

β -D-Galactoside Galactohydrolase of *Streptococcus thermophilus*: Induction, Purification, and Properties

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β -Galactosidase (β -D-galactoside galactohydrolase, EC 3.2.1.23) in *Streptococcus thermophilus* was induced by lactose and galactose but not by isopropyl- β -D-galactoside or methyl- β -D-thiogalactoside. Addition of glucose to induced cultures repressed further enzyme synthesis. The enzyme was purified 26-fold from cell-free extracts by DEAE-cellulose chromatography and Sephadex G-100 column chromatography. The molecular weight was estimated to be 105,000, and the optimum temperature and pH for activity were determined to be 55°C and pH 8.0, respectively. Magnesium ions, 2-mercaptoethanol, and dithiothreitol stimulated β -galactosidase activity. Ethylenediaminetetraacetic acid, *p*-hydroxymercuribenzoate, Ca²⁺, and Mn²⁺ were inhibitory. The K_m for *o*-nitrophenyl- β -D-galactopyranoside was 0.69 mM. *S. thermophilus* β -galactosidase was stable in aqueous solution in the presence of 10% (v/v) glycerol, retaining full activity at 4°C for at least 3-months.

INTRODUCTION

In mesophilic lactic streptococci (*Streptococcus lactis*, *S. cremoris*, and *S. diacetylactis*), the transmembrane transport of lactose involves modification of the sugar by a phosphoenolpyruvate (PEP)¹-dependent phosphotransferase system (PTS) and subsequent cleavage of lactose phosphate by β -D-phosphogalactoside galactohydrolase (β -Pgalactosidase) to glucose and galactose-6-phosphate (1-3). The components of the lactose-metabolizing system in *S. cremoris* are similar to those of carbohydrate transport systems found in other groups of procaryotic microorganisms (4, 5).

Of the known enzymes involved in lactose transport in lactic streptococci, the β -Pgalactosidase has been purified and characterized (6). The enzyme is present in all strains of lactic streptococci with the exception of *S. lactis* 7962 which contains β -galactosidase (7). The latter enzyme has also been purified and characterized (8, 9).

Information is limited on carbohydrate transport and metabolism in *S. thermophilus*, a related thermotolerant species that is important in the preparation

¹ Abbreviations used: PEP, phosphoenolpyruvate; PTS, phosphotransferase system; β -Pgalactosidase, β -D-phosphogalactoside galactohydrolase; ONPG, *o*-nitrophenyl- β -D-galactopyranoside; ONPGP, *o*-nitrophenyl- β -D-galactopyranoside-6-phosphate; SDS, sodium dodecyl sulfate; IPTG, isopropylthio- β -D-galactopyranoside; TMG, methyl- β -D-thiogalactopyranoside.

of fermented dairy foods and various cheeses. It was suggested by Reddy *et al.* (10) that lactose transport in *S. thermophilus* is catalyzed by PEP-PTS which implied the presence of β -Pgalactosidase in this group of streptococci. However, strains of *S. thermophilus* used by others apparently possessed an inducible β -galactosidase (2, 11, 12).

In this study, thirty one strains of *S. thermophilus* were surveyed for the presence of different types of lactose-metabolizing enzymes. Details of the purification, general properties, and the regulation of *S. thermophilus* β -galactosidase are reported in this paper.

MATERIALS AND METHODS

Cultures and growth conditions. *S. thermophilus* strains used in this study were obtained from private collections and commercial sources (13). The identity of each culture was verified by checking growth at 45°C in the presence and absence of 2% (w/v) NaCl, by maltose and mannitol fermentation tests, the arginine decarboxylase test (14), and a test for the presence of Lancefield's group N streptococcus antigen against Bacto Streptococcus antiserum (Difco), using a capillary tube method (15). On the basis of these tests, only two atypical strains were found (strains ST/AH and IS).

Cultures were maintained in 10% (w/v) reconstituted nonfat dry milk and also in Hogg-Jago broth (16), with 10 mM lactose, glucose, or galactose as the source of carbohydrate. The pH of each medium was 6.5 before sterilization by autoclaving.

Enzyme induction. Cultures of *S. thermophilus* were grown in both lactose and glucose media for 16 h at 37°C. Cells from 100 ml of each culture were pelleted by centrifugation and resuspended in 10 ml 50 mM phosphate buffer (pH 7.0). One milliliter of each cell suspension was transferred to 10 ml of medium containing lactose or glucose or a combination of the two sugars, as indicated later. During incubation at 37°C, samples were taken over a period of several hours and enzyme activity was determined in decryptified cells.

Preparation of decryptified cells. Each strain was grown in lactose, glucose, or galactose broth at 37°C to about 0.8 to 0.9 mg dry wt/ml. Cells were pelleted at 10,000g and 4°C in a Sorvall RC2-B centrifuge, resuspended in 50 mM potassium phosphate (pH 7.0) containing 25 μ g/ml chloramphenicol, or in 50 mM Tris/HCl buffer (pH 8.0), and recentrifuged as before. The membranes of washed cells were perturbed by addition of 50 μ l acetone-toluene (9:1, v/v) per milliliter of cell suspension and vortexing the mixture vigorously at 4-min intervals over a period of 20 min. Decryptified cell suspensions were kept at 4°C and used on the day of preparation.

Assay of β -galactosidase and β -Pgalactosidase. The chromogenic substrates *o*-nitrophenyl- β -D-galactopyranoside (ONPG) and *o*-nitrophenyl- β -D-galactopyranoside-6-phosphate (ONPGP) were used to measure β -galactosidase and β -Pgalactosidase activities, respectively. Reaction mixtures (2 ml total volume) containing decryptified cell suspension or cell-free enzyme extract (20 to 100 μ l) and 1 ml of 30 mM substrate in phosphate or Tris-HCl buffer (pH 8.0) were incubated at 37°C for 10 min. The reaction was terminated by adding 4 ml of ice-cold, 0.5 M sodium carbonate. When decryptified cells were used as enzyme source, cells were pelleted at 6000g for 10 min. The absorbance of the supernatant

solution was read at 420 nm and the amount of *o*-nitrophenol released from ONPG and ONPGP was determined from a standard curve. One unit of absorbance at 420 nm was equivalent to 213.2 nmol of *o*-nitrophenol. One unit (U) of β -galactosidase or β -Pgalactosidase activity was defined as the amount of enzyme catalyzing the release of 1 nmol of *o*-nitrophenol per minute under standard assay conditions. Specific activities were expressed as units of enzyme milligram of protein. Protein determinations were based on the Lowry method (17) with bovine serum albumin as the standard.

Enzyme purification. Unless otherwise indicated, all steps were carried out at 4 to 6°C. After a 16-h incubation period, lactose-grown cells of *S. thermophilus* 19258 from a 1-liter culture were harvested, washed, and resuspended in 15 ml 20 mM Tris-HCl buffer (pH 7.5) containing 10% (v/v) glycerol. The cell suspension was subjected to five 60-s bursts at maximum output in a Branson Model W185 sonifier. Cellular debris was removed by centrifugation at 45,000g for 20 min. The supernatant solution was designated as the crude cell-free extract (fraction I, Table I). The crude enzyme solution was applied to a column (1 × 16 cm) of DEAE-cellulose (Whatman DE-52), equilibrated with 20 mM Tris-HCl-glycerol buffer (pH 7.5). After washing with 150 ml of buffer, the column was eluted with a linear gradient of NaCl, ranging in concentration from 0 to 300 mM, in a total volume of 200 ml. Fractions of volume 3.5 ml were collected and 10 μ l of each was assayed for β -galactosidase activity. Protein was continuously monitored at 280 nm using an ISCO UV column monitor (Model UA-5), flow cell, and chart recorder. β -Galactosidase activity eluted at a NaCl concentration of 0.2 to 0.275 M. Active fractions were pooled (fraction II, Table I), and concentrated by ultrafiltration under nitrogen pressure (24 kg/cm²) in an Amicon Model 52 ultrafiltration cell using a UM-10 membrane. The concentrated enzyme solution was applied to a column (3 × 60 cm) of Sephadex G-100 (Pharmacia), equilibrated with 0.1 M Tris-HCl-glycerol buffer (pH 7.5) and eluted with the same buffer. Active fractions (5 ml/tube) were combined and concentrated to 4 ml by ultrafiltration as described above (fraction III, Table I).

Polyacrylamide gel electrophoresis. Analytical separations were performed in tubes (0.75 × 11.5 cm) with the discontinuous gel system (pH 8.3) of Davis (18). A 4-mA current was applied to each tube. After electrophoresis for approximately 3 h, gels were stained with 1% (w/v) Coomassie brilliant blue R250 (Eastman Kodak) in 7% (v/v) acetic acid for 30 min, and destained according to the procedure of Weber and Osborn (19).

Molecular weight determination. Samples of purified β -galactosidase containing 20 and 50 μ g of protein were dissociated in 1% (w/v) sodium dodecyl sulfate-1% (v/v) 2-mercaptoethanol by boiling for 2 min and subjected to electrophoresis. The sodium dodecyl sulfate-1% (v/v) 2-mercaptoethanol by boiling for 2 min and subjected to electrophoresis. The sodium dodecyl sulfate (SDS)-polyacrylamide gel method of Weber and Osborn was used (19). A 6-mA current per tube was applied for 5 h. Gels were stained with 1% Coomassie brilliant blue R250 and destained by diffusion.

Properties of β -galactosidase. The optimum temperature for enzyme activity was determined by activity determinations between 10 and 75°C, in phosphate buffer (pH 7.0). Optimum pH was determined by measuring enzyme activity in different buffers: Na₂HPO₄-citric acid (pH 2.5 to 7.5) and Tris-HCl (pH 7.5 to 10.0).

The effect of metal ions was measured in 50 mM Tris-HCl buffer (pH 8.0), by adding each metal salt (as the chloride) at 5 mM final concentration to the reaction mixture. The effects of other chemicals were tested at the following concentrations: EDTA, 1 and 5 mM; mercaptoethanol and dithiothreitol, 5 mM; glucose, galactose, and galactose 6-phosphate, 10 mM; D-galactal, isopropylthio- β -D-galactopyranoside (IPTG), and methyl- β -D-thiogalactopyranoside (TMG), 5 mM.

Chemicals. Carbohydrates, ONPG, ONPGP, and bovine serum albumin were products of Sigma Chemical Company, St. Louis, Missouri, IPTG, TMG, EDTA, Tris, and adenosine cyclic-3',5'-monophosphate (cAMP) were products of Calbiochem, San Diego, California. *N*-Ethylmaleimide, dithiothreitol, iodoacetamide, 2-mercaptoethanol, and *p*-hydroxymercuribenzoate were purchased from Eastman Kodak, Rochester, New York. D-Galactal was purchased from Koch-Light Laboratories, Colnbrook, Bucks, England. All reagents were used without further purification.

RESULTS

Distribution of β -Galactosidase and β -Pgalactosidase

Among the strains of *S. thermophilus* studied, 29 had β -galactosidase, one strain had β -Pgalactosidase, and one strain possessed both types of enzyme. The possible interference in the β -Pgalactosidase assay by cellular phosphatase activity (20, 21), resulting in the conversion of ONPGP into ONPG and P_i , was ruled out since the addition of 10 mM NaF to reaction mixtures failed to induce changes in *o*-nitrophenol release. The specific activity of β -galactosidase varied between strains and was up to 120-fold higher in cells grown in either lactose or galactose medium (1230–11,300 U/mg protein) than that found in glucose-grown cells (10–5400 U/mg protein) of the same strains.

Subcellular Location of β -Galactosidase

The distribution of β -galactosidase between the cell envelope and the cytoplasm was determined in four strains of *S. thermophilus* (4074, 6096, 14485, and 19987). After differential centrifugation of sonically disrupted cells, essentially all (90% or more) the β -galactosidase activity was found in the soluble fraction. In this respect, *S. thermophilus* is similar to other streptococci in which lactose-hydrolyzing enzymes are located in the cytoplasm (6, 22, 23).

Induction of β -Galactosidase

Induction studies were carried out with *S. thermophilus* strains 4074, 7024, 9353, and 19987. When cells were first grown in glucose medium and then transferred to lactose medium, the specific activity of β -galactosidase in the culture increased fivefold or more over a period of 6 h. Supplementation of the lactose medium with 25 mM glucose suppressed the increase in specific activity by 50 to 100% during the same period of incubation. The results of a β -galactosidase induction test with *S. thermophilus* 19987 are shown in Fig. 1, which is also representative of the data obtained with other strains of *S. thermophilus*.

To determine whether β -galactosidase could be induced by substances other than lactose, galactose and the possible gratuitous inducers IPTG and TMG were tried.

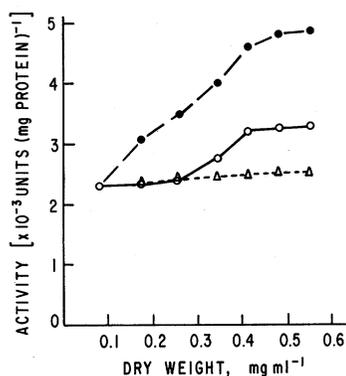


FIG. 1. Synthesis of β -galactosidase and cell mass in *S. thermophilus* 19987. Hogg-Jago broth and 10 mM glucose (Δ), 10 mM lactose (\bullet), and 10 mM lactose + 25 mM glucose (\circ) was inoculated with 16-h cells to an initial concentration of 70 μ g/ml.

IPTG and TMG failed to induce β -galactosidase. Galactose, however, was an excellent enzyme inducer and in some strains it was slightly more effective than lactose.

The effect of exogenous cAMP on the glucose-induced transient repression of β -galactosidase synthesis was tested in an early exponential phase population of *S. thermophilus* 4 growing in 10 mM lactose medium, containing 5 μ l toluene/ml as a cell permeabilizing agent. The specific growth rate of toluenized cell populations was much lower ($k = 0.29/h$) than that of the control population ($k = 0.81/h$) but still sufficient to demonstrate enzyme induction or repression. The addition of 25 mM glucose resulted in a decrease of β -galactosidase activity from 5300 to 1600 U/mg protein over a period of 3 h. When 2.5 mM cAMP was also added simultaneously or within 25 min, it nearly completely reversed the repressive effect of glucose, as evidenced by the persistence of high levels (4600 U/mg protein) of β -galactosidase in the culture. Thus, cAMP apparently overcame the transient repression of β -galactosidase synthesis. Similar results were found in experiments with *S. thermophilus* 19987.

Purification of β -Galactosidase

Preliminary tests showed that the β -galactosidase of *S. thermophilus* was unstable and lost nearly all of its activity (80% or more) during storage at 4°C for 96 h. However, addition of 10 to 20% (v/v) glycerol stabilized the enzyme so that it could be stored at 4°C for 3 months without any measurable loss of activity. Therefore, 10% glycerol was routinely incorporated in all buffer systems employed in the purification scheme. It was shown that at 10% concentration glycerol did not have a measurable effect on the activity of freshly prepared enzyme.

A summary of results from the purification procedure is given in Table I. A 26-fold purification was achieved with a yield of 85% of the original activity with a relatively simple procedure using DEAE-cellulose and Sephadex G-100 column chromatography (Fig. 2). The purified preparation appeared to be homogenous on discontinuous gel electrophoresis (Fig. 3).

TABLE I
Purification of β -galactosidase from *S. thermophilus* 19258^a

Step	Total protein (mg)	Total activity ($U \times 10^3$)	Specific activity (U/mg protein)	Purification (n-fold)	Yield (%)
I Crude cell-free extract	160	192	1,200	1	100
II DEAE-cellulose chromatography	28	171	6,107	5	89
III Sephadex G-100 chromatography	5.2	165	31,500	26	86

^a Details of the purification and assay procedures are presented under Materials and Methods.

Molecular Weight Determination

The molecular weight of *S. thermophilus* β -galactosidase was examined by SDS-polyacrylamide gel electrophoresis. Only one band of protein was observed with this procedure, indicating that the enzyme contains only one type of subunit. The molecular weight of β -galactosidase was calculated to be 105,000 using as molecular weight standards rabbit muscle myosin (210,000), *Escherichia coli* β -galactosidase (116,000), phosphorylase *a* (94,000), and bovine serum albumin (68,000).

Enzyme Properties

Decryptified cells of 16-h cultures of *S. thermophilus* strains 7, 7024, and 9353, and purified β -galactosidase from *S. thermophilus* 19258 were used to determine optimum pH and temperature requirements of the enzyme. The effect of pH is shown in Fig. 4. The enzyme was most active at pH 8.0 with a sharp decrease in activity above and below pH 8.0.

The effect of incubation temperature on β -galactosidase is shown in Fig. 5. The specific activity of the enzyme increased up to 55°C and decreased sharply above this temperature. The exception was the β -galactosidase of strain 9353, which was most active at 50°C.

The effect of substrate concentration on the velocity of ONPG hydrolysis was also determined. The data from low substrate concentrations were plotted by the method of Lineweaver and Burk (24) and a K_m value of 0.69 mM was determined.

The effect of cations and EDTA on the β -galactosidase of *S. thermophilus* strain 7 was determined in Tris-HCl buffer systems. The reaction mixtures were preincubated for 10 min at 37°C before the addition of substrate. The β -galactosidase activity in the absence of added chemical, taken as 100%, was 4500 U/mg protein. Enzyme activity was inhibited in the presence of metal ions in the following order of magnitude: zinc—97%, cobalt—90%, calcium—52%, and manganese—10%. On the other hand, β -galactosidase activity in the presence of 5 mM magnesium was 53% higher than the control. Monovalent cations (K^+ and Na^+) had no effect on the enzyme activity. Addition of EDTA (1 or 5 mM) to the assay mixture resulted in total loss of enzyme activity, 90% or more of which could be restored by adding excess magnesium. Dialysis of the pure enzyme from strain 19258 against 10 mM EDTA for 24 h at 4°C caused 90% loss of activity. After

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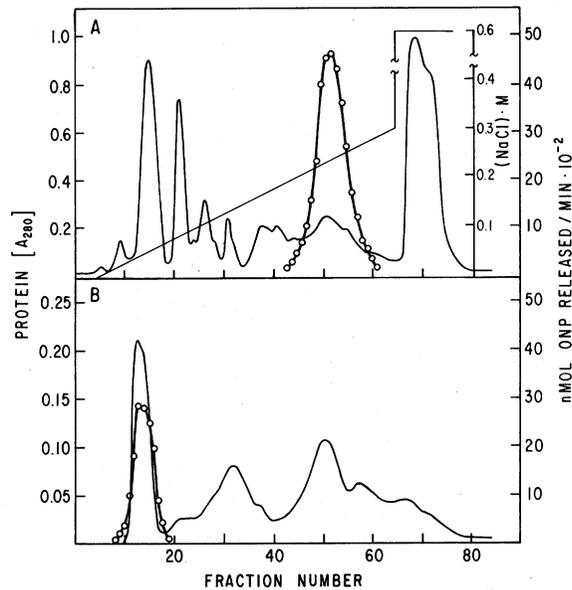


FIG. 2. Purification of β -galactosidase from *S. thermophilus* 19258 by column chromatography. Effluent fractions were assayed for β -galactosidase activity as described in the text. Symbols: β -galactosidase (\circ); absorbance at 280 nm (—). (A) DEAE-cellulose chromatography of fraction I (Table I). (B) Sephadex G-100 chromatography of fraction I (Table I). (B) Sephadex G-100 chromatography of fraction II (Table I).

removal of EDTA by dialysis against 50 mM Tris-HCl buffer (pH 8.0), enzyme activity was fully restored by the addition of 10 mM $MgCl_2$.

The effect of thiol group reagents was tested in the β -galactosidase of *S. thermophilus* strain 8. The enzyme was preincubated with each test compound for 15 min at room temperature before initiating the reaction with 15 mM ONPG. Enzyme activity in the absence of reagent was 5800 U/mg protein. Inhibition of the enzyme was most severe in the presence of iodoacetamide (95% loss), followed by *N*-ethylmaleimide (30% loss), and *p*-hydroxymercuribenzoate (23% loss). Dithiothreitol and mercaptoethanol stimulated β -galactosidase activity, causing 29 and 78% increase over the control value, respectively.

Data on the effects of carbohydrates and carbohydrate derivatives on β -galactosidase activity are summarized in Table II. Kinetic studies with lactose- or galactose-induced and decryptified cells as the enzyme source showed that galactose, IPTG, and TMG were competitive inhibitors of *S. thermophilus* β -galactosidase. The inhibition constants (K_i), determined from Dixon plots (25) of inhibitor saturation data, were 31, 3, and 12 mM for galactose, IPTG, and TMG, respectively, with ONPG as the substrate.

DISCUSSION

Our results established that β -galactosidase is the predominant lactose-hydrolyzing enzyme in nearly all strains of *S. thermophilus* used in this study. However, in the atypical strain IS, β -Pgalactosidase was found, and strain ST/AH apparently possessed both types of enzyme. Interestingly, the opposite situation is

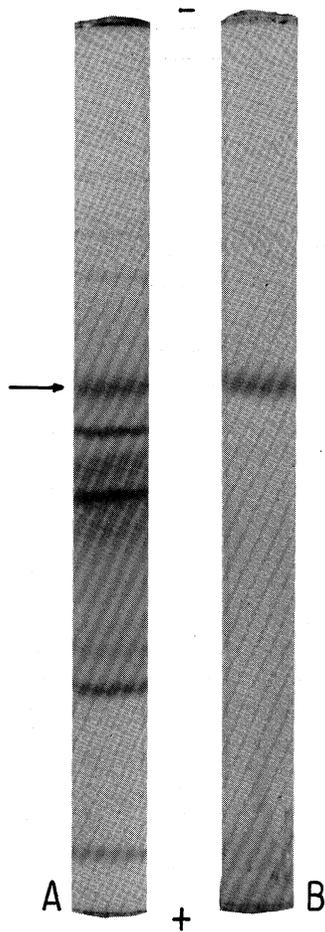


FIG. 3. Protein patterns on discontinuous gel electrophoresis of (A) crude enzyme, (B) active peak from Sephadex G-100.

known to exist among mesophilic lactic streptococci. In this group, β -Pgalactosidase may be regarded as ubiquitous since only the atypical strain 7962 of *S. lactis* possesses β -galactosidase (7).

Both lactose and glucose are apparently involved in regulating the level of β -galactosidase in *S. thermophilus*. This situation was evidenced by the findings that galactose and lactose induced the synthesis of this enzyme, whereas glucose inhibited it. In some strains galactose was the more efficient inducer. This phenomenon is similar to that previously reported in other bacteria, for example, *Lactobacillus plantarum* (26), *Bacillus megaterium* (27), and *S. lactis* (3). It was proposed by Yeung *et al.* (27) that glucose produced from the hydrolysis of lactose directly repressed β -galactosidase synthesis, a situation which may account for the reduced enzyme-inducing efficiency of lactose. We also found that IPTG and TMG were ineffective as gratuitous inducers of β -galactosidase in *S. thermophilus*.

Induction experiments with *S. thermophilus* strains 4 and 19987 indicated the possible involvement of cAMP in the regulation of β -galactosidase synthesis. The

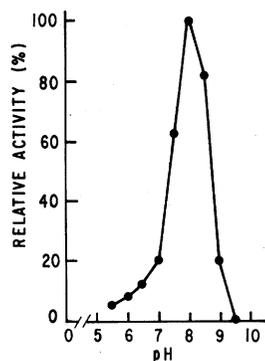


FIG. 4. Effect of pH on β -galactosidase activity. Purified enzyme from *S. thermophilus* 19258 was incubated with 2.5 mM ONPG at 37°C for 10 min in different buffers (see Materials and Methods).

versatility of cAMP in regulating the synthesis of inducible catabolic enzymes in Gram-negative bacteria is well known (28). In the case of Gram-positive bacteria, however, a similar role is still controversial. Ullmann reported in 1974 (29), that cAMP alleviated the glucose repression of β -galactosidase induction in *B. megaterium*, whereas Yeung *et al.* (27) failed to detect this phenomenon in the same species. Hasan and Durr (26) found that cAMP did not reverse the glucose effect in *L. plantarum*. On the other hand, the ability of cAMP to overcome glucose-induced transient repression of β -galactosidase synthesis was demonstrated recently in *S. salivarius* (20).

We have achieved a 26-fold purification of the β -galactosidase of *S. thermophilus*. Repeated gel filtration on Sephadex G-100 resulted in no further purification and the specific activity remained constant in fractions across the peak of activity. The final enzyme preparation was apparently homogenous as judged by discontinuous polyacrylamide gel electrophoresis. Numerically similar extents of purification have been reported in the isolation of homogenous preparations of enzymes involved in lactose hydrolysis in *S. cremoris* (6), *L. casei* (23), and *Staphylococcus aureus* (30). *S. thermophilus* β -galactosidase is extremely stable in

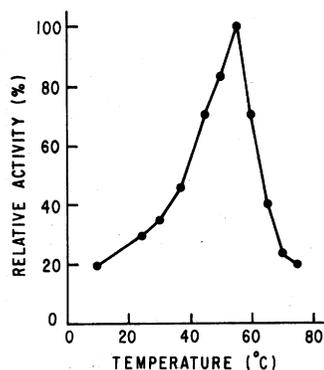


FIG. 5. Effect of temperature on β -galactosidase activity in *S. thermophilus* 9353. Decryptified cells were incubated in phosphate buffer (pH 8.0) with 2.5 mM ONPG at each temperature for 10 min.

TABLE II
Effect of Inhibitors on *S. thermophilus* β -Galactosidase^a

Strain	Inhibitor	Relative β -galactosidase activity (%)
7024	Galactose, 10 mM	47
404	Galactose-6-P, 10 mM	80
8	Glucose, 10 mM	100
8	D-Galactal, 5 mM	22
8	IPTG, 5 mM	26
8	TMG, 5 mM	35

^a The enzyme was preincubated with each inhibitor at 37°C for 15 min. Enzyme activity was measured by the standard assay procedure with ONPG (15 mM). β -Galactosidase activity is expressed as a percentage of the control activity measured in the absence of inhibitor.

4°C and below in the presence of 10 to 20% (v/v) glycerol for at least 3 months. A molecular weight of 105,000 \pm 5% was calculated for the enzyme by SDS-polyacrylamide gel electrophoresis. Thus the β -galactosidase of *S. thermophilus* is larger than the lactose-hydrolyzing enzymes from *S. mutans*, *Staph. aureus*, and *S. cremoris*, the molecular weights of which are estimated to be 40,000 (22), 50,000 (30), and 67,600 (6), respectively, but it is smaller than the enzyme of *S. lactis* (7) and *L. thermophilus* (23), that have been estimated at 500,000 and 540,000, respectively. The molecular weight of *S. thermophilus* β -galactosidase is quite similar to the value of 130,000 reported for the β -phosphogalactosidase of *L. casei* (23).

The purified β -galactosidase described here had a pH optimum of 8.0, the highest value reported so far for a bacterial β -galactosidase. Approximately pH 5.0 is optimal for the enzyme of *Thermus aquaticus* (31), pH 6.5 is optimal for the β -galactosidases from *L. thermophilus* (23) and *B. subtilis* (32), and a pH close to neutrality is preferred by the β -galactosidase of *L. bifidus* (33), *Pseudomonas* BAL-31 (34), *S. lactis* 7962 (8), and *E. coli* (35).

The β -galactosidase of *S. thermophilus* has an optimum temperature range of 50 to 55°C. In this regard, the enzyme is similar to the β -galactosidases from *B. subtilis* (32), *L. bifidus* (33), and *L. thermophilus* (23).

Of the cations tested, Mg²⁺ was the only one found to stimulate β -galactosidase activity. Another bacterial β -galactosidase showing enhancement of enzyme activity in the presence of magnesium is the β -galactosidase of *E. coli* (36).

The activity of β -galactosidase in all of the *S. thermophilus* strains studied was enhanced by 2-mercaptoethanol and dithiothreitol. Another bacterial β -galactosidase known to be stimulated by reducing agents is that from *T. aquaticus* (31). In *S. lactis* 7962, reducing compounds have a stabilizing effect on β -galactosidase activity (37). The sensitivity of the *S. thermophilus* β -galactosidase to thiol group reagents, especially iodoacetamide, further suggests sulfhydryl group involvement in enzyme catalysis, as in the case of the same enzyme from *S. lactis* 7962 (37), and *S. cremoris* (6), which are inactivated by *p*-hydroxymercuribenzoate. The β -galactosidase of *Pseudomonas* sp. BAL-31 is also sensitive to *N*-ethylmaleimide (34), whereas the β -galactosidase of *E. coli* is insensitive to this

reagent (38). The enzyme of *S. thermophilus* is inhibited by galactose, galactose 6-phosphate, D-galactal, IPTG, and TMG. The β -galactosidase of *E. coli* (39), *L. plantarum* (26), and *Pseudomonas* sp. BAL-31 (34) are also sensitive to IPTG. However, the enzyme from *L. plantarum* is not sensitive to TMG, and the *Pseudomonas* sp. BAL-31 is unaffected by galactose. The galactose analog D-galactal that exists in a planar half-chair conformation believed to be responsible for its inhibitory action on β -galactosidase enzymes from various sources including those from *E. coli* and *Clostridium perfringens* (40), is also a potent inhibitor of the β -galactosidase of *S. thermophilus*.

Our results suggest that *S. thermophilus* has a different mechanism for the dissimilation of lactose than do mesophilic lactic streptococci (1-3), and may use an active transport system followed by substrate hydrolysis with β -galactosidase, as in *S. lactis* 7962 (2, 3). The relatively high optimum temperature and excellent storage stability of the *S. thermophilus* β -galactosidase compare favorably with the corresponding properties of the enzyme of the yeast *Kluyveromyces fragilis* (formerly *Saccharomyces fragilis*), which is currently the most widely used β -galactosidase for food application (41), and impart definite advantages to the former enzyme.

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Mention of specific brand names does not imply endorsement by the authors or the U. S. Department of Agriculture to the exclusion of others not mentioned.

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