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REVIEW ARTICLE

Strategy in manipulating transglycosylation activity of glycosyl hydrolase for oligosaccharide production

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ABSTRACT

Background: The increasing market demand for oligosaccharides has intensified the need for efficient biocatalysts. Glycosyl hydrolases (GHs) are still gaining popularity as biocatalyst for oligosaccharides synthesis owing to its simple reaction and high selectivity.

Purpose: Over the years, research has advanced mainly directing to one goal; to reduce hydrolysis activity of GHs for increased transglycosylation activity in achieving high production of oligosaccharides.

Design and methods: This review concisely presents the strategies to increase transglycosylation activity of GHs for oligosaccharides synthesis, focusing on controlling the reaction equilibrium, and protein engineering. Various modifications of the subsites of GHs have been demonstrated to significantly modulate the hydrolysis and transglycosylation activity of the enzymes. The clear insight of the roles of each amino acid in these sites provides a platform for designing an enzyme that could synthesize a specific oligosaccharide product.

Conclusions: The key strategies presented here are important for future improvement of GHs as a biocatalyst for oligosaccharide synthesis.

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Oligosaccharide; glycosyl hydrolase; reaction equilibrium; protein engineering; oligosaccharide synthesis; hydrolysis; transglycosylation

Introduction

Demand for so-called "functional foods" is impressively growing due to the increasing health awareness amongst consumers. Oligosaccharides have attracted a lot of attention as it has the potential to be used as dietary carbohydrates and food additives to improve the nutritional value of the food. Oligosaccharides have been used as prebiotics for stimulating the growth and activity of beneficial intestinal microflora [1,2] that will assist in digestion, nutrient absorption, produce vitamins, enhance the immune system of the host, and suppress the growth of putrefactive pathogenic bacteria [3].

Oligosaccharides can be extracted from nature, but *in vitro* synthesis is preferred because it provides a high yield to fulfill the great market demand. The considerable development in oligosaccharides synthesis can be observed in recent years especially aiming at acquiring high yield, low cost, together with a robust and simple process. The shift from chemical synthesis to enzymatic synthesis for oligosaccharides production has been observed as the enzymatic method is more promising. The development in enzyme technology leads to innovation of improved enzymes to be used as biocatalysts for oligosaccharides synthesis. A major breakthrough in the protein engineering of glycosyl hydrolases (GHs) for oligosaccharides synthesis was the invention of glycosynthase, a mutant GH that catalyzes the synthesis of glycosidic linkages without hydrolyzing the newly formed glycosidic linkages. This has been extensively reviewed [4,5]. This review focuses on strategies to improve transglycosylation activity of GH for higher oligosaccharides synthesis using a reaction equilibrium control strategy and together with protein engineering.

Production of oligosaccharides

The world has witnessed a fast growth of the functional food market driven by an overwhelming consumer consciousness for healthier foods. According to the report by the Leatherhead Food Research, the global functional food market was predicted to increase 25% by 2017 compared to 2013, reaching USD 54 billion in value [6]. The United States, Europe, and Japan are

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