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RESEARCH ARTICLE

Some biochemical, catalytic, thermodynamic and kinetic properties of purified fructosyltransferase from wild and improved mutant-type *Aureobasidium pullulans* NAC8

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ABSTRACT

A low molecular weight intracellular and extracellular fructosyltransferase was purified from the wild (W_t) and improved mutant-type (M_t) *Aureobasidium pullulans* and its characteristics and thermodynamic properties determined. The W_t had been previously genetically modified by chemical mutagenesis. The purified fructosyltransferases from the W_t and M_t had subunit molecular weights between 13.4 and 17.0 kDa, respectively. The pH of the purified fructosyltransferase from the W_t and M_t were within 4.0–5.5. From the K_M values, the fructosyltransferase from M_t showed higher affinity for sucrose than W_t fructosyltransferase. The optimum temperature obtained for the M_t intracellular and extracellular fructosyltransferase were 80 and 70 °C, respectively, while the W_t extracellular and intracellular fructosyltransferase was 60 °C. Most metals enhanced fructosyltransferase activity in a concentration-dependent manner except for mercury. Triton X-100 and Tween-80 enhanced the fructosyltransferase activity. Organic solvents such as acetone, ethanol, methanol, toluene and dichloromethane enhanced the enzyme activity while dimethylformamide inhibited the enzyme. From the thermodynamic and kinetic parameters ($t_{1/2}$, ΔH^*, ΔS^*, ΔG^*) M_t fructosyltransferase was more stable to thermal inactivation than the W_t fructosyltransferase. Hence, it can be concluded that, after strain improvement of the W_t, the purified M_t fructosyltransferase was more stable to organic solvents, surfactants, and thermal denaturation, etc. compared to the W_t. This makes M_t fructosyltransferase more useful mostly in the food industry than the W_t.

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Fructosyltransferase; kinetics; purification; characterization; sucrose; *Aureobasidium pullulans* NAC8

1. Introduction

Fructosyltransferases (EC.2.1.4.9) are enzymes involved in the biotransformation of sucrose to yield fructooligosaccharides (Bali et al. 2015). Fructooligosaccharides (namely kestose, nystose, etc.) are fructose oligomers with a terminal glucose moiety (Altenbach et al. 2009). These fructooligosaccharides are classified as prebiotics (Flores-Maltos et al. 2014) because they serve important clinical functions and have numerous roles in the food industry. Fructooligosaccharides produced by this enzyme serves as prebiotics where they aid in the population of beneficial bacteria (Dominguez et al. 2013; Ganaie, Lateef, et al. 2014; Kovács et al. 2014); used as fortifiers in baby formulas and dairy products; and also, as a sweetener for diabetics instead of sucrose which is known to be cariogenic (Gibson and Roberfroid 1995; Guigoz et al. 2002; Peshev and den Ende 2014). Fructosyltransferases and or β-fructofuranosidases are produced for industrial use mostly from fungi of the genera Aureobasidium, Aspergillus, Fusarium, Xanthophyllomyces, etc. although some of this enzyme had been isolated from plants (Balasubramaniem et al. 2001; Biedrzycka and Bielecka 2004; Chen et al. 2011; Huang et al. 2016; Ademakinwa et al. 2018). Fructosyltransferases are remarkably known to be high molecular weight monomers with a molecular weight between 180 and 600 kDa (Rehm et al. 1998). There other known biochemical properties are that they have optimum pH values between 5 and 6.5 and optimum temperature between 50 and 60 °C for the biotransformation of sucrose to yield fructooligosaccharides. The biochemical and catalytic properties of fructosyltransferase from microorganisms vary from source to source. Also, variation is observed among similar strains (Antosova and Polakovic 2001). Production of fructosyltransferase by several strains of *Aureobasidium pullulans*

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Supplemental data for this article can be accessed here.