).

In order to obtain cell-free extracts, lysozyme was used to lyse the cells. (Noh, 1991). Enzyme activity was expressed as μ g of O-nitrophenol (ONP) produced during 10 min. incubation at 37⁰C per 1ml of cell suspension.

Results and Discussion

Table 1 shows the influence of different concentrations of oxgall on enzyme activity. At 0.15% oxgall, both strains showed the highest enzyme activities. However, at higher concentrations of oxgall, the activities decreased greatly. In case of *L. acidophilus*, the enzyme activities were increased significantly in the presence of 0.3% oxgall (Noh, 1991). The difference between the influence of oxgall on these two bacterial species likely was due to differences in bile tolerance. *L. acidophilus* is considered bile resistant whereas *L. bulgaricus* is not.

Cells were lysed to check the effect of oxgall on enzyme activities of cell-free extracts. The results are shown in Table 2. Enzyme activities in the cell-free extracts of both strains showed a significant decrease ($P < .05$) in the presence of oxgall. However, the enzyme activities did not show significant differences ($P > .05$) among 0.15 to 0.6% concentrations of oxgall.

From the results mentioned above, it can be postulated that the presence of oxgall changes the cellular permeability to allow more substrate to enter the cells, resulting in increased β -galactosidase activities. However, at higher concentration, oxgall may inhibit the enzyme activities.

Table 2. Effect of oxgall on β -galactosidase activities of cell-free extracts of *Lactobacillus bulgaricus*.

Oxgall Content	Enzyme Activity ^a	
	Lu 42	Alta Dena
0%	30.26 ^b	32.20 ^d
0.15%	22.58 ^c	26.58 ^e
0.3%	23.52 ^c	25.97 ^e
0.45%	22.80 ^c	26.50 ^e
0.6%	21.68 ^c	25.26 ^e

^a μ g ONP produced/10 min. incubation at 37⁰C/ml of cell suspension. Each value is an average of 3 trials.

^{b,c,d,e} Those in the same column with different superscripts differ significantly ($P < .05$).

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