Studies on production and application of alkaline protease from endophytic *Bacillus pseudofirmus*

Background

Proteases are enzymes produced by microorganisms, plants and animal tissues, and catalyze the hydrolysis of peptide bonds in proteins. Proteases are classified based on site of action *viz*., endopeptidase and exopeptidase, activity in the wide range of pH (acid, neutral and alkaline protease) and active site group of the enzyme, i.e. metallo- (EC.3.4.24), aspartic- (EC.3.4.23), cysteine- or sulphydryl- (EC.3.4.22), or serine-type (EC.3.4.21) (Kalisz, 1988; Rao *et al*., 1998). Alkaline proteases, active in neutral to alkaline pH range either have serine center (serine protease) or are of metallo-type (metalloprotease) (Ward, 1985) of which the alkaline serine proteases are the most important group of enzymes exploited commercially (Gupta *et al*., 2002a). Compared to animal and fungal proteases, bacterial alkaline proteases have more commercial importance due to their high production capacity and catalytic activity (Kumar and Takagi, 1999; Gupta *et al*., 2002a). Physical, biochemical, molecular and catalytic properties of alkaline proteases varies with the nature of the organism. Bacteria of the genus *Bacillus* are active producers of commercially available extracellular alkaline proteases (Kumar and Takagi, 1999; Gupta *et al*., 2002a; Haki and Rakshit, 2003). Although microbial alkaline proteases have been produced by solid-state and submerged fermentation, the latter has been more popular among researchers as found in literature. Alkaline proteases with high activity at different pH and at high temperatures have potential applications in pharma, diagnostic, detergent, tannery, amino acid production, contact-lens cleaning agents, effluent treatment, enzymatic debridement and supporting the natural healing process in the

skin ulcerations (Kumar and Takagi, 1999; Anwar and Saleemuddin, 2000; Gupta *et al*., 2002a; Sjodahl *et al*., 2002). The activity and stability are the most important parameters to understand utility of alkaline proteases in different sectors. In addition to robustness against solvents, surfactants and oxidants, the applications of alkaline proteases highly depends on their stability during isolation, purification and storage (Gupta *et al*., 2005; Najafi *et al*., 2005; El-Hadj-Ali *et al*., 2007; Sivasubramanian *et al*., 2008). Thus, in depth knowledge of kinetic and catalytic behavior of alkaline protease from any new strain is a prerequisite for evaluation of its biotechnological potential. Often, the wild type alkaline protease from any microbial strain is not suitable for industries and needs some improvement in their function in relation to activity and stability. Hence, there have been extensive studies on screening for new strains producing alkaline protease or/and protein engineering on the existing strains to obtain alkaline proteases suitable for commercial applications. Major industrial companies are continuously trying to identify enzymes that have potential industrial applications, either to use them directly or to create notified enzymes that have enhanced catalytic activity for well adapted large scale industrial processes (Glaser, 2000). However, these new enzymes would have to offer a competitive advantage over existing products. Details of the literature survey on different methods of protein engineering giving special emphasis on directed evolution to improve enzyme functions are presented elsewhere (Sen *et al*., 2007).

The present work focuses on screening and isolation of alkaline protease producing endophytic bacteria from fruits, identification of the most efficient producer among the isolates, maximization of the production of the alkaline protease from the chosen isolate and characterization of the alkaline protease to explore its potential for various industrial and environmental applications. The potential of the alkaline protease was tested for various applications *viz*., burnt-on protein removal from solid surface, goatskin dehairing, treatment of

effluents from tannery, dairy, domestic sewage and slaughterhouse and the antibacterial activity of the enzyme. An attempt was made to test its efficiency in comparison to some commercially available alkaline proteases.

Objectives:

1. Isolation, screening and identification of endophytic microorganism
2. Optimization of physical parameters to enhance the production of alkaline protease
3. Development of medium to maximize the production of alkaline protease
4. Purification and characterization
5. Potential application in food and tannery industries

Outcome:

A good knowledge from fermentation of enzyme production by scaling up of production is to be acquired to meet the required demands of the industries. Scaling up of the applications (dehairing and effluent treatment) may find potential use in the large scale industries.

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