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# Screening and Improved Production of an Extracellular β-Fructofuranosidase from Bacillus Sp

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**Abstract :** With the rising demand of sugar used today, it is proposed that world sugar is expected to escalate up to 203 million tonnes by 2021. Hydrolysis of sucrose (table sugar) into glucose and fructose equimolar mixture is catalyzed by β-D-fructofuranoside fructohydrolase (EC 3.2.1.26), commonly called as invertase. For fluid filled center in chocolates, preparation of artificial honey, as a sweetener and especially to ensure that food stuffs remain fresh, moist and soft for longer spans invertase is applied widely and is extensively being used. From an industrial perspective, properties such as increased solubility, osmotic pressure and prevention of crystallization of sugar in food products are highly desired. Screening for invertase does not involve plate assay/qualitative test to determine the enzyme production. In this study, we use a three-step screening strategy for identification of a novel bacterial isolate from soil which is positive for invertase production. The primary step was serial dilution of soil collected from sugarcane fields (black soil, Maddur region of Mandya district, Karnataka, India) was grown on a Czapek-Dox medium (pH 5.0) containing sucrose as the sole C-source. Only colonies with the capability to utilize/breakdown sucrose exhibited growth. Bacterial isolates released invertase in order to take up sucrose, splitting the disaccharide into simple sugars. Secondly, invertase activity was determined from cell free extract by measuring the glucose released in the medium at 540 nm. Morphological observation of the most potent bacteria was examined by several identification tests using Bergey's manual, which enabled us to know the genus of the isolate to be Bacillus. Furthermore, this potent bacterial colony was subjected to 16S rDNA PCR amplification and a single discrete PCR amplicon band of 1500 bp was observed. The 16S rDNA sequence was used to carry out BLAST alignment search tool of NCBI Genbank database to obtain maximum identity score of sequence. Molecular sequencing and identification was performed by Xcelris Labs Ltd. (Ahmedabad, India). The colony was identified as Bacillus sp. BAB-3434, indicating to be the first novel strain for extracellular invertase production. Molasses, a by-product of the sugarcane industry is a dark viscous liquid obtained upon crystallization of sugar. An enhanced invertase production and optimization studies were carried out by one-factor-at-a-time approach. Crucial parameters such as time course (24 h), pH (6.0), temperature (45 °C), inoculum size (2% v/v), N-source (yeast extract, 0.2% w/v) and C-source (molasses, 4% v/v) were found to be optimum demonstrating an increased yield. The findings of this study reveal a simple screening method of an extracellular invertase from a rapidly growing Bacillus sp., and selection of best factors that elevate enzyme activity especially utilization of molasses which served as an ideal substrate and also as C-source, results in a cost-effective production under submerged conditions. The invert mixture could be a replacement for table sugar which is an economic advantage and reduce the tedious work of sugar growers. On-going studies involve purification of extracellular invertase and determination of transfructosylating activity as at high concentration of sucrose, invertase produces fructooligosaccharides (FOS) which possesses probiotic properties.

**Keywords :** Bacillus sp., invertase, molasses, screening, submerged fermentation
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