Supporting Information

Short-chain cello-oligosaccharides: Intensification and scale-up of their enzymatic production and selective growth promotion among probiotic bacteria

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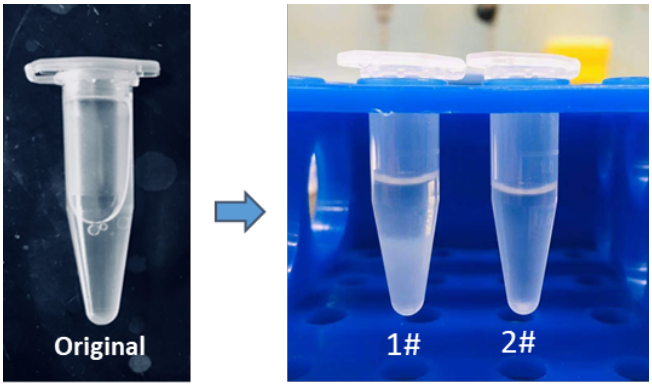
S1. Solubility of reaction mixture

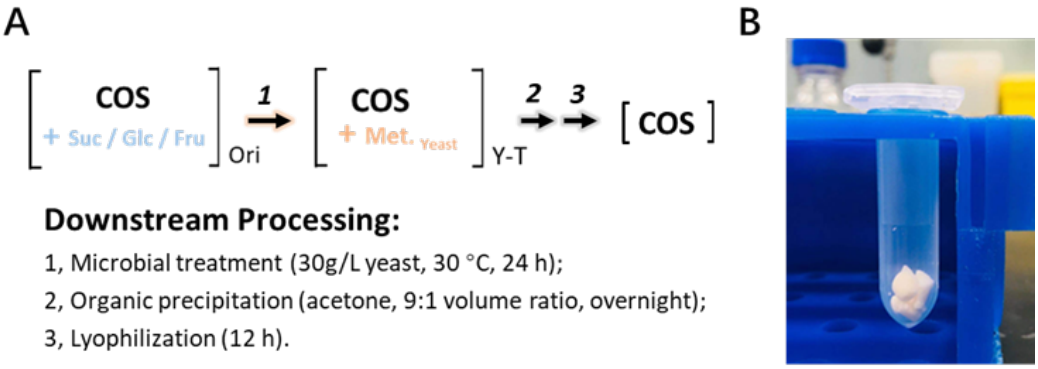
With a high carbohydrate concentration (~200 g/L) in the reaction mixture (Figure S1, Original), the apparent solubility of cello-oligosaccharide (COS) decreased.[1] A certain fraction of the total products gradually became insoluble and precipitated (Figure S1, 1#) after 2-day storage at room temperature.
In comparison, the reaction solution after the yeast treatment (30 g/L yeast, 30 °C, 24 h) showed better stability (almost no precipitation, Figure S1, 2#) over a two-week storage at room temperature.



Supporting Figure S1. Storage stability comparison of the original reaction mixture and the yeast treated solution. **1#**, reaction mixture after 2-day storage at room temperature. **2#**, yeast treated solution after 2-week storage at room temperature. The original solution was obtained from the reaction using 0.5 M sucrose, 0.15 M glucose, and 50 mM phosphate, 20 U/mL *Ba*ScP, 6 U/mL *Cu*CbP and 2 U/mL *Cc*CdP at 45 °C, pH 7.0 for 6 h.

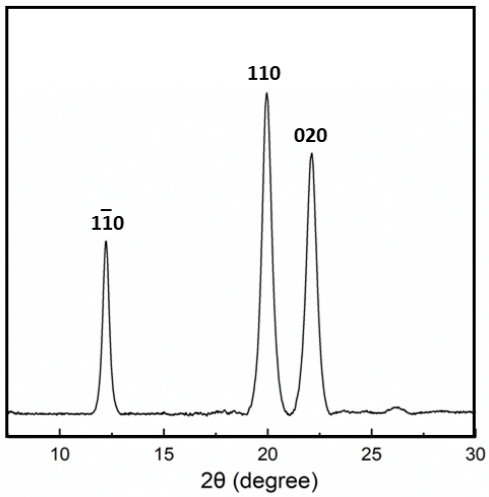
S2. Purification of COS from reaction mixture

Through a simple two-step downstream processing (microbial treatment and acetone precipitation) plus further lyophilization of the precipitated material (Figure S2A), the final product was obtained as a white powder (Figure S2B) with a high purity (≥ 95%) and excellent storage stability.



Supporting Figure S2. A) Scheme of downstream processing for purification of the COS products from reaction mixtures; **B**) Physical appearances of purified products (lyophilized). The original solution (Ori) was obtained from the reaction using 0.5 M sucrose, 0.15 M glucose, and 50 mM phosphate, 20 U/mL *Ba*ScP, 6 U/mL *Cu*CbP and 2 U/mL *Cc*CdP at 45 °C, pH 7.0, for 6 h.

S3. Crystal structure of insoluble product during enzymatic reaction



Supporting Figure S3. Wide-angle X-ray diffraction (WAXD) patterns of the insoluble product during enzymatic reaction. It was obtained from the reaction using 0.5 M sucrose, 0.15 M glucose, and 50 mM phosphate, 20 U/mL *Ba*ScP, 6 U/mL *Cu*CbP and 4 U/mL *Cc*CdP at 45 °C, pH 7.0, 8 h. The product was washed several times with water to remove enzymes and lyophilized before WAXD analysis.

S4. Genomic analysis of putative transport/enzyme systems for COS utilization

Genomic analysis of transporters and catabolic enzymes for COS in probiotic bacteria was carried out via genome searching on GenBank: https://www.ncbi.nlm.nih.gov/. Since the ABC transporter was reported for COS uptake in some bacteria, the genome searching was done using keywords "ABC transporter" or plus "sugar" when necessary. For catabolic enzyme searching, keyword of "phosphorylase" or "glucosidase" was used. The collective information was shown in Table S1.

Table S1. Transporters and enzymes for the COS utilization in bacteria: reported and putative

Microorganisms	DP of COS utilized	Transporter system (TS)*	Catabolic enzyme (CE)*	Genes (TS+CE) *	Ref.
Bifidobacterium breve UCC2003	2 - 5	ABC transporter	β-glucosidase	*cld*EFG + *cdl*C	2
Clostridium thermocellum	2 - 6	ABC transporter	CbP, CdP	*cbp*ABCD	3, 4
Ruminiclostridium cellulolyticum	2 - 5	ABC transporter	CbP**	*cua*ABCD + *cbp*A	5
Streptomyces reticuli	2 - 3	ABC transporter	β-glucosidase	*ceb*EFG + *bgl*C	6
Thermobifida fusca	2 - 3/4	ABC transporter	β-glucosidase	*bgl*AB + *bgl*C	7
Lactococcus lactis	2 - 6	Oligosaccharides ABC transporter	α/β-glucosidase	*ost*C, WP_003130089.1 + WP_021723033.1	8
C. butyricum	2 - 6	Sugar ABC transporter	CbP (+CdP)**	WP_058142197.1, WP_058146511.1 + WP_058146708.1	
Lactobacillus paracasei	2 - 6	Sugar ABC transporter	n.d***	WP_016376967.1, WP_003662470.1	
L. rhamnosus	2 - 6	Sugar ABC transporter	α/β-glucosidase	WP_005685292.1, WP_005684552.1	

* In the reported literature, the gene clusters were revealed to encode ABC transporter + catabolic enzymes responsible for COS utilization. For the probiotic bacteria used in this study, the genes encoding putative sugar ABC transporter including substrate binding protein and permease, and catabolic enzymes possibly related with COS or other oligosaccharides utilization were noted (GenBank: https://www.ncbi.nlm.nih.gov). Abbreviation: CbP, cellobiose phosphorylase; CdP, cellodextrin phosphorylase.

** Other unknown CdP or β-glucosidase is expected, but not revealed yet.[4, 5, 9]

*** No direct enzyme was detected, but a 6-phospho-β-glucosidase was noted that might potentially work as β-glucosidase or link to the PTS system.[10]

References

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