Screening and optimization of fermentation medium for β-galactosidase production from probiotic *Lactobacillus* strains

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INTRODUCTION

- β-galactosidase enzyme it is commonly known as lactase, because the main goal is to catalyze the hydrolysis of lactose to galactose and glucose.
- This enzyme is widely spread in the nature. It can be found in different plants like almonds, apricots, apples and also in animal organs, like the intestine, brain and placenta.
- Industrial importance has the β-galactosidase produced by different microorganisms, including yeast, fungi and bacteria.
- Commercial application of the enzyme can be applied in different fields, such as: production of hydrolyzed milk products, whey utilization and synthesis of oligosaccharides with prebiotic potential like the galactooligosaccharides.
- Of high importance for the food industry because of the GRAS (generally recognized as safe) status, has the β-galactosidase produced from probiotic bacteria.
- Enzyme produced from these sources can be used without extensive purification

AIM

- The aim of this study was to select the most promising probiotic *Lactobacillus* strains for β-galactosidase enzyme production.
- To determine the effect of quality and quantity of different carbon sources supplemented in the MRS medium on the enzymatic activity.

MATERIALS AND METHODS

Materials:

- The isolates of probiotic bacteria were obtained from Lallemand Health Solutions and Probiotical S.p.A.

Methods:

- **Enzyme fermentation:**
  - Enzyme fermentation was performed in 250 ml Erlenmeyer flasks supplemented with MRS medium modified according to the appropriate experimental settings for 16 and 24 hours at 37ºC.
  - The fermentations were started with 5 v/v % of previously prepared cell suspension.
- **Determination of enzyme activity:**
  - The principle of the assay is that β-galactosidase enzyme release p-nitrophenol from artificial substrate p-nitrophenyl-β-D-Galactopyranoside. The concentration of the product p-nitrophenol was measured by spectrophotometer at 405 nm. Predetermined optimal conditions for the enzyme activity assay were with temperature at 50ºC and optimal pH value of 6.5. The enzyme activity was calculated with the following equation:

$$U = \frac{(A_{sample} - A_{enzyme\ blank} - A_{substrate\ blank}) * dillution * reaction\ volume}{volume\ of\ enzyme * reaction\ time * 2.559}$$

RESULTS

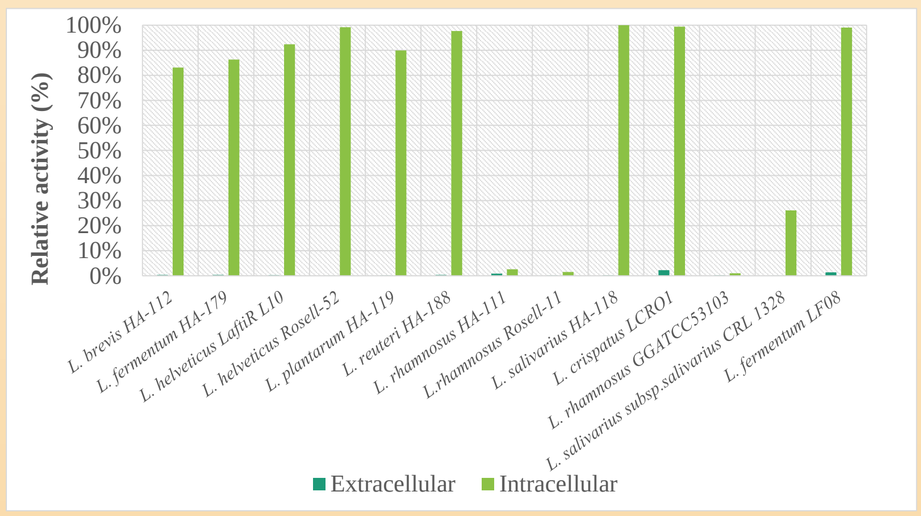

Figure 1:Enzymatic activity of intra and extracellular β-galactosidase obtained in different *Lactobacillus* strains

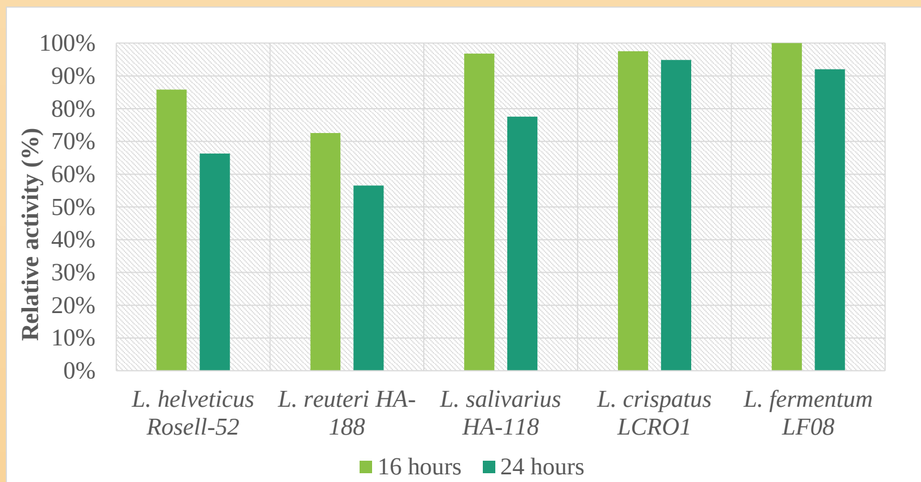

Figure 2: Intracellular β-galactosidase activity of *Lactobacillus* strains after 16 and 24 hours fermentation

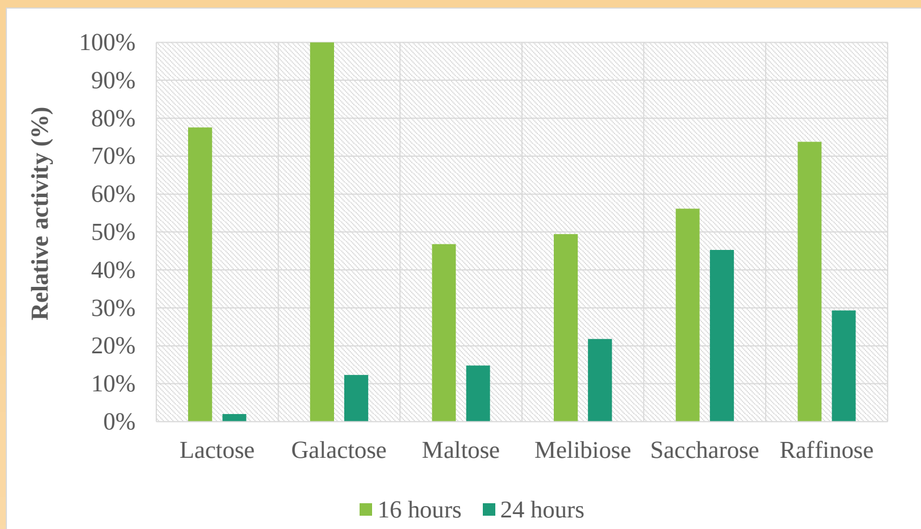

Figure 3: Effect of using different carbon source at 1 % concentration on the β-galactosidase activity (*L. fermentum* LF08)

13 different *Lactobacillus* strains were screened for β-galactosidase activity. It can be clearly observed that the β-galactosidase enzyme is produced intracellularly.

The obtained values for extracellular enzymatic activity were insignificant. It can be concluded that the extracellular enzyme may be derived from the lysed cells (**Figure 1**).

Highest enzymatic activity was obtained when *L. crispatus* LCR01 and *L. fermentum* LF08 were used under fermentation time of 16 hours.

It can be noted that 16 hours fermentation time was more favorable for the investigated strains **(Figure 2)**.

Further investigations were performed with *L. fermentum* LF08 strain

Effect of 6 different carbon sources were investigated. Highest enzymatic activity was determined at fermentation time of 16 hours, when lactose and galactose were supplemented in the medium.

The effect of maltose, melibiose, saccharose, raffinose was significantly lower compared to the lactose and galactose.

Fermentation time of 24 hours was proven to be not suitable for production of the enzyme at 1% concentration of the supplemented carbon source **(Figure 3)**.

Previously determined important carbon sources were investigated at different concentration level ranging from 0.5% to 6.0%.

In both cases, highest β-galactosidase was determined to be at 4.5% concentration. **(Figure 4 and 5)**.

Besides galactose and glucose as sole carbon source, the combination of carbon sources, such as: glucose and galactose and galactose and lactose were investigated.

Highest enzymatic activity was obtained when only galactose was used in the medium **(Figure 6)**.

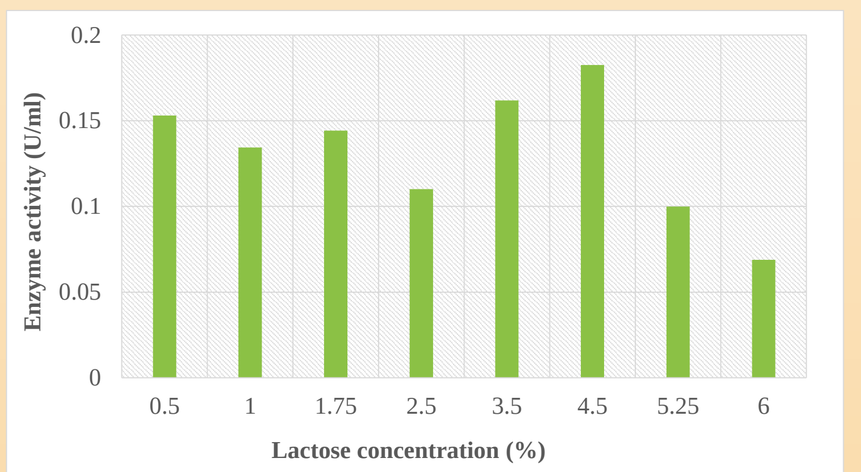

Figure 4: β-galactosidase activity under different concentrations of lactose (*L. fermentum* LF08)

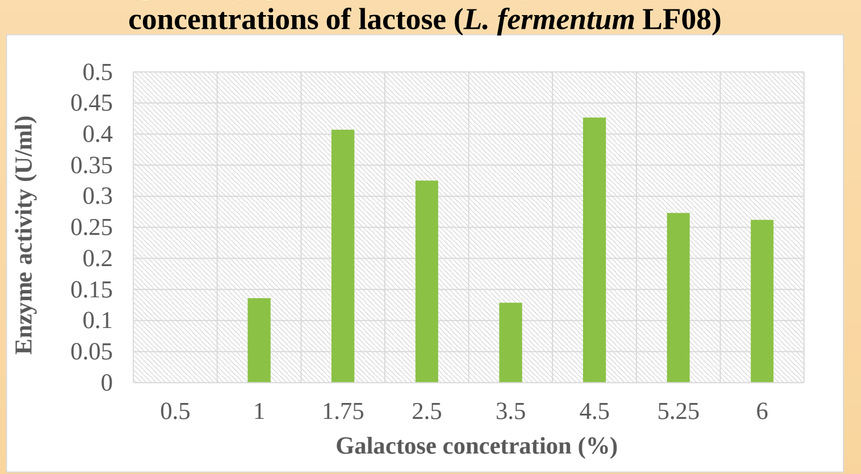

Figure 5: β-galactosidase activity under different concentrations of galactose (*L. fermentum* LF08)

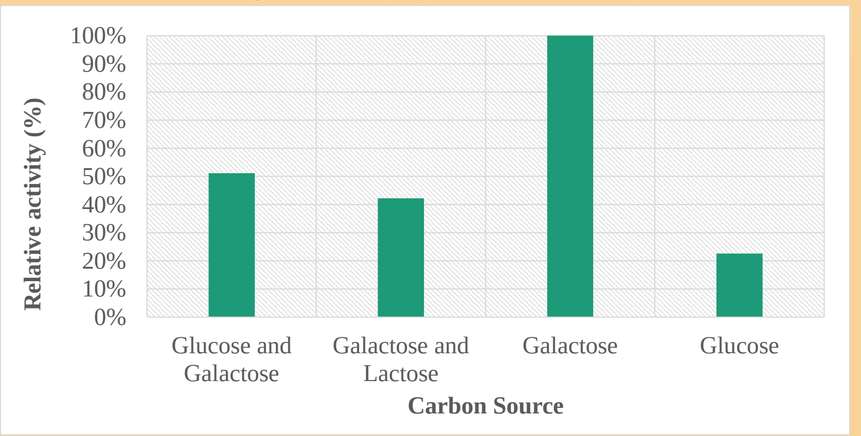

Figure 6: Effect of different carbon sources (maximal concentration of 4%) and their combination on the enzyme activity (*L. fermentum* LF08)

CONCLUSION

The presented results suggest that the β-galactosidase enzyme from the probiotic bacteria is produced intracellularly.

These results reveal the importance of the type and concentration of the carbon source provided in fermentation medium on the level of produced β-galactosidase enzyme.

Our results are preliminary but they can serve as a good basis for further optimization and experimentation in order to obtain higher final β-galactosidase activity.

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BIOSYSFOODENG 2021