Isolation, Partial Purification, and Characterisation of β-Galactosidase from *Limosilactobacillus fermentum* LF08

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Introduction

β-galactosidase, frequently called lactase, is an enzyme responsible for catalysing the hydrolysis of lactose to glucose and galactose. It has broad biotechnological application in dairy, pharmaceutical and functional food industries. Traditionally it is obtained from fungal and yeast source. However, there is growing interest in lactic acid bacteria (LAB) utilsiation as source, due to their probiotic properties and Generally Recognized as Safe (GRAS) status. Among the LAB, *Limosilactobacillus fermentum* LF08 strain, shows strong potential for β-galactosidase production. Nevertheless, further experimentation is required to gain a deeper understanding of the enzyme's specific properties and to optimize its purification.

Questions Addressed

- What are the main characteristics of the partially purified β-galactosidase enzyme?
- What knowledge can be gained from the optimized purification parameters and characterized properties to enhance its biological application?

Materials & Methods

Probiotic bacteria

Isolate of *Limosilactobacillus fermentum* LF08 obtained from Probiotical S.p.A.

Enzyme fermentation

- Modified De Man, Rogosa and Sharpe Medium with Galactose:Glucose as a carbon source, ratio 3:1
- Fermentation started with 3 v/v% inoculum
- 16 hours of fermentation time at a temperature of 37°C.

Cell disruption

French Press cell disruptor

Precipitation

Ammonium sulphate
75% saturation

Ion-Exchange chromatography

- Q-Sepharose column
- Flow rate: 4 mL/min
- Sample volume: 2 mL
- Fraction volume: 4 mL
- 50 mM Sorensen buffer (pH 6.5) +1M NaCl

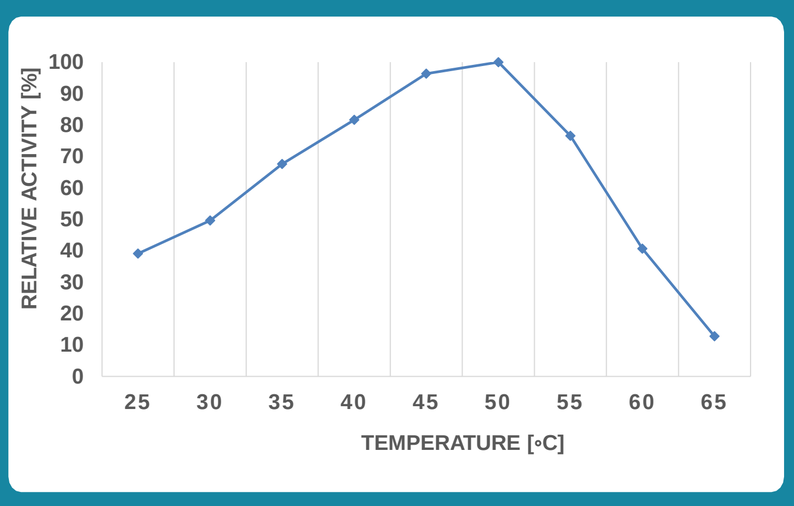
Characterisation of partially purified β-galactosidase

- pH and temperature optimum
- Effect of metal ions
- Enzyme stability
- Molecular weight

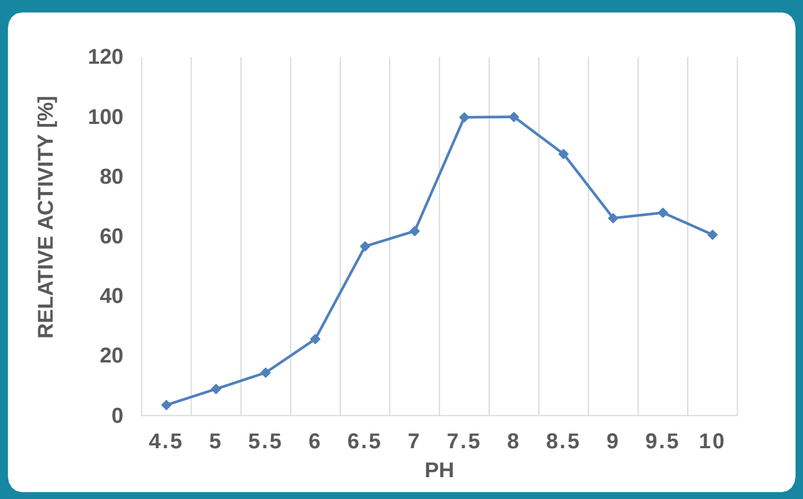
Enzyme activity was determined by using 4-nitrophenyl β-D-galactopyranoside substrate at 50◦C and Sorensen buffer pH (6.5). 0.1 N Na_2CO_3 was used for stopping the enzyme reaction.

RESULTS OF β-GALACTOSIDASE CHARACTERISATION FROM *L. FERMENTUM LF08*

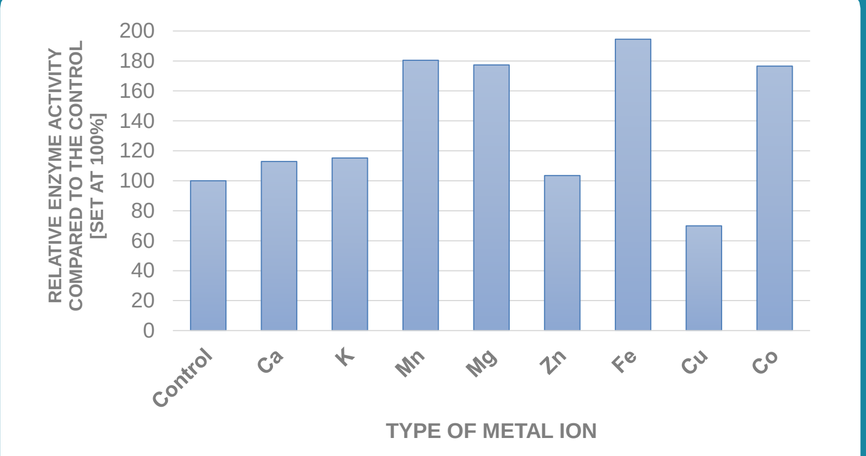
Effect of Temperature



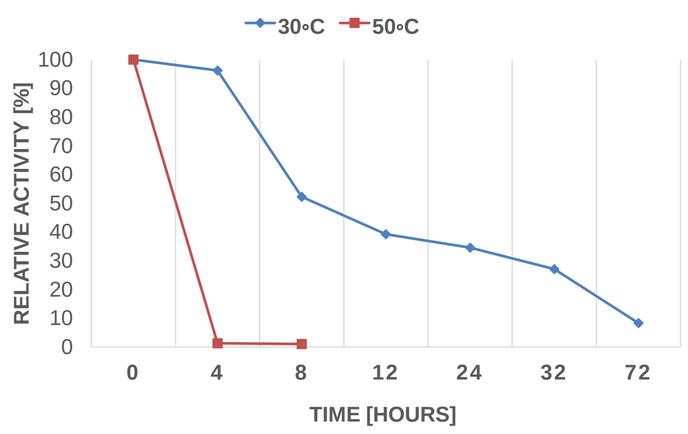
Effect of pH



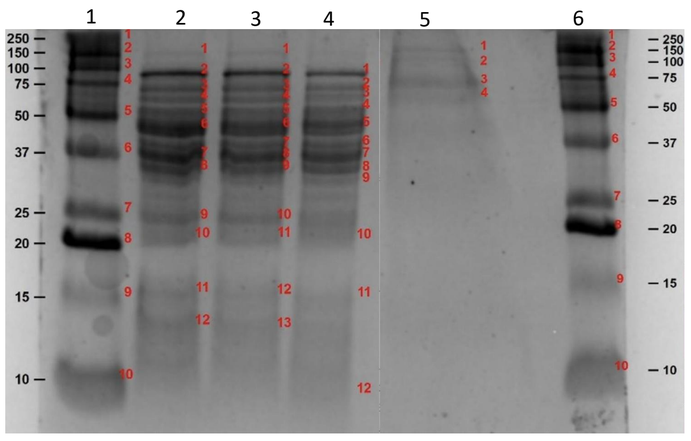
Effect of various metal ions



Thermal stability



SDS-PAGE analysis



1: molecular marker (10 µL)
2: sample after French Press cell disruption (10 µL)
3: sample after ammonium sulphate precipitation (10 µL)
4: sample after ultrafiltration (10 µL)
5: sample after ion-exchange chromatography (20 µL)
6: molecular marker (10 µL)

Main Observations and Conclusions

- ***Limosilactobacillus fermentum* LF08** cells were fermented for **16 hours** in MRS medium with a **3:1 galactose:glucose** ratio, and β-galactosidase was effectively released by **French Press.** With application of **ammonium sulfate precipitation, ultrafiltration, and ion-exchange chromatography,** enzyme suitable for further characterisation was obtained.
- The partially purified β-galactosidase enzyme exhibited optimal activity at **50°C** and **pH 7.5–8.5**. The optimal activity of the enzyme suggests promising potential for industrial and biotechnological applications.
- The presence of metal ions such as **Ca^{2+}, K^{+}, Mn^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}**, and **Co^{2+}** enhanced the activity, whereas **Cu^{2+}** slightly inhibits, which indicate possible interaction of this ion with the active site or structure of the enzyme.
- The molecular weight of enzyme protein was estimated to be **50–75 kDa** based on the **SDS-PAGE.**
- **The enzyme remained stable at 30°C for 72 hours, but lost activity at 50°C after 8 hours.** This points out the need of application of moderate temperature while handling the enzyme.

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