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Purification and characterization of maltooligosaccharide-forming α-amylase from moderately halophilic *Marinobacter* sp. EMB8

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ABSTRACT

Maltooligosaccharides especially maltotriose and maltotetraose producing amylases are highly desirable for application in bread making and other food industries. A maltotriose and maltotetraose producing amylase from moderately halophilic *Marinobacter* sp. EMB8 is described. Under optimized culture conditions, 48.0 IU/mL amylase was obtained. The enzyme was purified to homogeneity by ultrafiltration, DEAE cellulose and Sephadex G-75 column chromatography with 52% yield and 76-fold purification. It was a monomeric protein of 72 kDa. The amylase had many novel features viz. stability up to 20% NaCl, 80 °C temperature, pH 6.0–11.0 and in wide range of organic solvents at high concentrations. The enzyme efficiently hydrolyzed starch into maltooligosaccharides rich in maltotriose and maltotetraose. These novel properties make the *Marinobacter* sp. amylase a potentially useful enzyme.

1. Introduction

Maltooligosaccharides (MOS) have important industrial applications due to their high viscosity, water holding capacity and crystallization inhibition properties (Palacios et al., 2004). MOS with small oligosaccharides like maltotriose to maltopentaose prevent migration of moisture from starch granules, reduce retrogradation by inhibiting realigning of amylose and amylopectin chains and interfere with starch–gluten interaction (Min et al., 1998; Nagarajan et al., 2006). This makes them quite useful as antistaling agent in the bread industries. For these reasons, there has been a growing interest in amylases that can produce MOS rich in maltotriose and maltotetraose. Amylases with ability to synthesize MOS from starch have been reported from *Bacillus stearothermophilus* US100 (Ali et al., 2001), *Bacillus subtilis* (Messaoud et al., 2004), *Brachybacterium* sp. strain LB25 (Doukyu et al., 2007) and *Bacillus acidicola* (Sharma and Satyanarayana, 2010). Only few strains viz. *B. subtilis* (Takasaki, 1985) and *Bacillus* sp. GM8901 (Kim et al., 1995), produce maltotriose and maltotetraose. Hence, search continues for efficient maltotriose/maltotetraose producing amylases.

Oligosaccharide synthesis is favored in presence of organic solvents and at high temperature (Riva and Roda, 2000). Doukyu et al. (2007) have exploited the solvent stability of *Brachybacterium* sp. strain LB25 amylase, in DMSO to improve product selectivity. The present study aims at screening microbial strains producing solvent and heat stable amylase with maltotriose and maltotetraose forming ability.

Halophiles have been considered as potential source of enzymes, stable in salt and organic solvents (Karan and Khare, 2010; Margesin and Schinner, 2001; Ventosa et al., 1998). Since they inhabit in high salt surrounding which reduces water activity significantly, their enzymes are attuned to function in low water medium and exhibit stability towards organic solvents (Le Borgne et al., 2008). The solvent stable amylases have been reported from *Nesterenkonia* sp. strain F (Shafiei et al., 2011) and *Haloarcula* sp. (Fukushima et al., 2005). The MOS forming ability has not been investigated in any of these cases.

The present study describes a novel halophilic *Marinobacter* sp. EMB8 strain isolated from Kozhikode, India. The strain secretes a solvent and heat stable amylase. The production, purification, characterization of the amylase and its application in MOS synthesis are encompassed.

2. Methods

2.1. Microorganism

Marinobacter sp. EMB8 was isolated from the sea coast of Kozhikode (Kerala, 11°25′N 75°77′E) by salt enrichment. It was related to *Marinobacter* sp. by 16S rDNA sequence analysis and submitted in GenBank, NCBI, USA with accession number GU059908.

2.2. Amylase production

Amylase production was carried out under optimized conditions, viz. medium containing (g/L): starch, 50; casein enzyme

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