Construction of an *Aspergillus* enzyme library for the development of novel materials

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Research objective

Microorganisms produce many types of enzymes, such as glycosidases, proteases, and lipases, which degrade and assimilate biopolymers, including polysaccharides and proteins. For example, the genome of *Aspergillus oryzae*, an industrially important fungus, encodes more than 300 putative glycosidases. However, only 10%–20% of these putative glycosidases have been characterized, and most still remain uncharacterized. In previous studies, we identified and characterized some *A. oryzae* glycosidases, such as isoprimeverose-producing oligoxyloglucan hydrolase (IpeA)[1] and β-galactosidase (LacA)[2]. These glycosidases play vital roles in the degradation of xyloglucan, one of the major hemicellulosic polysaccharides in plant cell walls and seeds. Our previous research indicated that functional prediction of enzymes from amino acid sequences is an effective approach; nevertheless, *in vitro* assays are necessary to elucidate the actual roles of these enzymes.

In this study, an expression library of putative secretory proteins of *A. oryzae* was constructed to screen for novel enzymes.

Methods

The genome of *A. oryzae* (coding for approximately 11,000 proteins) was mined for secretory proteins carrying an N-terminal signal peptide using SignalP (http://www.cbs.dtu.dk/services/SignalP/)[3,4]. Transmembrane helices in the putative secretory proteins were predicted using the TMHMM server (http://www.cbs.dtu.dk/services/TMHMM/).

The coding sequences of genes encoding putative secretory proteins were amplified by polymerase chain reaction (PCR) using a cDNA mixture as template. The cDNA mixture was synthesized from total RNA of *A. oryzae* cells cultured under various conditions. Each amplified DNA fragment was cloned into the *Saccharomyces cerevisiae* 2-micron plasmid, containing a *TDH3* promoter, an N-terminal α-factor secretion signal sequence, and a *TDH3* terminator. The amplified DNA fragments were cloned in frame with the N-terminal α-factor secretion signal sequence and expressed as fusion proteins in *S. cerevisiae* cells.

Results

Approximately 1100 putative secretory proteins, carrying an N-terminal signal peptide for secretion, were identified in the *A. oryzae* genome. Proteins that were predicted to have multiple transmembrane domains were excluded from the analysis. We selected approximately 550 genes and designed oligo DNA primers to amplify them. Approximately 520 genes were successfully amplified by PCR using a cDNA mixture as template, among which approximately 500 genes could be cloned into the *S. cerevisiae* expression vector (Fig. 1).

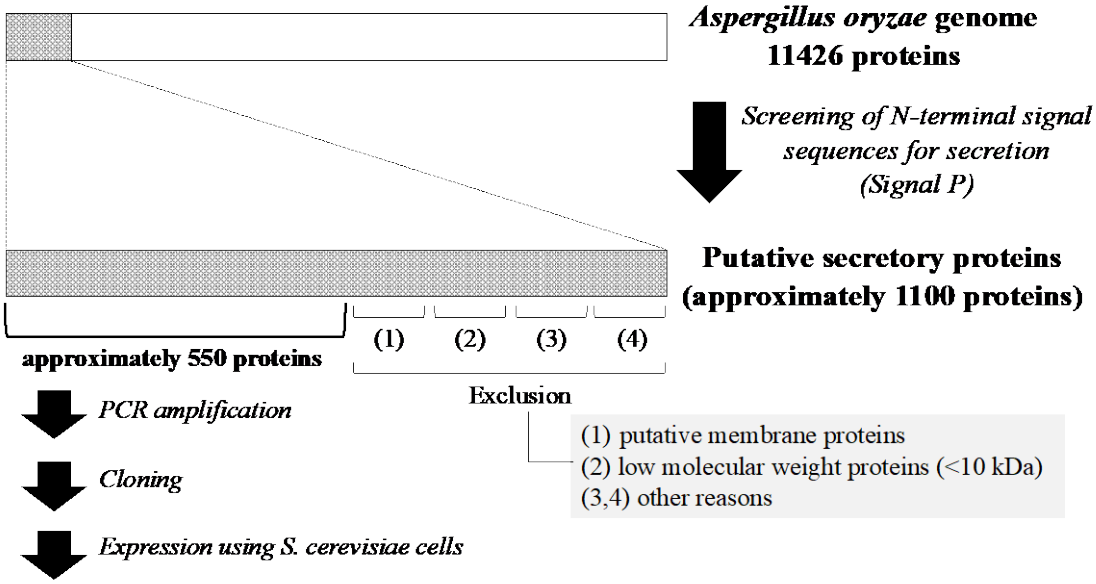

Figure 1. Procedure for the construction of the *A. oryzae* secretory protein library and for the performance of enzymatic assay.

Next, vectors containing the putative secretory protein genes of *A. oryzae* were introduced into *S. cerevisiae* cells. These were cultured in 96-well plates, and the culture supernatants were used for enzymatic activity assay. In this assay, insoluble chromogenic substrates, namely AZCL-xyloglucan, AZCL-xylan, and AZCL-arabinoxylan, and soluble chromogenic substrates, namely *p*-nirtophenyl (*p*NP) β-D-glucopyranoside, *p*NP β-D-galactopyranoside, *p*NP β-D-xylopyranoside, *p*NP α-D-glucopyranoside, *p*NP α-D-xylopyranoside, *p*NP α-L-arabinopyranoside, *p*NP α-L-fucopyranoside, and *p*NP α-L-rhamnopyranoside, were used for endo- and exo-type glycosidases, respectively. I found that some culture supernatants of *S. cerevisiae* cells expressing *A. oryzae* secretory proteins were able to degrade chromogenic substrates (Fig. 2), indicating that these *S. cerevisiae* cells harbored genes involved in oligo- and polysaccharide degradation.

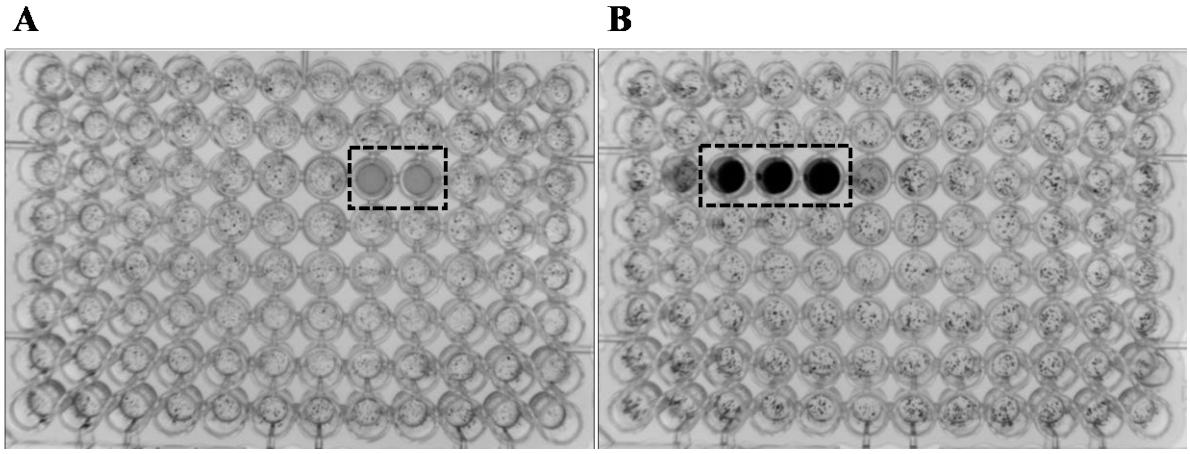

Figure 2. Screening of enzymes catalyzing endo-type polysaccharide degradation using insoluble chromogenic substrates. Culture supernatants of *S. cerevisiae* cells harboring an *A. oryzae* secretory protein library were incubated with AZCL-xyloglucan (A) or AZCL-xylan (B). The wells showing degradation activity toward these substrates are included in dashed rectangles.

Conclusion

In this study, I constructed a secretory protein library of *A. oryzae* and screened for novel enzymes. I believe that this library can be useful for protein screening; however, some concerns about its quality and comprehensiveness still remain. Therefore, our future efforts will focus on the improvement of this library and a more comprehensive screening of novel enzymes.

References

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