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Engineering of cellobiose phosphorylase for the synthesis of prebiotic cellotriose

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Cellodextrins are non-digestible oligosaccharides that have attracted interest from the food industry as potential prebiotics [1,2]. They are typically produced through the partial hydrolysis of cellulose, resulting in a complex mixture of oligosaccharides with a varying degree of polymerisation (DP). In our work [3], we explored the defined bottom-up synthesis of cellotriose as a product since this oligosaccharide is believed to be the most potent prebiotic in the mixture [1]. To that end, the cellobiose phosphorylase (CBP) from *Cellulomonas uda* and the cellodextrin phosphorylase (CDP) from *Clostridium cellulosi* were evaluated as biocatalysts, starting from cellobiose and α-glucose 1-phosphate as acceptor and donor substrate, respectively. The CDP enzyme was shown to rapidly elongate the chains towards higher DPs, even after extensive mutagenesis. In contrast, an optimised variant of CBP was found to convert cellobiose to cellotriose with a molar yield of 73%. The share of cellotriose within the final soluble cellodextrin mixture (DP2-5) was 82%, resulting in a cellotriose product with the highest purity reported to date. Remarkably, the reaction could even be initiated from glucose as an acceptor substrate, which could further decrease the production costs. Further research on the affordable and environmentally sustainable enzymatic synthesis of speciality carbohydrates such as cellotriose could result in the production of healthier carbohydrates (i.e., functional foods), now more relevant than ever due to the global rise in obesity and related health problems.

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