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Whole-cell catalyst by design enables efficient flux over three enzymatic steps for soluble cello-oligosaccharide production

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PURPOSE OF THE ABSTRACT

Soluble cello-oligosaccharides (COS, beta-1,4-D-gluco-oligosaccharides with degree of polymerization DP 2-6) receive increased attention in different industrial sectors, from food & feed to cosmetics [1-5]. Development of large-scale applications of the COS requires waste-generating and cost-effective technologies for their production [1,6-8]. Cascade biocatalysis by three enzymes (sucrose, cellobiose and cellodextrin phosphorylase) is promising because it enables bottom-up synthesis of the COS from expedient substrates (sucrose, glucose) [5,9,10]. A whole cell-derived catalyst that incorporates the required enzyme activities from suitable co-expression would represent an important step towards a more sustainable production [11,12]. The greenness as well as the economic impact of the whole-cell approach increases sharply with the number of enzymes involved in the cascade reaction, since each enzyme would require its separate production and workup [13,14]. Multi-enzyme co-expression in distinct activity ratios is challenging in general, but it receives special emphasis in the synthesis of COS: A finely tuned balancing between formation and elongation of the oligosaccharide precursor cellobiose is necessary to avoid product loss due to over-elongation into insoluble COS [10,15].

Gene expression of the three phosphorylases was pursued by either two separate plasmids or by a single co-expression plasmid. Activity ratios produced by the latter plasmid design were further optimized by rearranging co-expression cassettes towards up- instead of downregulating the expression levels of individual genes. The best effective strategy involved a self-constructed tricistronic plasmid that placed the genes for cellobiose phosphorylase (CbP), cellodextrin phosphorylase (CdP) and sucrose phosphorylase (ScP) in frame and in that order, with expression under control of the T7lacO promoter and strong ribosome binding sites (RBS). The whole cell catalyst achieved a recombinant protein yield of 46% of total intracellular protein in an optimal ScP:CbP:CdP activity ratio of 10:2.9:0.6, giving an overall activity of 315 U/g dry cell mass. Bioconversions catalyzed by a semi-permeabilized whole-cell catalyst achieved an industrial relevant COS product titer of 125 g/L and a space-time yield of 20 g/L/h. With CbP as the limiting enzyme, flux into higher oligosaccharides (DP above 6) was prevented and no insoluble products were formed after 6 hours of conversion.

A whole cell catalyst for COS synthesis was developed based on a coordinated strategy of three-enzyme co-expression to achieve high overall protein yields at balanced activities of the individual enzymes. In addition to the resource- and time-saving production route of whole-cell catalysts compared to free enzymes in general, the here designed whole cell catalyst even achieved similar performance metrics (yield, productivity, product concentration and quality) in the biotransformation compared to the optimized enzymatic one [5]. This, along with the flux control that minimizes the share of insolubles in total product, makes the whole cell synthesis promising for a greener production of COS in large-scale.

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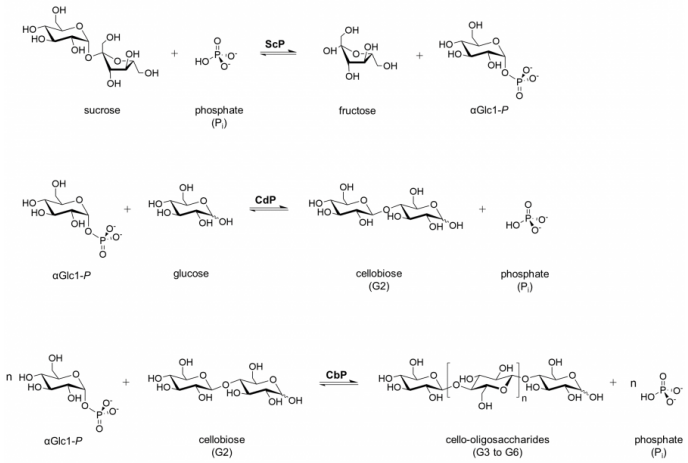
FIGURES



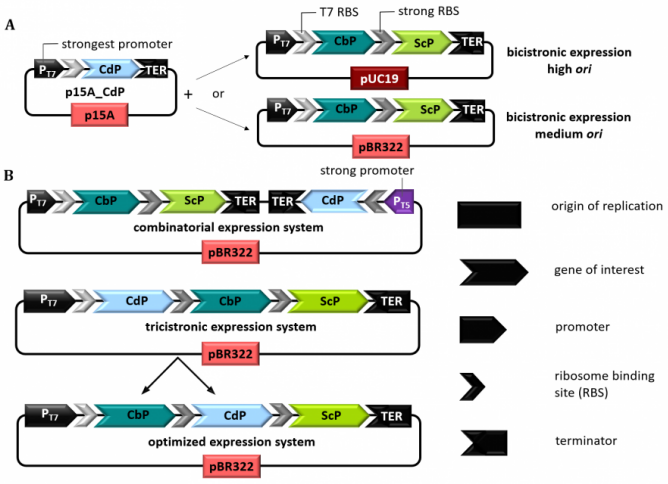


FIGURE 1

Enzymatic production of cello-oligosaccharides (COS).

Sucrose phosphorylase (ScP), cellobiose phosphorylase (CbP) and cellodextrin phosphorylase (CdP) catalyze the synthesis of soluble COS (DP 2-6, n is 1 to 4)

FIGURE 2

Plasmid desings for co-expression of three enzymes.

(A) Expression strategies using two plasmids: origins of replication p15A and pBR322 are medium copy, pUC19 is high copy. (B) Expression systems based on single plasmids and optimization steps.

KEYWORDS

cello-oligosaccharides | multi-enzymatic cascade | whole-cell catalyst | activity ratios

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