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Regulation mechanisms of cellulase gene expression in *Aspergillus aculeatus*

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Research aims

The filamentous fungus, *Aspergillus aculeatus* No. F-50, secretes a broad spectrum of polysaccharide-degrading enzymes that cooperatively hydrolyze pulp with those from *Hypocrea jecorina* (anamorph *Trichoderma reesei*), which is known to be one of the strongest cellulase producers.[1–3] Biochemical analyses of those enzymes revealed that hemicellulases and β-glucosidase secreted by *A. aculeatus* mainly contributed to effective saccharification of cellooligosaccharides to glucose. However, the regulation mechanisms of those genes are still insufficiently understood in *A. aculeatus*.

It has been revealed that Xyr1, which is a homolog of transcriptional activator XlnR of *Aspergillus species*, governed the inductions of all cellulase and hemicellulase gene expressions in *T. reesei*.[4] However, I revealed that an XlnR-independent signaling pathway existed and regulated cellobiohydrolase I (*cbhI*) and carboxymethyl cellulase 2 (*cmc2*) gene expressions in *A. aculeatus*. This XlnR-independent signaling pathway is supposed to be regulated by a novel transcription factor. To better understand regulation mechanisms of the XlnR-independent pathway in *A. aculeatus*, I attempted to identify physiological inducers and *cis*-elements involved in the XlnR-independent signaling pathway.

Methods

Gene expression profiles were investigated by semi-quantitative or quantitative RT-PCR. RNA was isolated from mycelia grown under the following conditions: *A. aculeatus* wild type was grown in minimal medium (MM) supplemented with 1% glucose for 24 hours at 30°C. Then, mycelia were harvested, washed with carbon free MM, and grown in fresh MM supplemented with various carbon sources for 3 hours. In the case of GUS assay, mycelia were grown in MM supplemented with 1% avicel or 1% arabinose for another 24 hours after 24 hours preincubation.

Aspergillus transformation was done by protoplast-PEG method. Reporter plasmids possessing modified *cbhI* promoters were integrated at *sC* locus of *A. aculeatus*. Activities of the GUS in transformants were assayed using pNP-β-glucuronide at 37°C. Proteins were extracted from mycelia using GUS assay buffer, after grinding with Micro Smash (Tomy, Japan). Protein levels were standardized using Protein assay reagent (Biorad, Japan).

Results

1) Effects of β-linked disaccharides on the *cbhI* gene expression

It is predicted that β-linked disaccharides derived from hydrolysis of cellulose could function as the physiological inducer for cellulase gene expression in filamentous fungi. Therefore, I investigated which β-linked disaccharides effectively induced the *cbhI* expression regulated via XlnR-independent pathway by quantitative RT-PCR. RNA was isolated from mycelia of *A. aculeatus* wild type grown for 3 hours in minimal medium supplemented with 3 mM glucose, 3 mM sophorose, 3 mM cellobiose, or 3 mM gentiobiose as a sole carbon source. To directly assess the effect of disaccharides on the *cbhI* expression, glucosidase inhibitor, nojirimycin, was added to culture during induction. Since addition of nojirimycin might cause carbon starvation, transcripts of the *cbhI* were also analyzed under carbon free condition. Among tested disaccharides, cellobiose induced the *cbhI* gene expression the most effectively. However, lactose did not induce the *cbhI* expression. These results suggest that the hydroxyl group on carbon 4 is crucial for the *cbhI* induction.

Although cellobiose effectively induced the *cbhI* expression, nojirimycin was necessary for the induction. Therefore, I assessed how much concentration of cellobiose was necessary for the sufficient *cbhI* expression with nojirimycin added. A significant induction of the *cbhI* was observed when more than 30 nM of cellobiose and nojirimycin were added. Km value of β-glucosidase for cellobiose is larger than hundred μM. These results suggest that the *cbhI* gene can be induced in response to cellobiose if a low concentration of cellobiose is supplied continuously.

2) Identification of *cis*-elements required for the *cbhI* induction

Next, I performed functional analysis of truncated and modified *cbhI* promoters using GUS as a reporter to identify *cis*-element(s) sufficient for the *cbhI* induction. Reporter plasmids possessing those modified promoters were introduced at *A. aculeatus sC* locus as a single copy. The attached −657 promoter region induced the GUS gene in response to an inducing carbon source, avicel. This expression level was 45-fold higher than that with a noninducing carbon source, arabinose. This result proved that *cis*-element(s) sufficient for the *cbhI* induction lay in the attached

promoter region. Although truncation of the promoter sequence to −469 had no effect on GUS expression, further truncation from −469 to −192 resulted in the reduction of gene expression level and the loss of inducibility. Conserved sequences were searched in this region and three conserved regions, namely A, B, and C, were found. Mutations of B and C region resulted in the reduction of the gene expression and induction level. However, the inducibility was lost only when all regions were mutated. These results indicated that three regions were required for complete gene induction by *cbhI* promoter. Common sequence among three conserved regions was identified as $CCGN_2CCN_7G$ which was quite similar to CeRE found as an element required for endoglucanase A gene induction in *A. nidulans*.[5] Now, we are planning to identify a factor recognizing the conserved sequence.

Conclusion

I revealed that cellobiose effectively induced the *cbhI* expression regulated via an XlnR-independent pathway in *A. aculeatus*. I assumed that cellobiose could not induce the *cbhI* expression since β-glucosidase would hydrolyze cellobiose immediately at first. However, my data suggested that cellobiose could be a physiological inducer since a low concentration of cellobiose effectively induces the *cbhI* expression. This signal is transmitted to the conserved sequence, namely, $CCGN_2CCN_7G$, in the *cbhI* promoter region and triggered the gene induction. Now, I try to identify the factors involved in this signal transduction pathway.

References

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