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Production and Purification of Lactase of Kluyveromyces Fragilis from Whey

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Summary

Two *Kluyveromyces fragilis* strains 397 & 665 were grown on whey as main natural culture media to produce Lactase (β-galactosidase). Strain 397 was choosen due to its ability to produce more enzyme. To extract enzyme from yeast cells as it is an intracellular enzyme different mechanical and chemical methods were used. Sonication as a mechanical method, due to it's better performance was chosen to obtain highly purified enzyme. Different biochemical systems were studied, salt precipitation and ion-exchange column chromatography using DEAE-sephadex A-50 were selected and highly purified (77.7%) lactase with high specific activity (64.05 unit/mg protein) was obtained. Lactase activity and stability was retained in 0.1M phosphate buffer saline containing 0.1mM $MnCl_2$, 0.5mM $MgSo_4$, pH 7 at 4°C for two months. In this process β-galactosidase retained its activity up to 95% . Molecular weight of this enzyme was found to be 150,000 daltons by SDS-PAGE and a single band proved its purity.

Key words: β-galactos dase, Lactase, Purification, Enzyme

Introduction

The complete name of β-galactosidase is β-D-galactoside galacto hydrolase EC, also called as lactase. This enzyme hydrolyse the β-D-galactoside bond between galactose and glucose of lactose which is called milk sugar. The importance of this enzyme as reported by scientists is that some children are sensitive to their mother's milk due to

the deficiency of this enzyme. This enzyme deficiency has also been reported in adults. It has been observed that 70-90% of Asian, African and South American, 2-15% American, 2-60% west European were sensitive to lactase, which suggested preparation of milk without lactose by addition of lactase. The enzyme is found in plants, animal and microbial cells. This enzyme was for the first time separated from microorganisms by Beijerinck is 1889. In this investigation whey was used as culture media for growing yeast to produce β-galactosidase which was then separated and purified.

Materials & Methods

Production of β-galactosidase: In this study, CBS-397 of *Kluyveromyces fragilis* strain obtained from microbiology Dept. Razi, Research Institute was used and transfered from solid culture to preculture media which contained 2% lactose, 0.3% $(NH_4)_2 SO_4$ and 0.3% K_2HPO_4 . Then it was transfered to whey as culture media and within 23 hours, it reached stationary phase. Yeast cells were separated from whey by centrifugation at 10000 rpm, for 30 min and sonicated by sonicator at 160W at 4 min intervals 3 times in ice and again centrifuged at 10000 rpm for 30 min and supernatent was collected.

Purification and characterisation of β-galactosidase: The crude enzyme solution was treated with 80% saturated $(NH_4)_2 SO_4$, and centrifuged at 10000 rpm for 30 min. The pellet was dissolved in PBS(0.1M, pH 7) and dialysed with several changes against PBS(0.1M, pH 7, 0.1mM $MnCl_2$ 0.5mM $MgSo_4$) for 12h at 4°C. For higher purification gel chromatography with different gels .i.e DEAE-sepharose, DEAE-cellulose and DEAE-sephadex A-50 were used. All the gels were washed and equilibrated with PBS (0.1M, pH 7, 0.1M $MnCl_2$, 0.02% NaN_3). For elution of enzyme from above gels same buffer with addition of 0.2M KCl were used. Different fractions (10ml pooled from each) obtained from these gels were collected and then O.D at 28Onm was recorded by spetrophotometer. Each fraction which showed enzme activity was treated by ONPG for choosing the enzyme fraction. The purified enzyme was treated again with 80% saturated ammonium sulphate and the pellet dissolved in 0.1M PBS, pH 7 and dialysed against same buffer with several changes. The protein of dialysate was measured with Lowry method(1951) and for observing purity and calculating the molecular weight of the obtained enzyme the SDS-PAGE (Laemmli, 1970) was done.

Results

All the results of purification of enzyme on DEAE-sepharose, DEAE-cellulose and DEAE-sephadex A-50 is shown in Fig. 1, 2 and 3 respectively. By comparing the results the enzyme purification with DEAE-sephadex A-50 (Fig .4) was chosen as the best method due to higher purity and better yield. The molecular weight of β-galactosidase was estimated as 150 KDa on SDS-PAGE and a single band on the gel proved its purity.

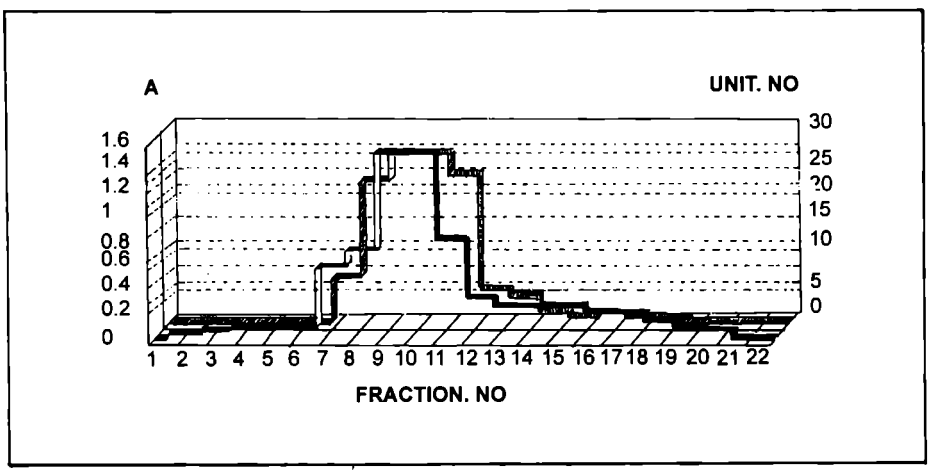

Fig. 1: Purification of β-galactosidase by DEAE-sepharose column.

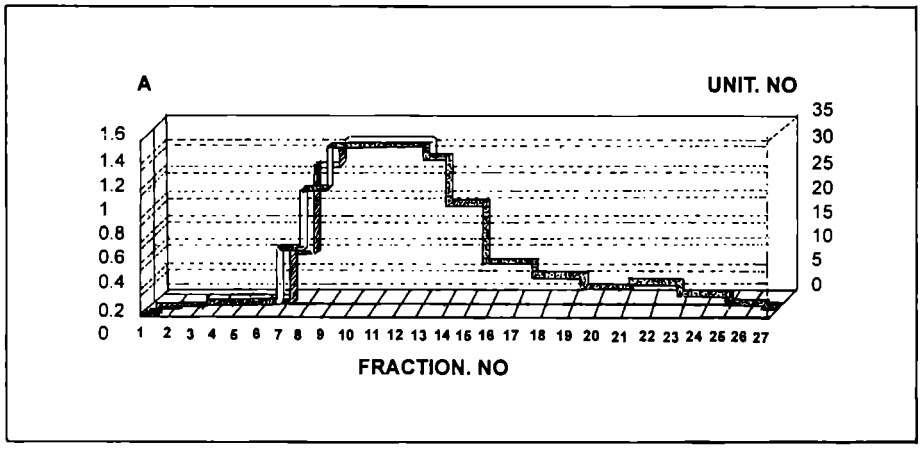

Fig. 2 : Purification of β-galactosidase by DEAE-cellulose column.

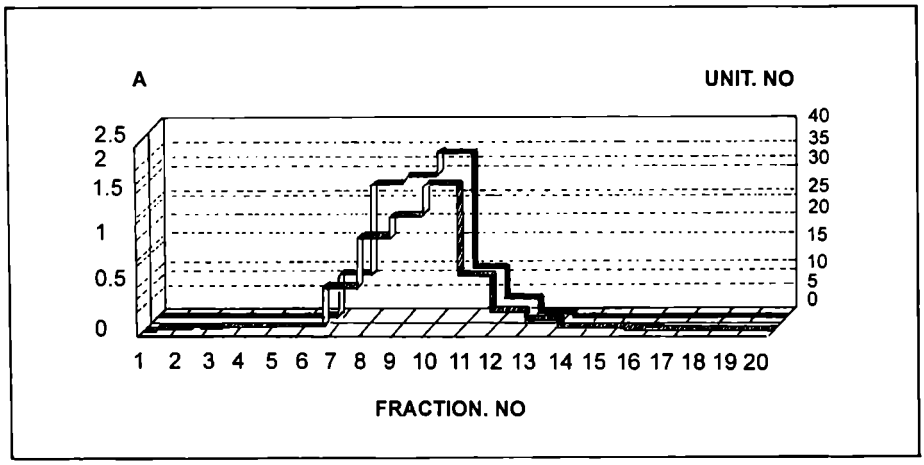

Fig. 3 : Purification of β-galactosidase by DEAE-sephadex A-50 column.

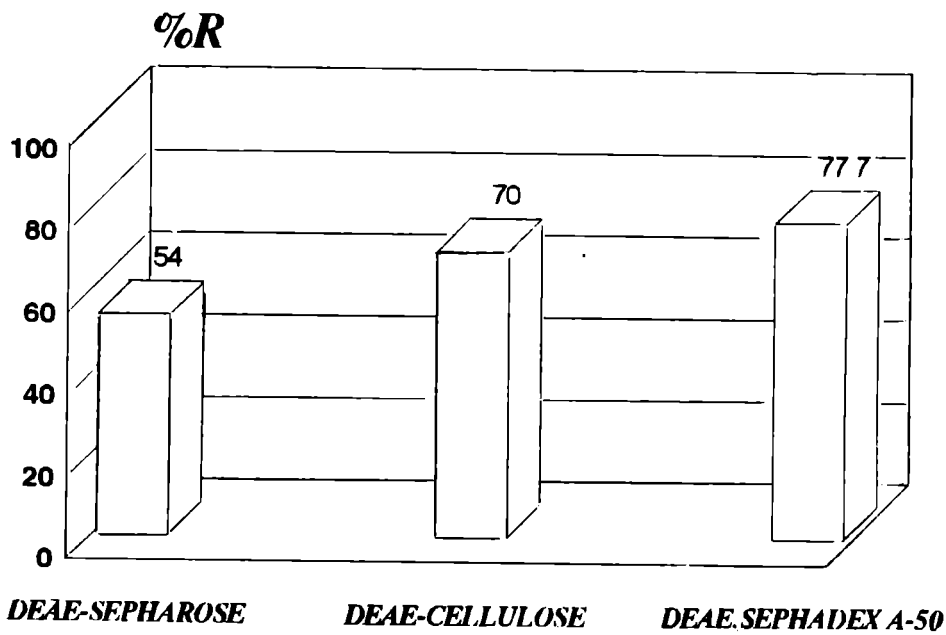

Fig. 4: Histogrames % of enzyme yields.

Table 1: Results of purified enzyme by DEAE-sepharose column.

Step	Procedure	Volume (ml)	Activity (U/ml)	Specific Activity (U/mg protein)	Total Activity	Fold purification	Yield %
1	cell-free extraction	153	29.6	4.93	4528.8	1	100
2	Ammonium sulfate	26	140	14	3640	2.84	80.37
3	DEAE-Sepharose	120	24	16	2760	3.25	60.94
4	Ammonium sulfate	15	170	53.96	2380	10.95	52.55

Table 2: Results of purified enzyme by DEAE-cellulose column.

Step	Procedure	Volume (ml)	Activity (U/ml)	Specific Activity (U/mg protein)	Total Activity	Fold purification	Yield %
1	cell-free extraction	153	29.6	4.93	4528.8	1	100
2	Ammonium sulfate	26	140	14	3640	2.84	80.37
3	DEAE-cellulose	120	29	15.26	3480	3.09	46.84
4	Ammonium sulfate	15	228	58.46	3420	11.85	75.51

Table 3: Results of purified enzyme by DEAE-sephadex A-50 colum

Step	Procedure	Volume (ml)	Activity (U/ml)	Specific Activity (U/mg protein)	Total Activity	Fold purification	Yield %
1	cell-free extraction	170	30.5	4.92	5185	1	100
2	Ammonium sulfate	30	142.5	13.84	4278	2.81	82.1
3	DEAE-Sephadex A-50	126	33	16.18	4158	3.28	80.19
4	Ammonium sulfate	17	237	64.05	4029	13	77.8

Discussion

In this study, *Kluyveromyces fragilis* producing lactase was used. For the growth, the conditions were optimized i.e pH was adjusted from 5.8 to 6.3 and temp 30°C. Similar conditions have been used by others (Wendorff and Amundson 1970). According to Mohammed and Laila (1988) lactose 2-15% in culture media is helpful for better growth and enhance enzyme production. Same condition can be seen in whey which contain 4.9% lactose and therefore selected as culture media. In this study, two ions i.e Mn^{++},K^{+} as co-factors for enzyme activity were used.

The activity of crude enzyme was 30.5unit/ml which was similar with the result of Kulikova, *et al.* (1978) Mahoney and Greenberg (1982) and Vogel, *et al.* (1994). The purity and specific activity of lactase was 77.7% and 64.05 unit/mg protein respectively, which is coherent with the results of Mahoney and Greenberg (1982).

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