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Biocatalytic potential of an alkalophilic and thermophilic dextranase as a remedial measure for dextran removal during sugar manufacture

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ABSTRACT

The present study is focused on dextranase from *Streptomyces* sp. NK458 with potential to remove dextran formed during sugar manufacture. The dextranase had molecular weight of 130 kDa and hydrolyzed 15–25 and 410 kDa dextran. Dextranase production was optimized using statistical designs and the enzyme was purified 1.8-fold with 55.5% recovery. It displayed maximum activity at pH 9.0 and 60 °C and was stable over a wide range of pH from 5.0 to 10.0. The k_m and V_{max} values were 3.05 mM and 17.97 mmol/ml/h, respectively. Ten units of dextranase could reduce dextran content by 67% in 24 h and 56% in 72 h from sugarcane juice of cane variety CoS 86032. The enzyme was stable up to 3 days at 30 °C beyond which its activity decreased and dextran removal could be retained by supplementation of 5 U of dextranase. These properties make it a promising biocatalyst for sugar industry.

1. Introduction

Dextran is a high molecular weight homoglycan comprising of α-D (1 → 6) linked glucose units with significant diversity in the branching of glucose (Khalikova et al., 2005). Due to this extensive branching in the glucose units, the physiochemical properties of the dextran are considerably affected in terms of viscosity and solubility. Although dextran has a number of biotechnological applications, it poses a major problem to the sugar industry. Dextrans are not indigenous to sugarcane juice but are formed from sucrose by resident microflora of sugarcane or by strains contaminating the juice during extraction process. *Leuconostoc mesenteroides* and *Lactobacillus* species are primarily responsible for these activities (Cuddihy et al., 2000). The presence of dextrans in the sugarcane juice used for sugar manufacture is undesirable as they impose a number of deleterious effects like increased viscosity of the sugarcane juice, blockage of the filters, poor yield and sugar of inferior quality (Christensen et al., 1998). Further, due to microbial activity, there is production of lactic and acetic acid from sucrose which lowers the pH considerably (Eggleston and Monge, 2005). To neutralize the increased acidity, high amounts of lime is required which brings about further loss of sucrose and blockage during filtration (Morel du Boil and Wienese, 2002). Presence of dextran falsely enhances polarization, leads to poor clarification and throughput, forms alcohol flocs and gummy sugars and causes distortion of hard candy thus bringing economic losses to the sugar industry. Physical methods such as vacuum filtration, membrane dialysis and reverse osmosis have proved to be useful to some extent but are economically not feasible (Jiménez, 2005). The only method most applicable in the sugar industry for dextran removal is its enzymatic hydrolysis. Dextranase (EC 3.2.1.11; 1,6-α-D-glucan-6-glucanohydrolase), hydrolyzes the α-D (1 → 6) linkages present in dextran resulting in lesser deleterious oligosaccharides. Dextranases in low amounts are usually employed prior to clarification which greatly reduces the viscosity of the sugar syrup. On the contrary, high amounts of dextranase are required during molasses processing (Jiménez, 2009).

During sugar manufacture the sugarcane juice is subjected to elevated temperatures and varying pH conditions indicating need of dextranases active and stable in wide pH and temperature ranges. Although a number of fungal and bacterial dextranases have been successfully purified, only few have reached industrial application. This can be attributed to various factors like high cost of the enzyme and insufficient harvesting techniques (Cuddihy and Day, 1999; Jiménez, 2005). Limitations in application of dextranase in sugar industry are also due to the cumbersomeness during culture preparation and dextranase purification (Decker et al., 2003). Extracellular dextranases have been previously reported from many bacteria (Kim and Kim, 2010) and fungi (Arnold et al., 1998). Most of the extracellular dextranases reported are active either over a broad range of pH or temperature (Khalikova et al.,

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