

## 4. Conclusion and future aspects

Particular *Ophiostoma* species such as *O. piliferum* are industrially and economically important microorganisms which have been widely used to prevent blue staining in the wood industry and to degrade wood extractives in the paper and pulping industry. Most previous studies have focused on the biochemical aspects rather than molecular genetics of sapstaining *Ophiostoma* spp. This work is the first attempt to develop a novel expression system using *Ophiostoma* as the expression host featuring a strong homologous *amy1* gene promoter isolated from *O. floccosum* as the driving force for the expression of recombinant proteins of interest.

Initially, the protein secretion capability of the parental strain *O. floccosum* J2026 was enhanced by five rounds of UV mutagenesis. Total protein concentration and amylase activity in the culture supernatant of the best mutant MQ.5.1 were increased by 6 and 240 times compared to the parental strain, respectively. It should be noted that even after this notable improvement of secreted proteins was obtained, *Ophiostoma* is not yet competitive with currently available fungal hosts applied in industry for bulk production of recombinant enzymes. However, *Ophiostoma* may found uses at a specific niche addressing *in situ* expression of recombinant proteins by a whole organism.

The series of mutants generated in this study have slightly different enzyme profiles. Proteinase profiles in the culture supernatants of the parental strain and selected key mutants were also characterized. Different strains may be used as hosts for the expression of recombinant proteins sensitive to particular proteinases to decrease potential degradation of the gene products.

Several dominant secreted proteins were identified in the culture supernatants of the parental strain and selected key mutants. The structural gene and the regulatory sequences of one of the dominant proteins  $\alpha$ -amylase were isolated and characterized with a view of using the  $\alpha$ -amylase (*amy1*) gene promoter for expression of gene products of interest. The predicted  $\alpha$ -amylase protein featured a catalytic domain at the N-terminus and a starch binding domain at the C-terminus, which are connected by a linker region. Detailed studies revealed that three *amy1* transcripts were generated from a single gene locus. The formation of three transcripts was due to the differential splicing events of introns. The *amy1* gene promoter and its regulatory sequences were

used further to construct a series of expression vectors. In this study, five expression vectors were constructed, however, several other dominant proteins identified, such as the glucoamylase and subtilisin-like serine proteinase, provide material for the construction of additional expression cassettes.

During this study, genetic transformation of *Ophiostoma* was the biggest challenge. There were two major difficulties, lack of a suitable selection marker and low transformation efficiency. Eventually, a modified protoplast transformation protocol based on previously published studies (Wang et al., 1999) was adopted and an alternative screening approach based on expression and detection of thermostable xylanase activity was developed to confirm *Ophiostoma* transformation. Notwithstanding this improvement, the transformation efficiency still remained low and would need to be optimized further. This seems possible based on recent efforts by several groups (Bernier, 2006; Hoffman and Breuil, 2004b) featuring application of *Agrobacterium* Ti plasmids and exploration of *Ophiostoma* transposons. Since the transformation efficiency seems different with different *Ophiostoma* strains, other mutant strains than MQ.5.1 or other *Ophiostoma* spp. may be explored for easier transformation.

Finally, a thermostable bacterial xylanase B was successfully expressed in *the* mutant MQ.5.1 under the *amyI* gene promoter. This approach also provided an alternative strategy for the detection of *Ophiostoma* transformants and provides apparent promise for the expression of foreign gene products in this new expression host, *Ophiostoma floccosum*.

In order to further improve the novel *Ophiostoma* expression system for industrial uses, promoters of genes encoding other dominant secreted proteins identified in this study can be isolated and used along with the *amyI* gene promoter. In addition, post-translational modification, such as glycosylation and phosphorylation of the secreted proteins can be investigated to better understand the nature and cellular mechanisms responsible for these modifications. This information may be valuable in assessing the ability of *Ophiostoma* to host the production of therapeutic proteins.

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