PREFACE

Gout is a disorder of hyperaccumulation of uric acid mainly in the form of monosodium urate (MSU) crystals. It is one of the leading causes of deaths in worldwide. This disease is also called as the “king of diseases and disease of Kings” because, most of the kings were having this disease due to their high consumption of red meat, seafoods, wine, beer etc. The major cause of hyperaccumulation of uric acid is due to the reduced renal clearance in the patients. The uric acid is the end product of purine metabolism. Recently it has been estimated that 0.3% population of India and above 8.3 million people in the United States at around 3% of the populations are suffering from an inflammatory disease called as ‘Gout’. The disease is most probably common in the middle-aged men and is increasing in the elder populations along with postmenopausal women.

Anti-inflammatory therapies including colchicines, non-steroidal anti-inflammatory drugs, and glucocorticoids are commonly used decades ago for the treatment. There are two possible approaches for the regulation of uric acid concentration in the body as

1] More than three decades ago allopurinol was considered as a first line drug which regulates uric acid synthesis by inhibiting xanthine oxidase (XO) enzyme. In addition to allopurinol, Febuxostat is also used to reduce the synthesis of uric acid by inhibiting the XO enzyme and is approved by the European Medical Agency in 2008 and US FDA in 2009. In contrast to allopurinol, it acts on both reduced as well as an oxidized form of XO. Partial relief from inflammation can be obtained by administration of hydroxychloroquine and non-steroidal anti-inflammatory drugs. However, all these drugs are prone to have enormous side effects, including severe kidney and heart-related disorders.

2] So in order to regulate the uric acid level in serum or soft tissues, enzymatic treatment is considered to be the better way without any side effects. The enzyme uricase is well known for the treatment of gout disease. Uricase (urate oxidase) EC 1.7.3.3 is a tetramer therapeutic enzyme which oxidatively opens the purine ring of uric acid and forms

allantoin, CO_2 and H_2O_2. Allantoin is a highly water-soluble compound compared to the uric acid. Uric acid is poorly water-soluble as ~ 11 mg/dL compared to the product allantoin which has solubility ~147 mg/dL. Hence allantoin is easily excreted through the urine. Uricase is also acting as the diagnostic enzyme used to determine the concentration of urate in serum and urine. Uricase enzyme is highly conserved present in mammals, plants, fungi, bacteria, and yeasts but is absent in humans due to evolutionary mutations in uricase gene.

In the current study, therefore, an effort has been made to purify an uricase enzyme from microbial source which would efficiently regulate uric acid concentration in animals. The uricase producers were selected and among them, the potential uricase producer was carried forward for further study. To enhance the uricase production efficiency of bacterium different medium component was optimized. Emphasis was more towards increasing the activity of uricase, so that even minimum dose of enzyme could be useful for the treatment of a patient. The results obtained in a batch mode using shake flask and in a bioreactor was presented in this thesis. The enzyme was purified to homogeneity in order to remove any impurity using different methods such ammonium sulphate fractionation, ion exchange and size-exclusion chromatography. To study the characteristics of purified enzyme at different conditions, the kinetic studies of the enzyme was performed. To understand the treatment efficiency of the uricase from *B. cereus GHMS* , enzyme was applied against hyperuricemic mice as a model. The immunological nature of the purified uricase was also studied.

The resulting subject matter, presented in this volume has been arranged in five chapters. Chapter 1 gives a brief idea introduction to gout, hyperuricemia, role of uricase, and objectives of the present investigation. Chapter 2 critically access the available literature published on uricase properties from other sources. Details of methodology for the experimentation are given in Chapter 3. Results obtained are presented and discussed in Chapter 4 and finally Chapter 5 concludes the results. The references are given at the end of the thesis.