GST-TAGGED α-GLUCOSIDE TRANSPORTER IN *SACCHAROMYCES CEREVISIAE*

da Silva, M.A.[1], Alves Jr., S.L.[2], Stambuk, B.U.[2] , de Araujo, P.S.[1]
[1]Departamento de Bioquímica – IQ – USP – São Paulo – Brasil
[2]Departamento de Bioquímica – CCB – UFSC – Florianópolis – Brasil

α-Glucoside is transported actively in yeasts through a H^+-symport mediated by the *AGT1* permease. The construction of Glutathione S-Transferase (GST) fusion proteins with a Thrombin cleavage site has been a very efficient approach to obtain purified proteins. To tag the *AGT1* permease with GST we used a standard PCR-mediated protocol developed for the modification of chromosomal genes. The strategy was to introduce the *AGT1-GST* gene in two strains. AGT1p is the only glucoside transporter in strain 340-2B, which depends on plasmid pJW5 containing the regulatory *MAL3* gene, for its expression. CEN.PK2-1C expresses maltose transporters besides AGT1p and can grow in rich media to produce cell mass. Both GST-strains grow on maltotriose thus indicating the correct insertion of the AGT1 region of the fusion protein which is functional in the plasma membrane, since maltotriose is taken up only by AGT1p in yeast cells. Five α-glucosides were effectively transported by both strains, with activities similar to the wild type strains when assayed by the H^+-cotransport pH-stat assay.
The GST region is very likely to stay in the yeast cell cytoplasm since negligible GST activity could be detected in intact cells. However, GST activity could be detected associated to a plasma membrane fraction.
The construction of these strains could lead to the purification of the AGT1p.

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