Co-production of enzyme uricase and alkaline protease in *Bacillus licheniformis*

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Uricase can be used medically as a diagnostic reagent for measurement of blood uric acid levels which is a significant application in clinical biochemistry. This enzyme catalyses the oxidation of uric acid to allantoin and finds its importance in purine metabolism. In the present work, seven local bacterial strains were screened for uricase production where *Bacillus licheniformis* has been recognized for its ability to produce high levels of the enzyme. In the literature, *Bacillus* species are well studied and reported for the production of industrially important enzyme alkaline protease[1]. However, there are limited literature available on the production of uricase using this strain to the best of our knowledge. The main purpose of this study is to optimize the culture conditions for maximum co-production of enzymes uricase and alkaline protease by *Bacillus licheniformis* using a single substrate. This work is the first report on the production of enzyme uricase by *Bacillus licheniformis* as well as on co-production of two enzymes uricase and alkaline protease using single substrate.

Primary screening of enzyme production is done by rapid plate assay method. Observation of zone of clearance around the colony confirms the enzyme production. Uricase and protease assay have been carried out by protocol as explained by Azab et al.,2005[2] and Kamoun et al.,2008[1], respectively. Here, the fermentation parameters affecting enzyme yields such as media (Nutrient broth (NB), Zepek dox, Mineral medium), incubation time (4hr, 8hr, 12hr, 16hr, 20hr, 24hr, 28hr, 32hr), inoculum concentration (1%, 2%, 5%, 10% (v/v)) and substrate concentration (0.03%, 0.05%, 0.1% & 0.2% (w/v)) have been studied alongwith other physical and chemical parameters which include temperature, agitation, pH, Carbon source and Nitrogen source.

It is observed that zone of clearance is obtained around the six agar wells inoculated with bacteria belonging to genus *Alcaligens*(2), *Bacillus*(1), *Azatobacter*(2), *Pseudomonas*(1) which confirms the uricase enzyme production, whereas no enzyme production is seen with bacterial genus *Lechevaliera*(1). Larger zone of clearance is seen with bacteria *Bacillus licheniformis*. Maximum enzyme activity of 3.4 U/ml uricase and 0.407 U/ml alkaline protease is obtained at 12hr incubation period, 2%(v/v) inoculum concentration and at 0.2%(w/v)uric acid concentration when organism is cultivated at 30°C and 150rpm. The results describing the optimized parameters in this study will be useful to develop an economic, commercially viable and scalable production process of uricase and protease enzymes.

**Keywords:** *Bacillus licheniformis*; Uricase; Protease

**References**
