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Isolation, identification, and characterization of a novel polysaccharide degradation enzyme for synthesis of novel oligosaccharides

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Research aims

A recent study identified a novel phosphorylase that acts on sophorose (Glc-*β*-1,2-Glc) in reverse reaction (OGP), thereby providing a simpler enzymatic method for the synthesis of *β*-1,2-glucan and *β*-1,2-glucooligosaccharides (Sop_ns) from sophorose. Sophorose, an expensive sugar, can be replaced by glucose as a starting material for the synthesis of Sop_ns. Although polymers are easily produced at an early stage of this reaction, the accumulation of Sop_ns is considerably slower when using glucose as a starting material, compared to sophorose. The addition of a *β*-1,2-glucan degrading enzyme to the reaction system would likely accelerate oligosaccharide synthesis; however, such an enzyme has yet to be identified. The aims of this study are described below:

1. Develop a method for the large-scale synthesis of *β*-1,2-glucan using OGP.
2. Isolate and identify an enzyme that degrades *β*-1,2-glucan, using the synthesized polymer.

Methods

We first developed a method for large-scale synthesis of *β*-1,2-glucan. As depicted in Fig. 1, two steps were combined to construct a one-pot reaction system: The phosphorolysis of sucrose by sucrose phosphorylase (SP),[1] generating fructose and glucose 1-phosphate (Glc1P), and the utilization of this Glc1P for *β*-1,2-glucan synthesis by OGP.

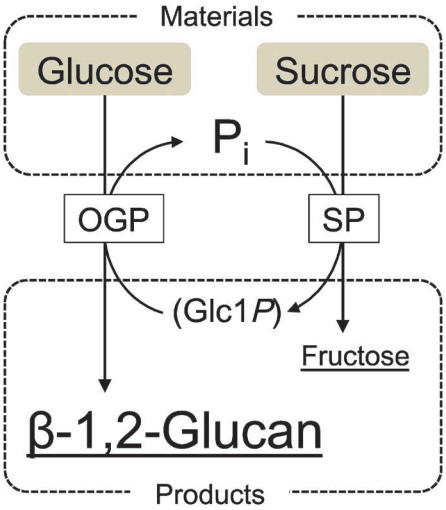

Fig. 1. A schematic of 1,2-*β*-glucan synthesis.
The parenthesis represents an intermediate of the reaction.

The optimal synthesis conditions were determined by measuring the levels of *β*-1,2-glucan production at an early stage of the reaction, using thin layer chromatography (TLC). The glucose concentration was optimized first, in the presence of fixed concentrations of sucrose and inorganic phosphate (1.0 M and 0.1 M, respectively), followed by the SP to OGP ratio. Large-scale synthesis reactions (2 L) were then prepared and incubated for 12 days until the available sucrose in the media was spent. Reaction enzymes were removed from the mixture using the anion-exchange resin DEAE cellulose, and the solution was treated with yeast to remove unnecessary sugars. *β*-1,2-Glucan was precipitated by mixing the reaction solution with an equal volume of ethanol, and the resulting precipitate was dried.

To identify *β*-1,2-glucan-degrading enzymes, five bacterial species were cultured using purified *β*-1,2-glucan as a sole carbon source: *Listeria innocua*, which possesses the *ogp* gene used in this study; *Enterococcus faecalis*, which possesses an *ogp* homolog, and whose genome sequence is available; *E. coli*; *Chitinophaga arvensicola*, the only bacterium previously reported to exhibit hydrolytic activity on *β*-1,2-glucan[2]; and *Chitinophaga pinensis*, a species related to *C. arvensicola*, and whose genome sequence is available. Culture filtrates and cell free extracts were assayed for degradation of *β*-1,2-glucan by TLC and detection of reducing activity. The *β*-1,2-glucan degrading enzyme was purified using three chromatography steps. Peptides extracted from the purified enzyme by in-gel digestion using trypsin or chymotrypsin were analyzed by LC/MS/MS. A pool of peptide sequences was constructed by *de novo* sequencing using PEAKS online, and high confidence peptides were selected from this pool. A candidate gene was selected by performing a BLAST search of the selected peptides. The selected gene was labelled with a His-tag sequence and expressed in *E. coli*. The recombinant gene product was purified by nickel column chromatography and assayed for substrate specificity and degradation of *β*-1,2-glucan.

Results

Large-scale synthesis of *β*-1,2-glucan

We first compared *β*-1,2-glucan synthesis in the presence of various concentrations of glucose, at an early stage of the reaction. *β*-1,2-glucan production increased in a glucose-dependent manner up to a concentration of 0.5 M. As glucose concentrations greater than 0.5 M failed to influence *β*-1,2-glucan production, 0.5 M was utilized as the optimal glucose concentration for the synthesis reaction. Next, we assessed the effects of various SP to OGP ratios on *β*-1,2-glucan synthesis. Reducing the SP concentration to one-fourth the concentration of OGP did not significantly affect the rate of *β*-1,2-glucan synthesis. As a result, a 1 : 4 ratio of OGP to SP was utilized. A large-scale reaction was then performed using the optimized conditions. The reaction was carried out for 12 days and yielded over 800 mM of *β*-1,2-glucan (glucose concentration equivalent). After precipitation, the final amount of purified *β*-1,2-glucan was approximately 167 g from 1 L of the reaction mixture.

Purification and identification of a *β*-1,2-glucan-degrading enzyme

Of the bacterial species tested, only *C. arvensicola* exhibited significant growth in media containing *β*-1,2-glucan as a sole carbon source. The degrading activity of *β*-1,2-glucan was significantly greater in the cell free extract than in the culture filtrate. As a result, the bacterial cell free extract was utilized to purify and identify the putative *β*-1,2-glucan-degrading enzyme. After separation by SDS-PAGE, a 45-kDa band was identified that was present only in fractions that exhibited hydrolytic activity on *β*-1,2-glucan. Peptides were extracted from this band by treatment with trypsin or chymotrypsin and analyzed by LC/MS/MS. Mascot analysis failed to obtain a confident candidate for a *β*-1,2-glucan degrading enzyme from *C. pinensis*, a related bacterium whose genome is available. We therefore generated a *de novo* sequence using the two results of LC/MS/MS analysis after trypsin and chymotrypsin treatment. Seven peptide sequences were common between the two digests and were selected for use in a BLAST search. Four of the seven peptides exhibited identity to the protein, SGL. The recombinant SGL degraded *β*-1,2-glucan, *in vitro*, with sufficient velocity to release oligosaccharides with DP of 2–5 as end-products. These results indicate that SGL is indeed a *β*-1,2-glucan-degrading enzyme.

Conclusion

We generated a large-scale method for production of *β*-1,2-glucan from sucrose and glucose. SGL was identified from the cell free extract of *C. arvensicola* cultured with *β*-1,2-glucan as a sole carbon source. While *in vitro* analyses verified that SGL is capable of degrading *β*-1,2-glucan, the amino acid sequence of SGL suggests it does not belong to any known glycoside hydrolase (GH) family and may therefore comprise a new family of GHs.

References

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