

Chapter I

Introduction

Uric acid is an end product from purine derivatives in human metabolism. When the uric acid levels increased in blood over than the normal value, it can cause renal failure and may contribute to a group of diseases such as gout, leukemia, toxemia of pregnancy, severe renal impairment and idiopathic calcium urate nephrolithiasis.

Uricase (urate oxidase EC 1.7.3.3, UC) catalyses the oxidation of uric acid (a final product of purine catabolism), to allantoin, which is more soluble and more easily to be excreted than the starting compound. Many organisms including higher plants and microorganisms are able to produce uricase. This enzyme is widely present in most vertebrates but is absent in humans.

In human and other higher primates, uricase gene is absent as a result of two mutations in the gene coding sequence. As a result, uric acid is the end product of purines metabolism, which is present largely as insoluble monosodium salt and in some individuals, uric acid can precipitate, leading to gout symptoms.

At the beginning, uricase prepared from an extract from ox-kidney mince. From 1 kg of ox kidneys, it is possible to obtain 620 mg of uricase. Scientists find

that under certain conditions true bacteria, actinomycetes, yeasts or fungi can produce or induce uricase in culture media containing uric acid as nitrogen source and inducer. Moreover, induction pattern of uricase in these different organisms are different. Uricase can also be produced in a large scale by cultivating a transformed microorganism thus obtained in appropriate medium under appropriate conditions. In this case, uricase can be produced effectively for example, by adding an inducer like isopropyl thiogalactoside. After cultivation, uricase was isolated by treating the cells with lysozyme or lysing the cells with supersonic waves followed by extraction, separation and purification of the culture broth.

The first important application discovered for uricase was in as a diagnostic reagent for measurement of uric acid in blood and other biological fluids. Determining the urate concentration in blood and urine is required for the diagnosis of gout as urate accumulation is a causative factor of gout in humans. It can be also used as protein drug for treatment of hyperuricemia, as Rasb Uricase.

An enzyme with the property of high thermal stability will have advantages in transportation, storage and clinical application. Therefore, isolation of a thermo stable bacterium has a great value in the medical application.

Many microorganisms such as *Arthrobacter globiformis*, *Bacillus subtilis*, *Nocardia farcinica* and *Microbacterium* sp. have been used to produce uricases. However, the uricases produced by these bacteria are not thermostable and at higher temperatures more than 60°C lost their activities in a short period.

The main objective of this research is to screen the available local bacterial strains for their uricase producing ability, select the most potent isolate and

optimize the culture conditions for maximum uricase production. Uricase production was improved using mutation and production of uricase by the obtained mutant was studied compared to control.