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Thesis Title	Production and characterization of uricase from locally isolated *Streptomyces Sp.*			
Year	2004			
Abstract	Enzyme uricase (EC 1.7.3.3) is a clinical enzyme normally used in clinical applications for the determination of uric acid in serum. Fifty local isolates of microorganisms were screened for production of uricase in solid and submerged cultures. The isolate ***Streptomyces sp.*** (L_{14s}) was selected based on its high production of enzyme and were chosen for the present study. The obtained results showed that uricase was produced intracellularly, and the extraction by ultrasonic waves with 0.1 M borate buffer at pH 8.5 showed the most effective procedure. The optimum condition for uricase production were 0.1% glucose, 0.5% uric acid and yeast extract 0.1% at pH 10.0 after 72 hr of incubation at 30 ^{o}C. Uricase was purified from ***Streptomyces sp.*** (L_{14s}) by several steps included precipitation by ethyl alcohol in a ratio 1:3 (v/v), the volume of crude extract to the volume of ethyl alcohol, then the enzyme were concentrated by dialysis and gel filtration on			

sephadex G-200 column. The obtained purification fold and recovery were 7.23 and 44.3, respectively.

The characterization of the purified enzyme showed that the optimum pH for its activity was 9.0 and the enzyme was most stable at pH 10.0. The maximum enzyme activity was observed at 35 ^{o}C, and the enzyme retained its original activity when incubated between (25-50) ^{o}C for 15 min, while it lost 80% of its activity after incubation for the same time at 60 ^{o}C, the remaining activity was 96% when incubated at 35 ^{o}C for 60 min. while the enzyme lost 26% of its activity when incubated for 80 min. at the same temperature. The activation energy for conversion of the substrate to product was 3.4 Kcal/mol.

The kinetic studies showed that the Michaelis constant (K_m) and maximum velocity (V_{max}) values for the enzyme using uric acid as a substrate were 2.8×10^{-2} mM and 0.199 $\mu M.min^{-1}$, respectively. The obtained values of turnover number (K_{cat}) was found to be 2.9×10^{-2} min^{-1}.

The effect of some inhibitors, reducing and chelating agents showed that the activity decreased by preincubating the enzyme with metal ions such as mercury, copper and iron. The enzyme lost 74% of its activity when preincubated with concentration of 10 mM of copper chloride. Furthermore, the enzyme lost all its activity in the presence of potassium cyanide in both concentrations 10 and 20 mM. The enzyme activity was affected only slightly or not at all by EDTA, 2-mercaptoethanol at a concentration 0.1 and 1 mM.

The enzyme was used in clinical application for the determination

	of uric acid in serum. The results were compatible with that done using ready kit from Biomaghreb Company.