World Academy of Science, Engineering and Technology
International Journal of Agricultural and Biosystems Engineering
Vol:9, No:05, 2015

Constitutive Flo1p Expression on Strains Bearing Deletions in Genes Involved in Cell Wall Biogenesis

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Abstract : The ability of yeast cell wall-derived mannoproteins (glycoproteins) to positively contribute to oenological properties has been a key factor that stimulates research initiatives into these industrially important glycoproteins. In addition, and from a fundamental research perspective, yeast cell wall glycoproteins are involved in a wide range of biological interactions. To date, and to the best of our knowledge, our understanding of the fine molecular structure of these mannoproteins is fairly limited. Generally, the amino acid sequences of their protein moieties have been established from structural and functional analysis of the genomic sequence of these yeasts whilst far less information is available on the glycosyl moieties of these mannoproteins. A novel strategy was devised in this study that entails the genetic engineering of yeast strains that over-express and release cell wall-associated glycoproteins into the liquid growth medium. To this end, the Flo1p mannoprotein was overexpressed in Saccharomyces cerevisiae laboratory strains bearing a specific deletion in KNR4 and GPI7 genes involved in cell wall biosynthesis that have been previously shown to extracellularly hyper-secrete cell wall-associated glycoproteins. A polymerase chain reaction (PCR) -based cloning strategy was employed to generate transgenic yeast strains in which the native cell wall FLO1 glycoprotein-encoding gene is brought under transcriptional control of the constitutive PGK1 promoter. The modified Helm's flocculation assay was employed to assess flocculation intensities of a Flo1p over-expressing wild type and deletion mutant as an indirect measure of their abilities to release the desired mannoprotein. The flocculation intensities of the transformed strains were assessed and all the strains showed similar intensities (>98% flocculation). To assess if mannoproteins were released into the growth medium, the supernatant of each strain was subjected to the BCA protein assay and the transformed Δknr4 strain showed a considerable increase in protein levels. This study has the potential to produce mannoproteins in sufficient quantities that may be employed in future investigations to understand their molecular structures and mechanisms of interaction to the benefit of both fundamental and industrial applications.

Keywords : glycoproteins, genetic engineering, flocculation, over-expression
Conference Title : ICBAE 2015 : International Conference on Biotechnology and Agricultural Engineering
Conference Location : Tokyo, Japan
Conference Dates : May 28-29, 2015