Introduction

CHAPTER 1 INTRODUCTION

Proteases are one of the most important categories of industrial enzymes. They account for more than 60-65% of the total industrial enzyme market (Banik and Prakash, 2004), which currently stands at over 3 billion US$ (Deng *et al.*, 2010). With the global demand for enzymes rising at the rate of 6.3 percent annually through 2013 (http://www.marketresearch.com/map/prod/2432362.html) the importance of this group of enzymes is also bound to increase exponentially. Protease constitute one of the three largest groups of the industrial enzymes and find application in a variety of industries such as detergents, leather, food, textile and pharmaceutical industries (Ajithkumar *et al.*, 2003; Bhaskar *et al.*, 2007, Jellouli *et al.*, 2009, Annapurna *et al.*, 2012). Besides this, they are used in waste treatment, peptide synthesis, diagnostic reagents and silver recovery from X-ray/photographic films (Rao *et al.*, 1998; Bhalla *et al.*, 1999; Upadhyay *et al.*, 2010).

In nature the proteases are widespread. Their importance in conducting the essential metabolic and regulatory functions is evident from their occurrence in all forms of living organisms (Rao *et al.*, 1998) and from the fact that in higher organisms about 2% of genes code for these enzymes (Barrett *et al.*, 2001; Marnett and Craik, 2005; Schilling and Overall, 2008). Though proteases are ubiquitous in nature, microbes serve as the most preferred source of these enzymes because of their rapid growth, limited space requirement for cultivation and the ease with which they can be genetically manipulated to generate new enzymes with altered properties that are desirable for their extended applications (Kocher and Mishra, 2009). Among microbes, bacteria and more specifically *Bacillus* is the most common source for the commercially produced proteases (Gupta *et al.*, 2002). Bacterial proteases are mostly extracellular, easily produced in larger amounts, thermostable, and active at a wider pH range. These properties make the bacterial proteases most suitable for wider industrial applications. Due to the growing market and various potential applications of proteases there is an ongoing interest in the isolation of new bacterial species that produce proteolytic enzymes with novel properties suitable for industrial applications. Underexploited regions and niche habitats are most likely to yield sources of such enzymes.

Proteases are classified as serine proteases, aspartic proteases, cysteine proteases and metalloproteases depending upon the nature of the functional group at the active site (Mahajan *et al.*, 2010). Among these the metalloproteases are the most diverse of the catalytic types of proteases. They are characterized by requiring a divalent metal ion like zinc, cobalt, manganese or nickel for their activity (Alvarez *et al.*, 2006). Various microbial strains such as *B. subtilis, B. thermoproteolyticus, B. megaterium, B. cereus, B. thuringiensis, L. monocytogenes* and *S. griseus* have been reported to produce neutral metalloproteases which are sensitive to metal chelating agents such as ethylenediaminetetraaceticacid (EDTA) and o-phenanthroline (Miyoshi and Shinoda, 2000). Neutral metalloproteases are of immense importance as they show specificity for hydrophobic amino acids generating less bitterness in hydrolyzed food proteins and hence are valuable in food industries, the low thermostability being an advantage for controlling their activity (Barrett, 1995; Rao *et al.*, 1998). Many of the thermostable proteases, namely, thermolysin, caldolysin and alkaline proteases from *Streptomyces* need calcium for their stability (Cowan and Daniel 1982; Zamost *et al.*, 1990). Therapeutically they selectively cleave fibronectin and type IV collagen and hence are important in dermatology (Stenn *et al.*, 1989). A metalloprotease from *B. thuringiensis* with the ability to degrade antibacterial proteins produced by the insect host has also been reported (Dalhammar and Steiner 1984). In addition, many extracellular metalloproteases also play an important role in pathogenesis (Miyoshi and Shinoda, 2000). Globally, 140 billion metric tons of agricultural wastes are generated every year. If managed carefully, however, solid state fermentation offers the best possible use of these agro-industrial wastes as substrate for metalloproteases production through different manipulations (Saxena and Singh, 2011). The high protein and moisture content of these underutilized wastes facilitate their use as substrate in SSF (Saxena and Singh, 2010). Advantages of solid-state fermentation include lower manufacturing costs with large volumes of production, less preprocessing energy and effluent generation, along with easy process management and better product recovery (Prakasham *et al.*, 2006; Oliveira *et al.*, 2006).

In wake of the wide ranged applications of microbial enzymes continuous efforts for their economic production to cope with industrial process is under focus. In the present study 221 bacterial isolates were isolated from soil samples collected from

various regions of Himachal Pradesh and tested for proteolytic activity. The isolate PPB-26 emerged as hyper producer of protease and was identified as *Serratia marcescens.*

Keeping in view the potential applications of the protease enzyme, research topic entitled, **"Protease from *Serratia marcescens*: Production, purification and characterization"** was undertaken with the following objectives:

1. To optimise process parameters for production of protease of *Serratia marcescens.*
2. To purify and characterize the protease of *Serratia marcescens.*
3. To optimize the reaction conditions for the assay of purified protease.