Optimisation of fermentation parameters and cell disruption methods for production of intracellular β-galactosidase by probiotic *Limosilactobacillus fermentum* LF08 bacterium

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INTRODUCTION

β-galactosidase or commonly known as lactase is an enzyme that catalyse the breakdown of lactose sugar. It is used in different industrial processes, such as whey utilisation, production of lactose-free products and synthesis of prebiotic galacto-oligosaccharides. This enzyme can be found in different plants, animal tissues and microorganisms. Industrial sources are usually obtained from *Aspergillus sp.* and *Kluyveromyces lactis.* Bacterial sources are also of huge importance for the food industry. There is a big difference in the functional properties of the enzyme depending on the source and differ also from strain to strain. β-galactosidase in the bacterial cells is produced intracellularly, and for that reason, efficient cell disruption method is of crucial importance. This study investigates the β-galactosidase from *Limosilactobacillus fermentum*, which is gram positive, heterofermentative bacterium.

AIM:

- To evaluate:
  - the optimal fermentation time and inoculum size
  - Effect of carbon sources
  - Most efficient cell disruption method

MATERIALS AND METHODS

Probiotic bacteria

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

Enzyme Fermentation

- **Fermentation time from 16 to 48 hours was evaluated**
- **1, 5 and 10% of inoculum were tested**
- **De Man, Rogosa and Sharpe Medium**
- **Optimised with different carbon sources (Glucose, Lactose, Galactose)**

Cell Lysis

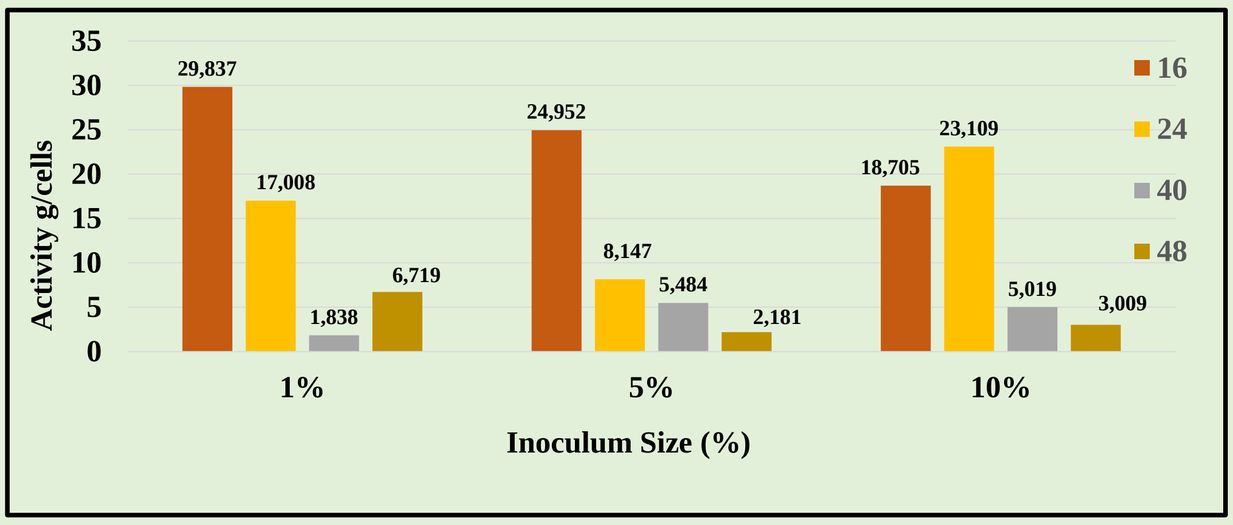
- **CTAB (cetyl-trimetyl-ammonium bromide) lysis buffer**
- **French Press Cell Disruptor**

Determination of Enzyme Activity

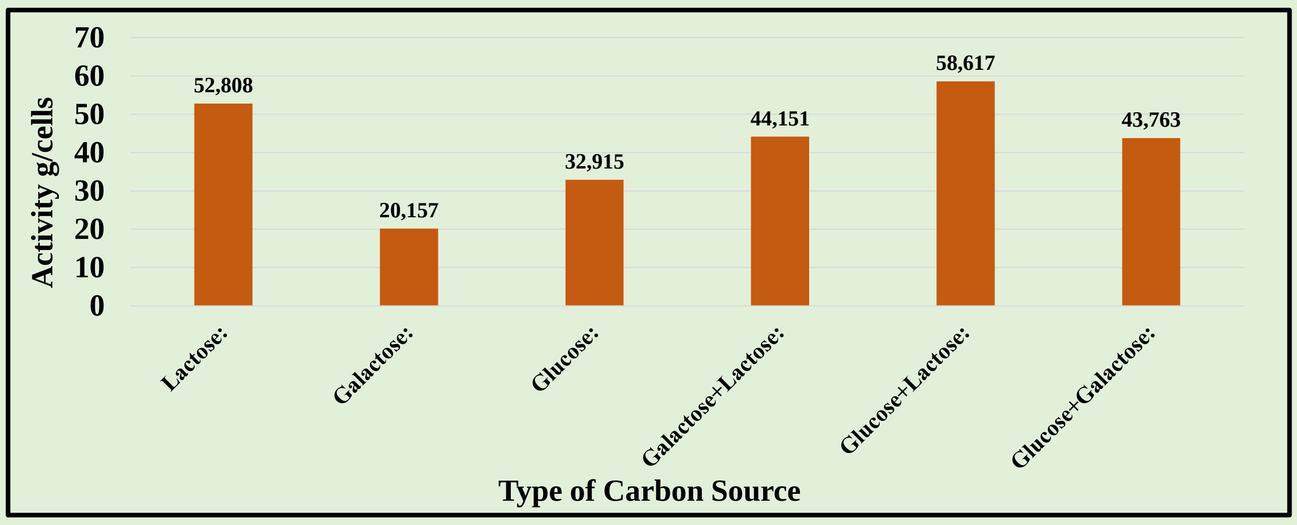
- **Optimal conditions:**
  - **pH 6.5 (Sorensen buffer)**
  - **Temperature 50°C**
- **Na_2CO_3 for stopping enzymatic reaction**
- **4-nitrophenyl β-D-galactopyranoside substrate**

RESULTS

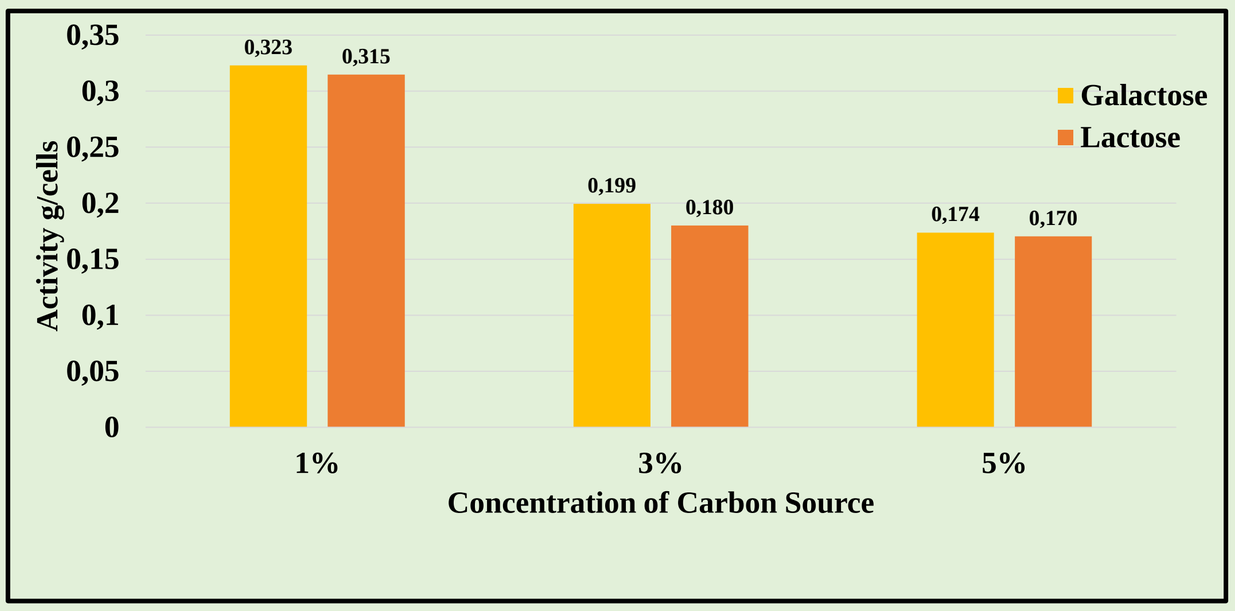
Evaluation of the fermentation time and inoculum size (Figure 1)



Effect of different carbon sources and their combination (Figure 2)



Effect of different concentrations of galactose and lactose (Figure 3)



Effect of different cell disruption methods (Table 1)

	CTAB (supernatant)	French Press (supernatant)	
		One Cycle	Two Cycles
Enzyme activity (U/ml)	0,003	0,671	0,553

MAIN OBSERVATIONS AND CONCLUSION

- Both, the fermentation time and inoculum size have an influence on the enzyme production. Highest enzymatic activity was observed at 1% inoculum size and 16 hours of fermentation (Figure 1)
- Another significant factor for the β-galactosidase activity is the chosen carbon source. When combination of carbon sources was used, higher enzymatic activity was obtained in combination between glucose and lactose, followed by lactose as a sole carbon source (Figure 2). On the other hand, different tested concentrations of lactose and galactose (1,3,5%) resulted with similar results (Figure 3)
- Among the two tested cell disruption techniques, better results were obtained with using of the French Press cell disruptor, however increasing the number of the cycles did not result with greater activity (Table 1)
- Obtained results are preliminary, but they can serve as a good basis for further research and optimisation

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