P - 310

Purification and Characterization of β-Galactosidase of *Lactobacillus salivarus* subsp. *salivarus* CNU27

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Introduction

β-Galactosidase are known to occur widely in plants, animals, fungi and bacteria and have been studied most widely plants and fungi. The β-galactosidase catalyzes the breakdown of the substrate lactose to two products, galactose and glucose, compounds which readily feed into the glycolytic pathway (Martin, 1996).

β-galactosidase deficiency, due to biochemical or genetic aberrations leads to flatulence, diarrhea, and bleeding. Various populations, particularly those of East Asia and Africa, suffer from lactase deficiency (Hoyoux *et al.*, 2001). The enzyme β-galactosidase has two main biotechnological uses in the dairy industry- the removal of lactose from milk for lactose tolerant persons and the production of galactooligosaccharides for use in probiotic foodstuffs (Boon *et al.*, 1999). In this paper we purified an β-galactosidase associated with *Lactobacillus salivarus* subsp. *salivarus* CNU27 from human feces, and present some of its biochemical characteristics(Bae and Nam, 2001).

Materials and Methods

4L of MRS broth was inoculated with 40ml of bacteria and grown at 37℃ for 24 hours. Cultured cells were collected by centrifugation (7,000rpm, 15min) and cells were broken with Bead Beater with 0.1mm diameter of glass beads. Extract solution was collected by centrifugation (10,000rpm, 15min) and supernatant was used as source of crude enzyme. β-galactosidase purification was carried out 2 step by DEAE-Sepharose-anion exchange chromatography and FPLC Mono Q ion exchange column chromatography. Electrophoresis of enzymes was carried out using 12% separating gel at 50V for 80min. Enzyme activity of β-galactosidase was determined by the rate of hydrolysis of 5mg/ml ONPG at 40℃ and pH 7.0 (5mM NaH_2PO_4 buffer). The effect of pH was measured a in range pH 1.0~12.0 and

enzyme stability was determined in the range pH 1.0~12.0. After incubation of the enzyme for 1 hour, the pH was adjusted to 7.0 with 5mM NaH_2PO_4 buffer containing 5mg/ml ONPG. The optimum temperature was determined by performing the standard assay at temperature ranging 5 to 100℃. Thermal stability was determined by assaying for residual β-galactosidase activity after incubation of the enzyme in 5mM NaH2PO4 buffer (7.0) at 40, 45, 50, 55, 60℃ and 65℃ and residual enzyme activity determined from 0 to 60 minutes by 10 min intervals. The effect of metal ions on the β-galactosidase was examined by adding 1mM salt (Ca^{2+}, Cu^{2+}, Fe^{3+}, K^{+}, Li^{+}, Mg^{2+}, Mn^{2+}, Zn^{2+}) and enzyme activity was checked after incubating the culture at 40℃ for 1 hour.

Result and Discussion

Lactobacillus salivarus subsp. *salivarus* CNU27 possess a high level of β-galactosidase activity. Purified β-galactosidase was obtained after sonication cell pellet followed by DEAE-Sepharose and Mono-Q anion exchange chromatography and β-galactosidase was eluted withh 0.25M NaCl at 2 step chromatography. The specific activity of the purified enzyme was 5312.0unit/mg.

β-galactosidase, which is purified using DEAE-Sepharose chromatography, used to further experiments is optimum temperature, temperature stability, optimum pH, pH stability and effect of metal ions. The molecular weight of native β-galactosidase was about 30,000 dalton in the SDS-PAGE. The optimum temperature and optimum pH were 50℃ and 5.0, respectively. Temperature stability was between 35 and 60℃. β-galactosidase activity was lost rapidly below pH 3.0, above pH 7.0 and above 65℃ after 10min incubation. Ca^{2+} and Zn^{2+} metal ions increased β-galactosidase activity by 64.09% and 27.37%, and Cu^{2+}, Fe^{3+} and Mn^{2+} metal ions decreased β-galactosidase activity by

Table 1. Effect of different steps in the purification of β-galactosidase of *Lactobacillus salivarus* subsp. *salivarus* CNU 27

Purification step	Volume (ml)	Protein concentration (mg/ml)	Total protein (mg)	Activity (unit/ml)	Specific activity (unit/mg)	Total enzyme activity (unit)	Fold purification	Yield (%)
Cell extract	230	4.78	1.100	2.57	20.16	22.184.0	1	100
DEAE chromatography	26	2.36	11	2.51	565.56	6.221.17	28.05	28.04
MonoQ chromatography	3	0.16	0.5	9.165	5,312.0	2.656.0	263.5	11.97

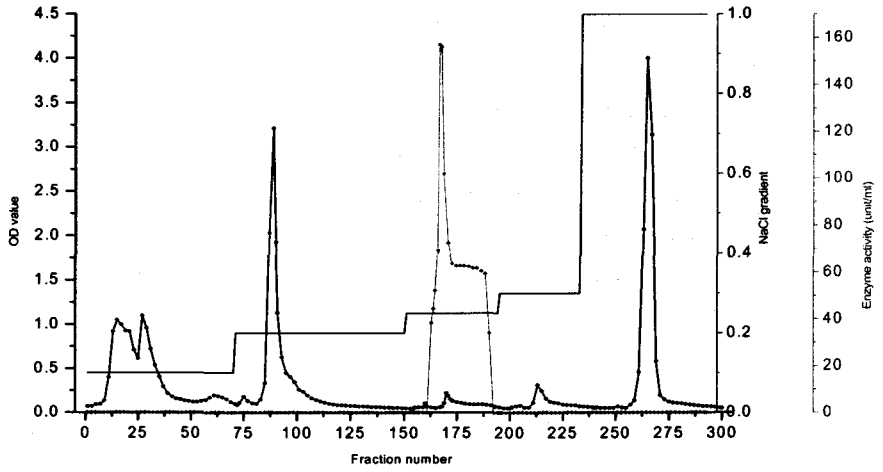

Fig. 2 DEAE-Sepharose anion exchange chromatography of crude enzyme from *Lactobacillus salivarius* subsp. *salivarius* CNU27. Fractions (167 to 195) was showing β-galactosidase activity and fractions 171, 172 was showed high activity.

—— NaCl gradient, ——● OD value, ——▲ Enzyme activity (unit/ml)

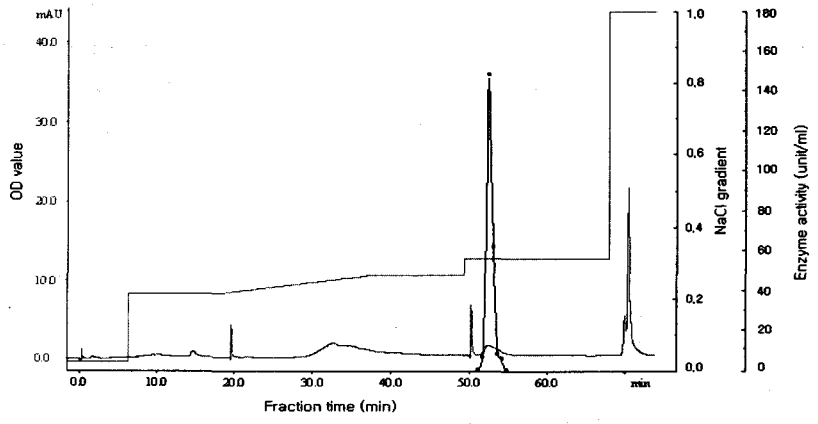

Fig. 3. Mono Q Anion exchange chromatography of β-galactosidase of DEAE- Sepharose fractions with enzyme activity. Fractions (44 to 49) were showed β-galactosidase activity and 45, 46 fractions was maximum enzyme activity within fractions with activity.

—— OD value (unit/ml) ——● NaCl gradient ——▲ Enzyme activity (unit/ml)

41.71%, 14.9% and 22.34%, respectively. Other metal ions not affect to β-galactosidase activity significantly.

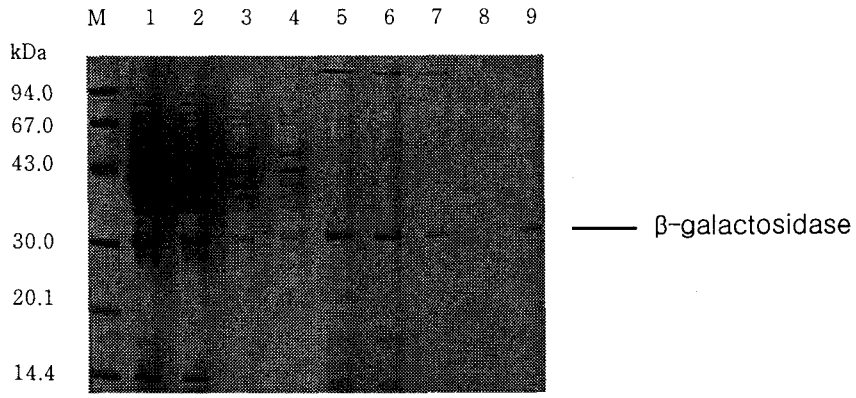

Fig. 4 SDS electrophoresis in 12% polyacrylamide gel of the different steps in the purification of β-galactosidase from *Lactobacillus salivarus* subsp. *salivarus* CNU27.

M: molecular mass of standard proteins in range from 14,400 to 94,000.
Lane 1 to 4: supernatant after breakage of cells with glass bead mill.
Lane 5 to 8: Fraction of DEAE-Sepharose anion exchange chromatography.
(Lanes (1-8) : different by loading volume).
9 : Mono Q chromatography fraction.

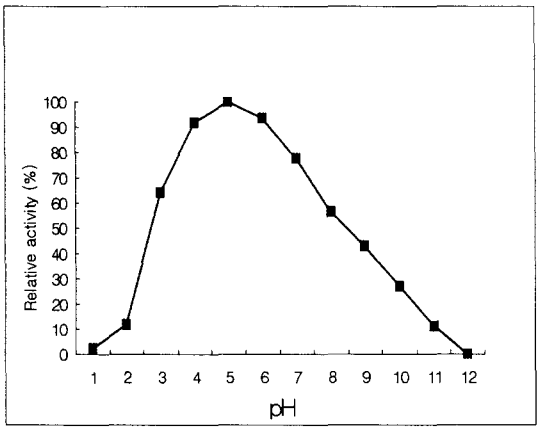

Fig. 5. Optimum pH of β-galactosidase of *Lactobacillus salivarius* subsp. *salivarius* CNU27. β-galactosidase was 70% activity at pH range from 3.0 to 7.0.

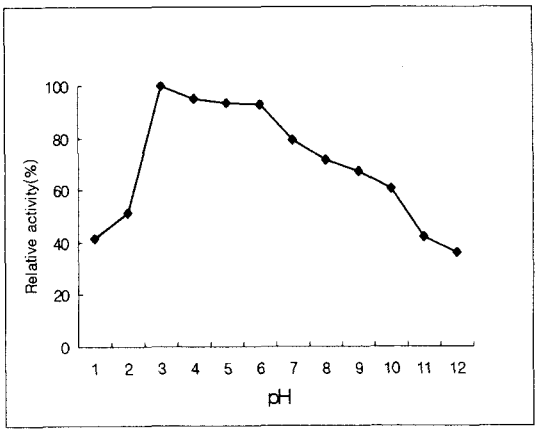

Fig. 6. pH stability of β-galactosidase of *Lactobacillus salivarius* subsp. *salivarius* CNU27. β-galactosidase was more stable at pH 3.0 or acidic conditions.

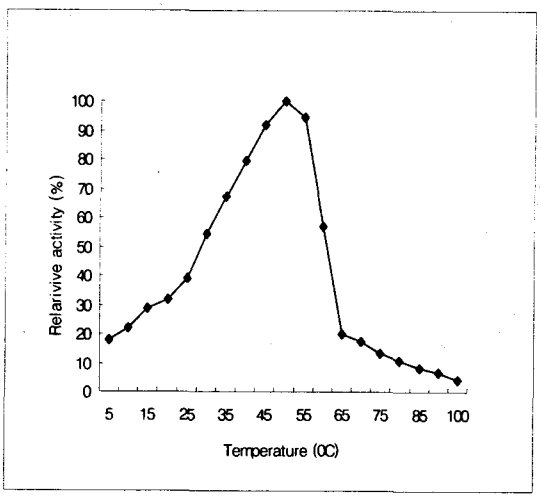

Fig. 7. Effect of temperature on β-galactosidase activity of *Lactobacillus salivarus* subsp. *salivarus* CNU 27. High activity of β-galactosidase was showed at 50℃ and β-galactosidase rapidly lost enzyme activity at above 65℃.

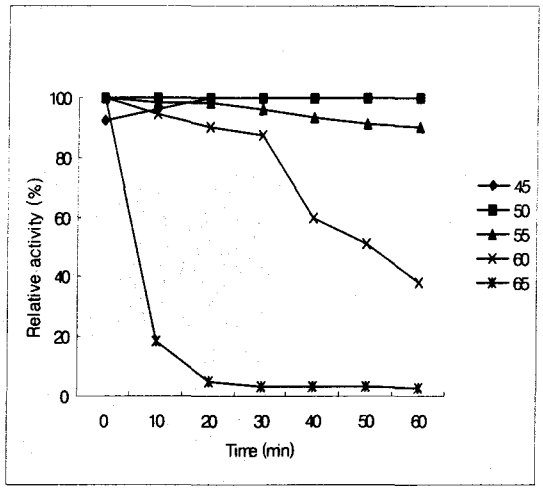

Fig. 8. Effect of temperature on stability. β-galactosidase was retained 100% activity at 50℃ and 55℃ after 60 minute incubation.

References

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