In-Vitro Dextran Synthesis and Characterization of an Intracellular Glucosyltransferase from Leuconostoc Mesenteroides AA1

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Abstract : Dextransucrase [EC 2.4.1.5] is a glucosyltransferase that catalysis the biosynthesis of a natural biopolymer called dextran. It can catalyze the transfer of D-glucopyranosyl residues from sucrose to the main chain of dextran. This unique biopolymer has multiple applications in several industries and the key utilization of dextran lies on its molecular weight and the type of branching. Extracellular dextransucrase from Leuconostoc mesenteroides is most extensively studied and characterized. Limited data is available regarding cell-bound or intracellular dextransucrase and on the characterization of dextran produced by in-vitro reaction of intracellular dextransucrase. L. mesenteroides AA1 is reported to produce extracellular dextransucrase that catalyzes biosynthesis of a high molecular weight dextran with only α -(1 \rightarrow 6) linkage. Current study deals with the characterization of an intracellular dextransucrase and in vitro biosynthesis of low molecular weight dextran from L. mesenteroides AA1. Intracellular dextransucrase was extracted from cytoplasm and purified to homogeneity for characterization. Kinetic constants, molecular weight and N-terminal sequence analysis of intracellular dextransucrase reveal unique variation with previously reported extracellular dextransucrase from the same strain. In vitro synthesized biopolymer was characterized using NMR spectroscopic techniques. Intracellular dextransucrase exhibited Vmax and Km values of 130.8 DSU ml-1 hr-1 and 221.3 mM, respectively. Optimum catalytic activity was detected at 35°C in 0.15 M citrate phosphate buffer (pH-5.5) in 05 minutes. Molecular mass of purified intracellular dextransucrase is approximately 220.0 kDa on SDS-PAGE. N-terminal sequence of the intracellular enzyme is: GLPGYFGVN that showed no homology with previously reported sequence for the extracellular dextransucrase. This intracellular dextransucrase is capable of in vitro synthesis of dextran under specific conditions. This intracellular dextransucrase is capable of in vitro synthesis of dextran under specific conditions and this biopolymer can be hydrolyzed into different molecular weight fractions for various applications. **Keywords :** characterization, dextran, dextransucrase, leuconostoc mesenteroides

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