

**BIOCHEMICAL STUDIES ON DEXTRANASE ENZYME
PRODUCED BY MICROBIAL ISOLATES FROM
HONEY BEES**

By

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دراسات بيوكيميائية على إنزيم الديكسترانيز المنتج بواسطة عزلات ميكروبية من عسل النحل

رسالة مقدمة من

الطالبة/ سارة هلال على منصور
بكالوريوس العلوم (ميكروبيولوجى – كيمياء ٢٠٠٧)

للحصول على درجة الماجستير فى فلسفة العلوم
(ميكروبيولوجى)

قسم الميكروبيولوجى
كلية العلوم
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ABSTRACT

Screening of five different isolates from different sources of honey was carried out for the production of extracellular dextranase enzyme. Among these tested isolates, a gram- positive, sporulating halophilic bacteria was found to be the most potent microorganism for dextranase production. Primary identification of this isolate was carried out using 16S rRNA analysis and identified by 16-23S intergenic region as *Bacillus subtilis* NRC-B233. This strain was able to produce dextranase in high concentration (258 U/ml). Screening of different types of wastes and other cheap materials for their suitability for dextranase production under the two conditions, shaking and static fermentation techniques showed that corn flour gave the best yield when used in production medium under both conditions shaking and solid state fermentation (SSF) (75.276 U/ml , & 61.323 U/g , respectively). Because the difference between shaking and SSF was not high, SSF was chosen as a good condition for dextranase production to save energy. Corn flour is considered as a new dextranase production medium. The optimized culture conditions for dextranase production using solid state fermentation were obtained at 37 °C ; after 32 hrs. ; at pH (10) and 20 (v/w) moisture content. A unique character of *Bacillus subtilis* NRC-B233 is its

ability to produce steady dextranase irrespective to the presence of NaCl in medium. The addition of 0.175M CrCl₃ increased the enzyme production about 4.5 fold. Among different nitrogen sources studied, peptone at a concentration of 2g/l yielded the highest dextranase production (1225.031 U/g). The optimum carbon source was obtained at concentration of 10g/l starch (1553.364 U/g). Further improvement enzyme production was achieved by simple UV mutation which increased the enzyme production up to 2842.3 U/g. This mutant was developed after 15 minutes of exposure of the wild type strain to UV lamp. The crude enzyme was highly tolerant to repeated freezing and thawing, the remaining activity was 100% after three months.

The crude *Bacillus subtilis* NRC-B233 dextranase was partially purified by ultrafiltration then fractional precipitation with ethanol and acetone. The use of ultrafiltration for downstream processing would result in one-step, cost-effective method of recovery for dextranase. The molecular weight of the partially purified enzyme was less than 10 KDa. Acetone at fraction 70% was proved to be the best method for partial purification of dextranase and the purification fold reached to 112.2.

The partially purified dextranase showed its maximum activity at pH 9.2 and 70 °C. It retained full activity (100%) at 75 °C for one hour. Dextranase activity increased about 4 fold in the presence of 10% NaCl.

On the other hand, CaCl_2 (0.050M), EDTA (0.100M) and KCl (0.100M) showed high influence on enzyme activity. The calculated values of K_m and V_{\max} using different concentrations of dextran (150,000 M.wt) as a substrate were found to be 4.46 mg/ml and 41.66 V_{\max} , respectively. The enzyme showed variable degradation effect on different types of dextran and various carbohydrates.

These results suggest that the dextranase secreted by *Bacillus subtilis* NRC-B233 is industrially important from the perspectives of its activity at broad pH ranged from 5.2-10.2. In addition, thermostability, halophilic characteristics and ability to degrade different types of α -1,4 and α -1,6 glycosidic linkages, are more attractive characteristics for application of this enzyme at industrial scale.

In this study we focused on isolation of halophilic bacteria from honey as new source for dextranase production. The mutagenic honey isolate produced a novel halophilic low molecular weight constitutive dextranase characterized by unique features, like thermostability and pH stability. Further, cheap medium like corn flour would be a superior alternative to the already available expensive dextran, since 30-40% of the production cost of industrial enzymes is accounted by the cost of the growth medium.

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