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Isolation, partial purification, characterization and inhibition of urease (E.C. 3.5.1.5) enzyme from the *Cajanus cajan* seeds

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Urease (urea amidohydrolase, E.C. 3.5.1.5), a nickel dependent metalloenzyme, catalyzes the hydrolysis of urea and one molecule of urea results in the release of two molecules of ammonia and one molecule of carbon dioxide. The objective of present study was to characterize urease enzyme from *Cajanus cajan*. The partial purification of urease enzyme was done by acetone fractionation method. The optimum temperature for urease enzyme in the present study was found to be 60°C and the optimum pH was 7.5. Partial purification of *Cajanus cajan* showed the fold purification to be 5.17 and the per cent recovery was found to be 56.6. Further, the effect of various inhibitors including $CuSO_4$, $AgNO_3$, $SnCl_2$ and $HgCl_2$ on the activity of urease enzyme was determined.

Key words : *Cajanus cajan*, Urease, Partial purification, Inhibition

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INTRODUCTION

Urease is a nickel dependent metalloenzyme which catalyzes the hydrolysis of urea to yield ammonia and carbamate, the latter compound spontaneously hydrolyzes to form carbonic acid and another molecule of ammonia (Andrews *et al*., 1984). The best-studied urease is that from jack bean (Blakeley and Zerner, 1984), which was identified as the first nickel metalloenzyme (Dixon *et al*., 1975) and urease from jack bean (*Canavalia ensiformis*) was the first enzyme to be crystallized. Sumner (1926) showed that urease is a protein. Urease is found in bacteria, yeast, and several higher plants. Urease is a cytosolic enzyme. Its major activity with some exceptions is associated with the soluble fractions of the cells (Mobley *et al*., 1995). The best genetic data of plant ureases are available for soybean (*Glycine max*) (Polacco and Holland, 1993; 1994). Two urease isoenzymes, a tissue-ubiquitous and embryo-specific encoded by two separate genes, as well as regulatory proteins encoded by unlinked genes were identified in soybean (Meyer-Bothling and Polacco, 1987; Torisky *et al*., 1994). The embryo-specific urease is an abundant seed protein in many plant species, including soybean, jack bean (Polacco and Holland, 1994) and *Arabidopsis* (Zonia *et al*., 1995), while the other type of urease (called ubiquitous) is found in lower amounts in vegetative tissues of most plants (Hogan *et al*., 1983).

For activation, urease needs to bind two nickel ions per subunit (Benini *et al*., 1999). The enzyme inhibitors can interact with enzymes and block their activity towards natural substrates (Amtul *et al*., 2002). Because of instability of enzyme, its use is limited. This problem can be overcome by recent developments in the field of biotechnology for immobilizing enzymes. Many methods exist for the immobilization of enzymes but usually used methods include entrapment; physical adsorption; co-polymerization; and covalent attachment. The immobilization of urease in nylon tubes, carboxymethyl-cellulose, polyacrylamide and gelatin has been done. Calcium alginate is commonly used for enzyme entrapment (Sunger *et al*., 1992; Das *et al*., 1998).

A number of medical and ecological significances of ureases have been described. The significance of the enzyme includes: to serve as a virulence factor in human and animal infections of the urinary and gastrointestinal tracts, play role in recycling of nitrogenous wastes in the rumens of domestic livestock, and its application in environmental transformations of nitrogenous compounds, involve urea based fertilizers (Mobley and Hausinger, 1989). During storage of wines the wines treated with preparation of killed cells containing an acid urease, remove urea from wine which is the potential source of ethyl carbamate (carcinogen) and thus prevent the