Production of Beta-Galactosidase from *Streptococcus thermophilus* Grown in Whey

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β-D-Galactosidase (EC 3.2.1.23) was extracted from *Streptococcus thermophilus* grown in deproteinized cheese whey. Cultural conditions optimum for maximum enzyme production were pH 7.0, 40°C, and 24 h. Proteose peptone (2.0%, wt/vol) and corn steep liquor (2.8%, wt/vol) were highly stimulatory, increasing the enzyme units available in their absence from 660 U/liter of medium to 18,200 and 10,000 U/liter of medium, respectively, in their presence. There was an insignificant increase in the production of enzyme in the presence of added inorganic nitrogen and phosphorus sources. Enzymatic hydrolysis for reduction of lactose content in aqueous solution and in skim milk was studied.

The importance of lactose in imparting characteristic flavor and texture to milk and milk products, as well as its contribution towards their nutritive value, is well recognized. On the other hand, the problems associated with whey disposal, lactose crystallization in frozen concentrated desserts, and milk consumption by lactose-intolerant populations of the world have drawn the attention of several research workers. This has led to the survey of many microorganisms (1, 7, 11) with a view to selecting microorganisms with high potentials for producing β-galactosidase, the enzyme that hydrolyzes lactose into its component monosaccharide units. Enzyme preparations from selected isolates (1) as well as from *Saccharomyces fragilis*, which is the most widely used source of enzyme for food application (7, 8, 10), suffer from the disadvantages of being heat labile and having low storage stability. We surveyed a large group of microorganisms to select a high-yielding source of stable β-galactosidase. The β-galactosidase from *Streptococcus thermophilus* (strain I) exhibited twice the activity of enzyme preparations from *S. fragilis*, being stable at −4°C for more than a year and having high heat stability. The present report deals with cultural conditions optimum for the production of enzyme from *S. thermophilus* grown in whey.

**MATERIALS AND METHODS**

Organism. *S. thermophilus* (strain I) was obtained from the culture collection of the National Dairy Research Institute and was maintained in sterile litmus milk. It was subcultured once a week and stored in a refrigerator at 4°C.

Preparation of cell-free extracts. Whey was obtained from the Experimental Dairy of the Institute. It was deproteinized by heating at 90°C and pH 4.5 for 10 min, filtered through Whatman no. 1 filter paper to remove the coagulated protein, adjusted to pH 7.0, and sterilized at 121°C for 15 min. The sterile whey was inoculated with a 1% active culture of the organism and incubated at 40°C for 24 h. At the end of the incubation period, the cells were harvested by centrifuging at 3,500 × g for 20 min. They were washed twice with phosphate buffer (0.1 M, pH 7.0) and disintegrated by using a Sonifier B-12 (Branson Sonic Power Co.) at 60 W and 4°C. The cell debris was removed by centrifugation at 20,000 × g for 15 min, and the cell-free extract thus obtained was used for assay of the enzyme.

Assay of enzyme activity. β-D-Galactosidase was assayed at 45°C, with p-nitrophenyl-β-D-galactopyranoside (Sigma) as the substrate, by a modified procedure of Wendorff and Amundson (9). A unit of enzyme activity was defined as the amount of enzyme required to liberate 1 μmol of p-nitrophenol in 10 min.

The determination of β-galactosidase activity was also conducted with lactose as a substrate (5% lactose in 0.1 M, pH 7.0 phosphate buffer and in pasteurized, reconstituted skim milk containing 10% solids) at 45°C (5). Deproteinization of the sample was accomplished by using Somogyi zinc-alkali reagent. The released glucose was estimated with Glucostat reagent, obtained from Worthington Biochemicals Corp., Freehold, N.J.

Samples withdrawn from the culture flask were titrated to the phenolphthalein end point with 0.1 N NaOH, and the percentages of developed acidity were calculated as lactic acid.

**RESULTS**

Whey. Acid whey (HCl and citric acid) from cow, buffalo, goat, and mixed milk (cow and buffalo) and cheese whey from these sources
were used to determine the effect of different types of whey on β-galactosidase production. The activities for all samples were in the range of 630 to 678 U/liter of medium, and the percent acidities ranged from 0.31 to 0.33. Spray-dried cheese whey powder, obtained from the Experimental Dairy of the Institute, was used for all subsequent studies, since all types of whey supported the growth and enzyme production to a similar extent. When the whey was reconstituted to different levels of total solids, the normal level of total solids present in whey (ca. 6 to 7%) supported maximum enzyme production. Different heat treatments of whey (pasteurization, 63°C for 30 min; steaming, 30 min; and sterilization, 121°C for 15 min) did not affect enzyme production.

**pH of whey.** The effect of the initial pH of the medium on enzyme production was studied over a pH range of 4.0 to 9.0. The pH was adjusted with 0.1 N NaOH or HCl. Maximum enzyme production was observed between pH 6.5 and 7.5. Cells lowered the pH from an initial pH of 7.0 to 4.5 after 24 h of growth.

**Period and temperature of incubation.** β-Galactosidase production by *S. thermophilus* was studied up to a period of 60 h. A progressive increase in enzyme units available was observed up to 24 h of incubation, after which it became constant. The optimum temperature for enzyme production coincided with the optimum temperature for the growth of the organism, about 40°C.

**Nitrogen and phosphorus sources.** Various inorganic and organic nitrogen supplements were examined to determine their effect on enzyme production. The stimulation in enzyme production by inorganic nitrogen (Fig. 1) was much lower than that by organic nitrogen supplements (Fig. 2). Proteose peptone (2.0%, wt/vol) stimulated enzyme production almost twice as much as the other organic nitrogen sources and nearly 30 times more than the control. Among the mono-, di-, and tribasic salts of phosphate tested, monobasic salt (0.8%, wt/vol) stimulated enzyme production to the maximum extent (Fig. 3).

**Growth factors.** Various growth factors (beef extract, yeast extract, malt extract, corn steep liquor, and molasses) were tried (Fig. 4). The addition of corn steep liquor (2.8%, wt/vol) and beef extract (1.6%, wt/vol) resulted in maximum enzyme production (10,000 and 9,100 U/liter, respectively).

**Metal salts.** Mono- and divalent salts (MgCl₂, MnCl₂, KCl, NaCl, and CaCl₂) were studied to determine their effect on enzyme production (Table 1). Divalent salts exerted a
S. THERMOPHILUS LACTASE

as well as in skim milk at 45°C and pH 7.0. The lactose in solution was hydrolyzed completely within 120 min, and the lactose in skim milk was hydrolyzed within 90 min (Fig. 5).

**DISCUSSION**

Optimum conditions for β-galactosidase production from *S. fragilis* has been the subject of study by several workers (2-4, 8, 10; H. Young and R. P. Healey, U.S. Patent 2,776,928, 1957). Young and Healey (U.S. Patent 2,776,928, 1957) reported that the optimum conditions for β-galactosidase production in *S. fragilis* need not be the same as those for growth. In the present study, the optimum pH and temperature for maximum enzyme production were nearly the same as for optimum growth. The observations that there were no significant differences in enzyme production in whey from different sources and in whey subjected to different heat treatments are important to those

**TABLE 1. Effect of metal salts on enzyme production**

<table>
<thead>
<tr>
<th>Metal salts</th>
<th>Conc (M)</th>
<th>U/liter</th>
<th>Developed acidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>660</td>
<td>0.32</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.018</td>
<td>1,166</td>
<td>0.55</td>
</tr>
<tr>
<td>MgCl₂·6H₂O</td>
<td>0.009</td>
<td>1,650</td>
<td>0.58</td>
</tr>
<tr>
<td>MnCl₂·4H₂O</td>
<td>0.010</td>
<td>1,760</td>
<td>0.65</td>
</tr>
<tr>
<td>KCl</td>
<td>0.026</td>
<td>825</td>
<td>0.60</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.034</td>
<td>990</td>
<td>0.55</td>
</tr>
</tbody>
</table>

slight stimulatory effect, comparable to stimulation by inorganic nitrogen sources.

**Lactose hydrolysis.** The crude enzyme preparations (1.5 mg of protein per ml) obtained from cells produced under optimal production conditions were used to determine the rate and extent of lactose hydrolysis in lactose solution

![Fig. 3](http://aem.asm.org/)

**Fig. 3. Effect of phosphorus on enzyme production.** Symbols: NaH₂PO₄ (○); Na₂HPO₄ (▽); Na₃PO₄ (▽).

![Fig. 4](http://aem.asm.org/)

**Fig. 4. Effect of growth factors on enzyme production.** Symbols: Beef extract (○); yeast extract (▽); malt extract (●); corn steep liquor (▽); molasses (×).

![Fig. 5](http://aem.asm.org/)

**Fig. 5. Rate of hydrolysis of lactose in skim milk (○) and in solution (▽).**

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interested in commercial development of the process. The poor stimulation by inorganic nitrogen and phosphorus salts in the present study is similar to that of *S. fragilis* (10). The stimulatory effect of peptone, yeast extract, casein digest, etc., has been similarly observed with *S. fragilis* (8, 10). The stimulatory effect of corn steep liquor and especially of proteose peptone is interesting from the economic point of view, because the former is inexpensive and a significant increase in enzyme production was caused by the latter. Very little, but significant, stimulation by metal salts indicates that the levels of these salts in whey were sufficient.

Finally, the enzyme has a potential for use under practical conditions because it can hydrolyze all of the lactose present in milk in a short period at the relatively high temperature of 45°C. The period of complete lactose hydrolysis may be reduced by using high concentrations of enzyme after purification and increasing the incubation temperature. The methods of extraction of the intracellular enzyme and its purification and properties are currently being studied and will be reported later.

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LITERATURE CITED