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Entitled

BIOCHEMICAL CHARACTERIZATION OF A FRUCTOSYLTRANSFERASE ENZYME FROM A HALOPHILIC ARCHAEON, HALOARCULA MARISMOTUI, FOR BIOTECHNOLOGICAL APPLICATIONS

by

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Date & Venue

10:00 am

Friday, 10 November 2023

Room 021, F3 Building

Abstract

Fructosyltransferase enzymes are involved in the production of fructans, which are polymers of fructose and naturally present in many foods like fruits, vegetables, legumes, and cereals. Fructans with short chain lengths are called Fructooligosaccharides (FOS), which are small dietary fibers having low caloric values and are known as “prebiotics”. Fructans have a broad range of applications in the food, pharmaceutical, cosmetics, and chemical industries. They have unique physiochemical properties and functionalities depending on the source of enzyme used for their production. The present study aimed at the utilization of a recently discovered unique fructosyltransferase enzyme from an extremely halophilic microorganism, *Haloarcula marismortui,* to produce novel fructans for biotechnological applications. A 1.4 kb fructosyltransferase (*ftf*) gene from *Haloarcula marismortui* was cloned in a pET15b vector having a 6xHis Tag at its N-terminus and expressed in the mesophilic host, *E.coli*, under different growth conditions to optimize the expression of the recombinant enzyme. The expression of the *ftf* gene in the *E.coli* BL21(DE3) strain produced an insoluble protein in the form of inclusion bodies. However, soluble, and active recombinant protein has been successfully obtained in the *E.coli* BL21(DE3) Rosetta strain, which provides rare codons for the expression of recombinant proteins in a heterologous host. Almost 85% pure enzyme was obtained by affinity purification using Ni-NTA column. The detailed biochemical characterization (pH dependence, salt dependence, thermostability, and metal ions stability) of the recombinant enzyme showed that the enzyme works optimally in 2.5 M NaCl and is stable in 1 M, NaCl for 10 days at 4°C. The pH and temperature optima are 7 in KPi buffer and 40˚C, respectively. The enzyme lost its activity and stability when heated at 60°C and above for 5 minutes. None of the metals tested supported the enzyme activity. The enzyme synthesized more product in the presence of 1M sucrose compared to 500mM under optimum assay conditions. The TLC analysis of the product exhibited the presence of a polymer which was found to be inulin by further analysis through NMR. Production of the recombinant halophilic enzyme in a larger quantity to obtain more product for the detailed analysis of linkages among fructose monomers to explore its biotechnological potential remains a challenge for the future.

Keywords: Prebiotics, Fructooligosaccharides, Fructosyltransferase, Halophiles.