Abstract

The present work is focused on the isolation, identification, cloning and expression of uricase gene in *Escherichia coli* from *Bacillus subtilis* which was isolated from soil sample of pharmaceutical industry. The uricase resistant bacteria was isolated from uric acid containing plates. The isolated bacteria was identified as *Bacillus subtilis*. For molecular detection of uricase gene, the gene was selected and primers were designed. The uricase gene of ~1.5kb was amplified by polymerase chain reaction and ligated in pTZ57R/T cloning vector. The recombinant vector was transformed to competent cell of *E. coli* DH5α and confirmed by sequencing of uricase gene. The gene was cloned in expression vector pET21a(+) and on SDS-PAGE ~60kDa band was detected. It was observed that uricase give the highest activity when precipitated with 70% of ammonium sulphate i. e 29 U/mL/min. in case of lysate and supernatant 25 U/mL/min at 60 % ammonium sulphate saturation. The enzyme was further characterized, optimization of temperature and pH revealed that the bacterial strain gave its maximum activity at 55 °C and pH 7.0 when metal ion effect was checked it observed that Ca^{2+} and Mg^{2+} greatly enhanced the activity of enzyme.