Construction, expression and application of microbial β-1,3-glucanase for β-1,3-glucan oligosaccharide production

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Abstract

β-1,3-Glucan oligosaccharides produced by enzymatic hydrolysis of β-1,3-glucans are of great interest, because they possess biological functionalities such as regulating intestinal health, improving immunity and anti-tumor. A significant qualification for the industrial production of glucan oligosaccharides is β-1,3-glucanase with high catalytic ability. These enzymes are widely present in fungi, bacteria, plants and invertebrates, they are separated into glycoside hydrolase families 3, 5, 16, 17, 55, 64, 72, 81, 128 and 132. This report presents the research progress on the construction and expression of β-1,3-glucanases in Pichia pastoris and Escherichia coli from different microorganisms. Culture conditions and strategies to improve enzyme activity in these hosts are summarized. The application of β-1,3-glucanases in different β-1,3-glucans (curdlan, scleroglucan, laminarin, and schizophyllan) degradation and their hydrolysate analysis are reviewed. This review provides an important reference for further modification and utilization of microbial β-1,3-glucanases in large-scale production of multi-functional oligosaccharides.

May 14, 2022

To,

Editor-in-Chief

Biotechnology Journal

Subject: Manuscript submission

Dear Prof. Alois Jungbauer:

Please consider our manuscript entitled "**ὀνστρυςτιον, εξπρεσσιον ανδ αππλιςατιον οφ μιςροβιαλ β-1,3-γλυςαναση φορ β-1,3-γλυςαν ολιγοσαςςηαριδε προδυςτιον** " for publication as a Review Article in**Biotechnology Journal** .

β-1,3-Glucan oligosaccharides produced by enzymatic hydrolysis of β-1,3-glucans have attracted great interest, because they possess biological functionalities such as disease-resistance in plants, immuno-modulating activities in animals and anti-tumor properties. β-1,3-Glucanase with high specific activity is a prerequisite for the industrial preparation of glucan oligosaccharides. These enzymes are widely distributed in bacteria, fungi, plants and invertebrates, and are classified into glycoside hydrolase families 3, 5, 16, 17, 55, 64, 72, 81, 128 and 132. Different endo-β-1,3-glucanases produce various hydrolysates with different degrees of polymerization, which can be used not only to prepare oligosaccharides with multiple biological functions, but also to understand chemical structures and biological functions of polysaccharides.

The early research of our team focused on the fermentation condition optimization for the production of β-1,3-glucanase by wild-type*Trichoderma harzianum* GIM 3.442. Coupled fermentation systems of*T.*

harzianum GIM 3.442 and *Agrobacterium* sp. ATCC 31749/*Sclerotium rolfsii* WSH-G01/*Schizophyllum commune*GDMCC 5.43 were established for producing linear and branched β-1,3-glucooligosaccharides. Then, endo-β-1,3-glucanase gene (*BGN*) from *T. harzianum* was expressed in *P. pastoris* GS115 with promoter optimization (pGAP), and a mutant endo-β-1,3-glucanase was obtained by implementing error-prone PCR technology, and the specific activity towards curdlan was much higher than the original enzyme (292 U/mg) and other reported enzymes, such as recombinant Blg32 from *Bacillus lehensis* (233.01 U/mg) and Endo23 from *Trichoderma teesei* GIMCC 3.498(1.52 U/mg). One-step production of functional branched glucan oligosaccharides was established with coupled fermentation of *P. pastoris* and *S. rolfsii* , the maximum yield of β- glucan oligosaccharides (DP 2–17) was 12.71 g/L in a 7-L bioreactor.

In order to improve the endo-β-1,3-glucanase catalytic efficiency and expression level, great efforts have been made to develop effective enzyme engineering and fermentation strategies. Numerous genes encoding endo-β-1,3-glucanases have been isolated and characterized from various organisms, and can be produced in high yields by recombinant expression methods. This report presents the research progress on the construction and expression of β-1,3-glucanase in *Escherichia coli* and*P. pastoris* from different microorganisms. Culture conditions and strategies to improve enzyme activity in these hosts are summarized. The application of β-1,3-glucanase in degradation of different β-1,3-glucans (curdlan, scleroglucan, laminarin, and schizophyllan) and their hydrolysate analysis are reviewed. It also summarizes the structural differences and healthy functional activities of different types of oligosaccharides. This study provides an important reference for further modification and utilization of microbial β-1,3-glucanases in large-scale production of multi-functional oligosaccharides.

I, as the corresponding author, on behalf of all co-authors of the paper declare that this manuscript has not been previously published and is not currently submitted for review to any other journal. All authors declare no conflict of interest. We would greatly appreciate your kind attention on this submission and look forward to the helpful comments from you and other referees.

Please let us know if we can provide any additional information to facilitate your assessment of the suitability of our work for publication in *BTJ* .

Sincerely yours,

Minjie Gao

The list of SCI journals papers published by the corresponding and first authors

1. Gao, M. J., Xu, Y., Yang, G. S., Jin, S. X., Hu, X. Y., Jiang, Y., Zhu, L., Li, Z. T., & Zhan, X. B. (2021). One-step production of functional branched oligoglucosides with coupled fermentation of*Pichia pastoris* GS115 and *Sclerotium rolfsii* WSH-G01.***Bioresource Technology***, 335* , 125286.
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