Note

 β -Galactosidase in Alkalophilic *Bacillus*

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Received December 5, 1978

In the preceding paper,¹⁾ we have reported the isolation and identification of alkalophilic strain of a bacterium which produced β -galactosidase inducibly. This bacterium, *Bacillus* No. C-125 produced the enzyme not only in alkaline media but also in neutral media. Comparative studies on the β -galactosidases produced in alkaline and neutral media were investigated from a view point of the effects of pH value of the media.

Bacillus No. C-125 was cultured in a neutral (containing 0.2 M NaCl instead of Na_2CO_3 , pH 7.6) or an alkaline (containing 0.1 M Na_2CO_3 , pH 10.2) medium containing 1.5% lactose, as described previously.¹⁾ The cells at the end of log phase were collected by centrifugation and washed with 20 mM Na-K phosphate buffer (pH 7.0) containing 10 mM MgCl_2 . To the cell suspension (20 mM phosphate buffer pH 7.0 containing 10 mM MgCl_2 and 12% sucrose) was added lysozyme (200 $\mu\text{g}/\text{ml}$), and the suspension was incubated for 15 min at 37°C. Spheroplasts were collected by centrifugation at 12,000 $\times g$ for 10 min and washed once with the same buffer. 97% of the total β -galactosidase activity was included in the spheroplasts. To the washed spheroplasts suspended in 50 times volume of 10 mM phosphate buffer containing 2 mM mercaptoethanol, Triton X-100 was added at 1%. More than 95% of the enzyme was found to be soluble as judged from the activity distribution. The enzyme solution was concentrated with polyethyleneglycol and dialyzed against *P*-buffer (10 mM Na-K phosphate buffer, pH 7.3 containing 2 mM mercaptoethanol). This crude enzyme solution was treated with streptomycin (0.7 mg/ml) and the supernatant fluid was fractionated with ammonium sulfate (25 to 50% saturation). The precipitate was collected by centrifugation, dissolved in *P*-buffer and dialyzed against the same buffer for 24 hr. The enzyme solution was loaded on to a DEAE-cellulose column (15 $\times$ 300 mm) equilibrated with *P*-buffer. The column was thoroughly washed with *P*-buffer containing 0.2 M NaCl and the enzyme was eluted with *P*-buffer containing 0.3 M NaCl. The active fraction was poured into a cellulose tube and concentrated to about 12 ml by dipping into Ficoll (Pharmacia, Sweden). The con-

FIG. 1. Optimum pH of β -Galactosidase of *Bacillus* No. C-125.

The following buffer systems were used: 0.1 M acetate buffer (pH 4.5~5.5); 0.1 M phosphate buffer (pH 6.0~8.2); 0.1 M NaHCO_3 - Na_2CO_3 buffer (pH 9.0). Other conditions are described in the text. ●—●, pH 7.6 enzyme; ×—×, pH 10.2 enzyme.

FIG. 2. Optimum Temperature of β -Galactosidase of *Bacillus* No. C-125.

Reaction mixture and other conditions are described in the text. ●—●, pH 7.6 enzyme; ×—×, pH 10.2 enzyme.

centrated enzyme solution was applied onto a Sephadex G-200 column (15 $\times$ 450 mm) equilibrated with *P*-buffer and eluted with the same buffer at a flow rate of 60 ml per hr. A 20-fold purified enzyme was obtained from the cells grown at pH 7.6 (pH 7.6 enzyme) and a 27-fold purified enzyme from the cells grown at pH 10.2 (pH 10.2 enzyme). Disc electrophoresis on polyacrylamide gels was performed according to the method described by Davis.²⁾ Electrophoresis was carried out at 4°C with a current of 3 mA per tube. The gels were incubated with ONPG-buffer solution

TABLE I. EFFECT OF SUGARS AND METAL IONS.

	Conc. (mM)	Remaining activity (%)
1) Sugars		
Raffinose	10	98.9
Lactose	10	95.8
Melibiose	10	51.1
Sucrose	10	94.7
Cellobiose	10	97.8
Glucose	10	85.1
Galactose	10	40.1
Mannose	10	85.3
Fructose	10	95.2
Arabinose	10	95.2
Xylose	10	104.0
2) Metal ions		
Hg ²⁺	10 ⁻¹	0
Ag ²⁺	10 ⁻¹	0
Pb ²⁺	1	0
Ni ²⁺	1	97
Ba ²⁺	1	119
Zn ²⁺	1	10
Co ²⁺	1	112
Mn ²⁺	1	113
Cu ²⁺	1	5
Ca ²⁺	1	107
Fe ²⁺	1	62
Mg ²⁺	1	102

Sugar or metal ion and enzyme were mixed in 50 mM phosphate buffer and allowed to stand for 10 min at 37°C. To the reaction mixture was added the ONPG solutions and activity was determined.

and colored in 1 M Na₂CO₃ solution. Only one peak was observed in the both enzyme preparations. Enzyme was assayed by the method described in the preceding paper.¹⁾ The enzyme was most active at pH 6.5. pH stability after treatment for 10 min at various pH's at 37°C was also determined, and more than 85% of the activity was found to remain at pH's from 5.5 to 9.0 using either enzyme preparation. Effect of temperature on the enzyme activity was examined. As shown in Fig. 2, the temperature optimum was exhibited at about 45°C. Residual activity after treatment for 10 min at pH 6.5 was also measured. The enzyme was stable up to 38°C, but lost almost all of the activity at 55°C. Molecular weight of the enzyme

was determined by the gel filtration method described by Andrews.³⁾ The molecular weight of both pH 7.6 enzyme and pH 10.2 enzyme were calculated to be about 185,000. The *K_m* values on ONPG were 9.26×10^{-3} M for pH 7.6 enzyme and 9.35×10^{-3} M for pH 10.2 enzyme. *K_m* on lactose was calculated by measuring the amount of glucose liberated.⁴⁾ Those on lactose was 5.05×10^{-2} M for pH 7.6 enzyme and 4.88×10^{-2} M for pH 10.2 enzyme. Then the effect of sugar and metal ions on the pH 7.6 enzyme was examined. As shown in Table I, galactose and melibiose at 10 mM inhibited the activity and heavy metal ions such as Hg²⁺, Ag²⁺, Pb²⁺, Zn²⁺ and Cu²⁺ almost completely inhibited the enzyme activity. Effects on pH 10.2 enzyme were almost the same. These data shows that two enzymes seem to be identical.

Up to now β -galactosidase from some species of genus *Bacillus* have been reported. Optimum pH's of β -galactosidases from *Bacillus subtilis*,⁵⁾ *Bacillus megaterium*⁶⁾ and *Bacillus stearothermophilus*⁷⁾ were 6.5~7.5. Enzymatic properties of β -galactosidase of *Bacillus* No. C-125 were similar to those of neutrophilic *Bacillus*. There is no information on the location of the β -galactosidase in the cells of alkalophilic *Bacillus*, it is of interest that β -galactosidase of alkalophilic *Bacillus* has an optimum pH at 6.5 and is stable at neutral pH value. It was reported⁸⁾ the protein synthesizing system of alkalophilic *Bacillus* was similar to that of neutrophilic bacteria. These results strongly suggest that the differences of alkalophilic and neutrophilic bacteria do not exist in inside of the cells but mainly in outersurface.

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