NISR Research GRANT
2009 Young Investigator Research Grant

Identification and Application of New Genes Involved in Glycosylation in Filamentous Fungi

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Research aims

Most of the enzymes from *Aspergillus*, including *koji* mold, are glycosylated, which improves both the functioning and efficacy of these enzymes. Although galactofuranose and glucose residues have been observed to be attached to the *O*-glycans and *N*-glycans of proteins in *Aspergillus*, the glycosyltransferase genes involved in the biosynthesis of these sugar residues have not yet been identified and the function of these sugar moieties also is not yet known[1].

The ability of filamentous fungi to secrete large quantities of proteins means that they are well suited for use as expression hosts for extragenous genes in industrial applications. However, since the sugar moieties of glycoproteins from filamentous fungi are antigenic in humans, they cannot be used for medicinal applications, such as antibody preparation. Consequently, a method for altering the structure of these sugar moieties needs to be developed so that a protein expression system using filamentous fungi can be developed.

We therefore sought to identify the genes involved in the biosynthesis of these sugar moieties in filamentous fungi, and to elucidate the function of these sugar moieties in fungal growth and protein secretion.

Methods

Using the Carbohydrate Active Enzymes (CAZy) database, approximately 90 putative glycosyltransferase genes were identified in the *Aspergillus nidulans* genome[2,3]. After excluding those genes having high sequence homology with genes of known function in other organisms, 19 putative candidate glycosyltransferase genes remained (Table 1). Each of the 19 glycosyltransferase genes were then disrupted and the resulting phenotypes were examined.

The Δ*nkuB* strain of *A. nidulans* (AKU89), which has a high gene-targeting frequency, was used as the host[4]. For each of the 19 target genes, a gene replacement cassette, in which a selection marker gene (*argB*$^+$) was flanked by the 5′- and 3′-flanking sequences of the target gene, was constructed by recombinant PCR. First, the 5′- and 3′- flanking regions of the *A. nidulans* genomic DNA were amplified using primer pairs FC/R1 and F3/RC, respectively, and *argB* of pDC1 (Fungal Genetics Stock Center) was amplified using primers F2 and R2. Then, after the three purified DNA fragments were combined as a template and the gene replacement cassette was amplified using primers F1 and R3 using Phusion DNA polymerase (Finnzymes). The amplified DNA fragments were then transformed into strain AKU89 using the protoplast-PEG method (Fig. 1). Transformants were analyzed for correct gene replacement by PCR and Southern blot analysis.

Results

To analyze the function of the putative glycosyltransferase genes, we disrupted each of the putative genes in *A. nidulans* AKU89 by gene replacement with *argB*$^+$.

Strain ΔAN8677 formed a fragile colony after incubation on minimal medium (MM medium) at 30°C for 3 days. Growth defects was not almost recovered on MM medium in the presence of 0.6 M KCl. The growth rate of strain ΔAN8677 (0.24 mm/hr) was 66% of that for the wild-type strain (0.35 mm/hr). The conidiation efficiency of strain ΔAN8677 (6.6×10^7/mm^2) was reduced to approximately 10% of that for the wild-type strain (6.0×10^8/mm^2). Similarly, monosaccharide analysis of the cell wall revealed that the galactose residues in the ΔAN8677 stain were reduced to 63.7% compared to the wild-type strain. It therefore seems likely that the AN8677 gene is involved in the

Table 1. List of putative glycosyltransferase genes

Systematic Name	GT family No.
AN2923	GT25
AN5058	GT25
AN6460	GT25
AN10604	GT25
AN2015	GT31
AN5663	GT31
AN8677	GT31
AN4824	GT31
AN7535	GT31
AN8627	GT31
AN11144	GT31
AN11697	GT31
AN4092	GT69
AN4738	GT69
AN7483	GT69
AN6571	GT71
AN6857	GT71
AN0246	GT90
AN4084	GT90

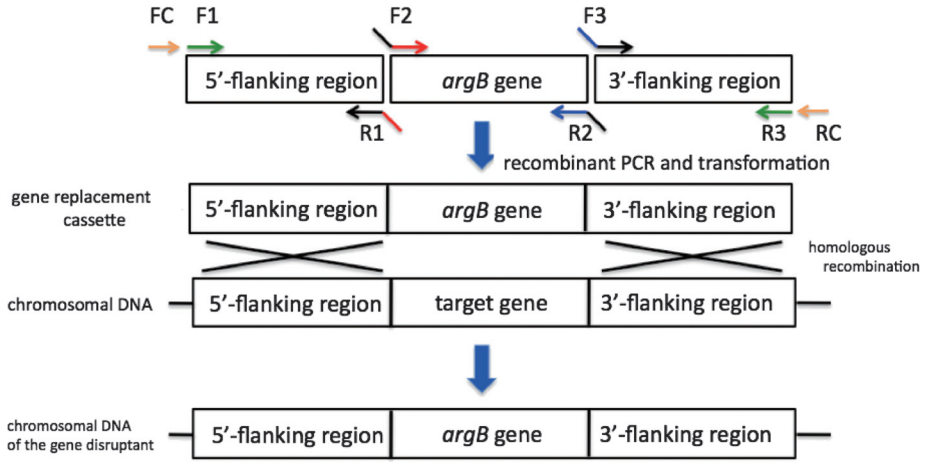

Fig. 1. Strategy of the gene disruption

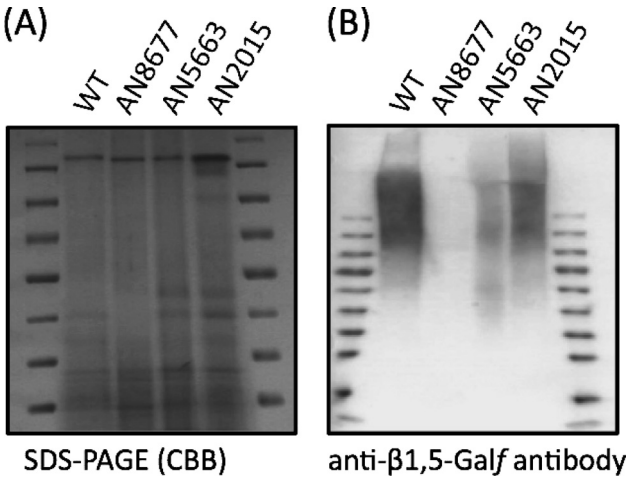

Fig. 2. Western blotting analysis of the cell wall protein fraction

biosynthesis of glycans in the cell wall.

Cell wall proteins were then extracted from strain ΔAN8677 and the disruptants of orthologs AN5663 and AN2015. Western blotting analysis of the cell wall protein fraction using anti-β1,5-galactofuranose antibody revealed that β1,5-galactofuranose residues were not present in the cell wall of the strain ΔAN8677 (Fig. 2).

Conclusion

Of the 19 disruptants, a reduction in the growth rate and conidia formation was observed in strain ΔAN8677. In addition, no β1,5-galactofuranose residues were observed in the cell wall proteins of the mutant. These results demonstrated that the AN8677 gene is involved in the biosynthesis of β1,5-galactofuranose residues.

References

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