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A Commentary on Development of Efficient Enzyme Cocktails for the Bioconversion of Hemicelluloses

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Abstract

The construction of minimum enzymes cocktails could facilitate the bioconversion of specified feedstocks into monosaccharides or XOS. In recent reported researches, the hemicelluloses and recombinant hemicellulases, including endo-ß-1,4-xylanases, ß-xylosidases, α-L-arabinofuranosidases, acetyl xylan esterase, feruloyl esterase and mannanase as well as their interaction mechanisms were investigated by enzymatic hydrolysis. Four representative interesting works on exploring the synergistic mechanisms and effects between enzymes were reported in this paper. Recombinant enzymes expressed from *Pichia pastoris* were characterized to reveal the mechanism during the process. This study contributes to the development of efficient enzyme cocktails for the bioconversion of hemicelluloses into monosaccharides and XOS.

Keywords: Hemicelluloses; Bioconversion; Enzyme cocktails; Xylo-oligosaccharides; Recombinant enzymes

Introduction

Hemicellulose in biomass is a long chain polymer of monosaccharide units with an easily hydrolyzed amorphous structure. Hemicellulose consists mainly of xylan, which combines cellulose microfibrils through hydrogen bonds and cross-links with lignin to form lignin-carbohydrate complex [1,2]. Xylan is characterized by a linear ß-1,4-linked backbone of xylosyl residues substituted by arabinose or 4-O-methylglucuronic acid [3,4]. It is reported that xylo-oligosaccharides (XOS) and arabinoxylo-oligosaccharides (AXOS), as the intermediate products of hemicellulose hydrolysis, are value-added food ingredients that can be produced through enzymatic hydrolysis of arabinoxylan-containing biomass [5,6]. Xylanase were widely used in application for degrading hemicellulose. The efficient process on enzymatic hydrolysis of hemicelluloses into XOS or monosaccharides with the possible minimum enzyme loading is of great importance for the biorefinery industry [7-9].

The hydrolysis of hemicelluloses by enzymes from fungus such as *Aspergillus niger*, *Hypocrea orientalis* and *Trichoderma reesei* were previously compared [10]. The enzymes from *A. niger* showed a high efficiency in producing monosaccharides, whereas the enzymes from some fungi, such as *T. reesei* and *H. orientalis*, were more preferable for obtaining XOS, arabinoxylo-oligosaccharides or ferulic acid [11]. However, considering the complex structure of hemicelluloses and the wide range of glycosyl hydrolases (GH) in crude enzymes, the interaction mechanisms between hemicelluloses and enzymes in the hydrolysis process were difficult to be researched with characterized hemicelluloses and crude enzymes [12]. Determination of the exact chemical structure of hemicelluloses and the investigation of the interactions between hemicelluloses and hemicellulases could facilitate the construction of minimum enzymes cocktails for the bioconversion of specified feedstocks into monosaccharides or XOS [13]. The coordinated action of enzymes could significantly improve the hydrolysis of hemicellulose. Here we give four representative interesting examples in the recent researches.

The first favorable study is that hemicellulases including endo-ß-1,4-xylanases (HoXyn11A and AnXyn10C), ß-xylosidases (AnXln3D) and α-L-arabinofuranosidases (AnAxh62A) from *H. orientalis* and *A. niger* were heterologously expressed at a high level and well characterized [14]. The popular tools for the structural characterization of isolated hemicelluloses were reverse-phase high-performance liquid chromatography with ultraviolet detection (RP-HPLC-UV), Fourier transform infrared spectroscopy (FT-IR) and nuclear magnetic resonance (NMR) thin-layer chromatography (TLC) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). The end products released from the hemicellulose by the action of the enzymes were different. AnXyn10C released shorter end products than HoXyn11A from isolated hemicelluloses. AnAxh62A was able to release all single-substituted α-L-arabinofuranosyl residues from hemicellulose. AnXyn10C and HoXyn11A were able to directly act on holocellulose, whereas AnAxh62A and AnXln3D did not. On the basis above, the synergistic hydrolysis of hemicellulose by these recombinant enzymes was investigated. The recombinant enzymes exhibited different synergistic effects. The combination of HoXyn11A and AnAxh62A produced the highest xylo-oligosaccharides (XOS) yield from hemicellulose, whereas AnXyn10C alone produced the highest XOS yield from holocellulose.

Another example, the research on synergistic cooperation between a novel acetyl xylan esterase (heterologous expressed at high levels in this study) and four other xylan-degrading enzymes (reported previously) were performed in our previous study [15]. The acetyl xylan esterase (AnAxe) gene was cloned from *Aspergillus niger* and expressed in *Pichia pastoris*. The AnAxe was expressed with a molecular weight of 31 kDa and exhibited maximal specific activity of 480.2 IU/mg at pH 7.0 and 40°C. A significant synergistic effect was determined between AnAxe and the other four xylan-degrading enzymes, including endo-ß-1,4-xylanases, ß-xylosidases, α-L-

arabinofuranosidases and α-glucuronidases. The highest degree of synergism was obtained between AnAxe and endo-ß-1,4-xylanases/ß-xylosidases. The prominent synergistic effect between AnAxe and xylan-degrading enzymes may provide one method to obtain high sugar yields with low enzyme dosage.

The third classical work reported here is the ferulic acid (FA) and xylooligosaccharides (XOS) generated based on the synergistic action of two xylan-degrading enzymes, xylanase (AnXyn11A) and feruloyl esterase (AnFaeA), which were cloned from Aspergillus niger and heterologously expressed at high levels in Pichia pastoris [16]. AnXyn11A exhibited a maximal activity of 240 U mL^{-1} and AnFaeA showed a maximal activity of 21 U mL^{-1}. Both of them displayed high specific activity and thermostability at 60. The ratio of FA released from destarched wheat bran (DSWB) under the synergistic action of AnXyn11A and AnFaeA increased 70%, compared to using AnFaeA alone. The XOS yield was almost doubled in the optimum level of enzyme synergistic cooperation. Therefore, the synergistic cooperation between AnXyn11A and AnFaeA provide promising value-added product efficiencies.

The fourth example was reported by Guo, the construction of chimeras of xylanase and mannanase expressed in *Pichia pastoris* to enhance the synergistic action of enzymes on hemicellulose [17]. The results presented that the types of peptide linker, the length of linker and the integration order of two enzymes had great effects on the synergistic efficiencies toward the hydrolysis. It revealed that the synergistic action of enzymes on hemicelluloses could be strengthened by constructing proper chimeras.

Therefore, the synergistic action between various enzymes played an important role. Future potential research directions are mainly focused on the heterologous expression of hemicellulases and its synergistic effect in hydrolyzing hemicellulose for co-production of high value-added products. It is interesting and meaningful to research the heterologous expression of acetyl xylan esterase and its synergistic cooperation with other side-chain degrading enzymes in degrading hemicellulose with a complex structure. It has been verified that acetyl xylan esterases cooperating with other side-chain degrading enzymes showed excellent sugar yields with less enzyme dosage [15]. The cleavage of the side groups may contribute to the relaxation of the cell wall structures and reduce the steric hindrance, resulting in more binding sites for hemicellulases. It must be useful for developing a promising process for hemicellulose hydrolysis.

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