

Efficient Immobilization of β -galactosidase using solid-binding peptides to produce galactooligosaccharides from whey

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Background

Galactooligosaccharides (GOS) are oligosaccharides composed of 2-9 different galactosyl residues and a terminal glucose linked by β -glycosidic bonds. GOS exhibit prebiotic effect, favorably affecting the human intestinal microbiota. In addition, the excellent technological properties make GOS convenient for use in the food industry, making it possible to obtain functional products with their addition. GOS can be obtained from lactose in transgalactosylation reaction catalyzed by a number of enzymes (Figure 1). Among the enzymes, beta-galactosidases obtained from *Lactobacilli* and *bifidobacteria* are of particular interest, since they are natural inhabitants of the human intestine and potentially GOS obtained from them should have superior prebiotic effect.

Significant studies have been conducted regarding the synthesis of GOS from various *Lactobacilli* and *bifidobacteria*. Among which, *Lactobacillus reuteri* L103 is one of the most studied. Previous studies reported that the maximum yield of GOS obtained was about 30% of the total amount of sugars at an initial lactose concentration about 200 g/l using β -galactosidase from *L. reuteri*. The final GOS mixture consisted of oligosaccharides with degree of polymerization (DP) between 2 and 4 and linked via β -1,3 and β -1,6-bonds. These structures are promising as potential prebiotics. Although, β -galactosidase from this organism is of interest for the synthesis of GOS, several challenges remain that need to be addressed. For example, the use of free enzymes in industrial processes is complicated due to enzyme storage instability, thermal effects and difficulty and cost of enzyme separation from reagents and reaction products. Several of the drawbacks presented by free enzymes can be eliminated by using immobilized enzymes. Enzyme immobilization allows them to be used repeatedly in batch and continuous biological processes (cost reduction), quick termination of reactions, controlled product formation and provide flexibility in industrial biological processes.

β -galactosidase from *L. reuteri* L103 has been immobilized on chitin carriers using a carbohydrate-binding domain from a chitinolytic enzyme. The immobilization yield reached 99.3% and the conversion factor was maintained at 80% for 8 cycles. However, the residual activity of the immobilized enzyme was reduced considerably to only 9.19 to 25.89 % of their initial activity. In another report, the β -galactosidase from this organism was immobilized on the cell surface of four *Lactobacillus* strain using the peptidoglycan-binding motif LysM. The immobilization yield was only 6.53 % and activities on the cell surface were 3.06 %. Thus, effective immobilization strategies for this enzyme that are capable of supporting high immobilization yields and high enzyme activities are still lacking. So, there is an unmet need for the development of effective and industrially-feasible methods for the immobilization of β -galactosidase from *L. reuteri* L103 for the efficient production GOS.

Proposal

There is a facile and effective enzyme immobilization technology based on solid-binding peptides (SBP) (Figure 2). Solid-binding peptides (SBPs) are short amino acid sequences that can act as molecular linkers to direct the orientated immobilization of biomolecules onto solid materials without impeding their biological activity. Many insoluble materials can be used as carriers for the immobilization of enzymes. However, the mode of interaction between the enzyme and the carrier can have a critical effect on the stability and catalytic efficiency of the immobilized enzyme. Silica-based materials like silica and zeolite are considered to be suitable matrices for enzyme immobilization in industrial processes. They offer high mechanical strength and stability, are chemically inert and can be deployed over a wide range of operating conditions. They have exploited the property of one SBP that binds to materials that contain silica and have constructed functional fusion proteins displaying high binding affinity to silica and zeolite whilst retaining high levels of enzyme activity.

The use of SBP to immobilize β -galactosidases to industrially-acceptable and inexpensive matrices would probably allow the development of an efficient method for GOS production. Moreover, the current increase in cheese consumption and production has led to a steady increase in lactose-rich permeates which are available as substrate for GOS production.

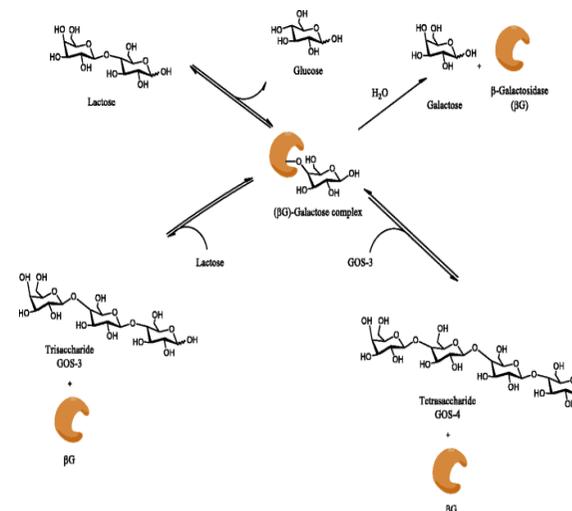


Fig. 1. Transgalactosylation reaction for GOS synthesis

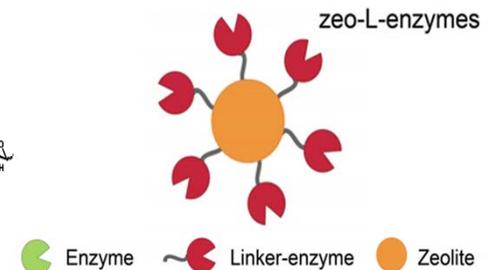


Fig. 2. Diagram representing immobilized enzymes with SBP

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