CHAPTER V
APPLICATIONS OF PROTEASE

5.1. INTRODUCTION

Production of proteases has been reported from a wide range of microbes, especially a large proportion of the commercially available proteases are derived from *Bacillus* spp. (Mehrotra et al., 1999). *Bacillus* strains are preferred because of their ability to produce extracellular enzymes in a short time (Maurer, 2004). In order to produce proteases in a cost effective manner, attempts have been made to clone and express them in *Escherichia coli* (Fu et al., 2003; Karbalaei-Heidari *et al.*, 2008).

Also for effective washing, a good detergent protease must compatible and stable with all commonly used detergent compounds such as surfactants, bleaches, oxidizing agents and other additives, which might be present in the formulation (Gupta *et al.*, 2002). The stability towards SDS is essential because enzymes with SDS stability are not generally available (Oberoi *et al.*, 2001; Sellami-Kamoun *et al.*, 2008). Among all proteases, alkaline proteases are primarily used as detergent additives, the use of enzyme - based detergents is preferred over the conventional synthetic ones in view of their cleaning properties, better performance at lower washing temperature (Krik *et al.*, 2002). The majority of the commercially available enzymes are not stable in the presence of oxidizing agents. Many bacterial alkaline proteases with better stability towards surfactants and oxidizing agents have been produced, using site
directed mutagenesis and protein engineering by the replacement of certain amino acid residues (Yang et al., 2000). The alkaline proteases known as “green chemicals” have made their way as key-ingredients in detergent formulations (Rai et al., 2009). The parameters involved in the selection of an ideal detergent protease are: compatibility with the detergent components, good activity at relevant washing pH and temperature, and oxidant and bleach stability (Manni et al., 2010).

Proteases in organic synthesis have attracted a great deal of attention in recent years because of their advantages associated with the application of enzymes for the synthesis of peptides and esters (Gupta and Roy, 2004; Kumar and Bhalla, 2005). Hence, organic solvent tolerant bacteria are being explored for their potential in industrial and environmental biotechnology, considering their enzyme retaining activity in the presence of very high concentrations of organic solvents (Sardessai and Bhosle, 2004). Since Inoue and Horikoshi first reported that a toluene-tolerant Pseudomonas putida strain was able to grow at high concentration of benzene in the late 1980s, some bacteria have been discovered more tolerant to toxic solvents than most microorganisms (Inoue and Horikoshi, 1989; Sardessai and Bhosle, 2004). In recent years, neutral proteases having organic solvent-stability have been isolated and reported from B. cereus, B. subtilis, Pseudomonas sp., B. pumilus, and B. licheniformis (Shimogaki et al., 1991; Rahman et al., 2005; Gupta and Khare, 2006; Rahman et al., 2007; Mahanta et al., 2008; Sareen and Mishra, 2008).
Enzymatic dehairing in tanneries has been envisaged as an alternative to sulfides (Beynon and Bond, 1989; Altschul et al., 1997). The use of enzyme based products is currently being explored in many areas of leather making process, with increasing importance in the dehairing process, thus eliminating the use of hazardous sodium sulfide (Thanikaivelan et al., 2004). Due to the increasing demand of enzyme in the leather industry, there arises a need for new proteases (Shrinivas and Naik, 2011). Alkaline proteases can be used which enables the swelling of hair roots, and the subsequent attack of protease on the hair follicle protein allowing easy removal of the hair (Gupta et al., 2002). Leather proteases find great applications at various steps of leather processing. The neutral proteases are widely used in soaking process (Deshpande et al., 2004), alkaline proteases are involved in dehairing (Dayanandan et al., 2003), and acid proteases play critical role in bating process (Padmavathi et al., 1995).

Keratinases are widely distributed in nature and secreted by variety of organisms belonging to bacteria, actinomycetes and fungi. Keratinases are active over a wide pH of 7-12 and temperature range of 30-80°C with optimal activity at temperatures up to 50°C (Gradisar et al., 2005). The molecular masses of the enzymes range from 20-60 kDa and mainly belong to serine or metalloproteases. A thermostable, alkaline keratinolytic proteinase was produced by Chrysosporium keratinophilum (Dozie et al., 1994). Traditionally keratinases have been in use for production of feather meal, fertilizers, glues etc. Further their applications have been extended to other areas such as detergent formulations, cosmetics, leather, medicine and animal feed.
It is known that photographic and X-ray films are partly prepared using silver compounds. Black metallic silver spread on gelatin emulsion layer of waste X-ray films is about 1.5-2.0% silver by weight. Burning of X-ray films, and striping methods are conventionally used to recover silver. But, the conventional burning and chemical alkali methods result in the generation of foul smell and cause pollution. To overcome this problem, alkaline proteases can be used and it could degrade gelatine within few minutes to recover silver, it has been reported by Fujiwara et al. (1991) and Nakiboglu et al. (2001). Nakiboglu et al. (2001) reported that the treatment with such enzyme resulted with the achievement of about 99% pure silver recovery from X-ray films. In addition to recovery of silver, recovery of base film made of polyester is also possible using alkaline proteases (Gupta et al., 2002).

Removal of proteinaceous stains of blood, milk, egg, grass and sauces is very difficult using conventional surfactants. Removal of such a stain is now a day achieved by using alkaline proteases (Rao et al., 1998; Maurer, 2004). To be used as detergent additives proteases need to be active and stable at an alkaline pH, be stable in presence of chelating agents, and must possess broad substrate specificity. In addition, stability and residual activity in the presence of detergent additives such as surfactants, builders, bleaching agents, bleach activating agents, fillers, and fabric softeners are also required (Maurer, 2004). Considering the applications of proteases in various industries, in the present study the crude enzymes obtained from three potent bacterial strains were subjected to various industrial applications for the possible industrial use.
5.2. MATERIALS AND METHODS

5.2.1. Effect of surfactants on enzyme stability

Stability of protease towards various surfactants such as sodium dodecyl sulphate (SDS), Tween 20, Tween 40, Tween 60, Tween 80, Triton X 100 and polyethylene glycol (PEG) was evaluated individually with 1% (v/v) level each. For this, the surfactant solutions were prepared and mixed with 0.1 ml of crude enzyme produced by the three potent bacterial isolates *B. subtilis*, *P. luteoviolacea* and *L. fusiformis* were added and it was incubated for 1 h at room temperature (30 ± 1 °C). Then the protease activity was estimated under standard assay procedure. The enzyme activity of a control sample (without surfactant) was taken as 100%.

5.2.2. Effect of detergents on enzyme stability

The stability of obtained proteases towards various detergents was also studied. The detergents used were: Henko, Ujala, Tide+, Ariel, Sunlight, Mr. White, and Surf excel. These detergents were individually taken with 1% (v/v) level each. The detergent solutions were prepared in double distilled water then the crude enzyme produced by the three potent bacterial isolates *B. subtilis*, *P. luteoviolacea* and *L. fusiformis* were mixed to these and incubated for 1 h at room temperature (30 ± 1 °C). The protease activity was estimated under standard assay procedure. The relative activity was expressed in percentage activity considering the activity of control as 100% (Mukherjee *et al.*, 2009).
5.2.3. Effect of solvent on enzyme stability

The stability of protease produced by the three potent bacterial isolates *B. subtilis*, *P. luteoviolacea* and *L. fusiformis* towards various solvent was studied by incubating in individual solvents such as acetonitrile, benzene, chloroform, toluene, hexane and acetone along with 0.1 ml crude sample. The mixture was incubated for 1 h at room temperature (30 ± 1 °C) and the protease activity was estimated under standard assay procedure. The enzyme activity of a control sample (without solvent) was taken as 100%.

5.2.4. De-hairing properties of protease

De-hairing properties of protease produced by the three potent bacterial isolates *B. subtilis*, *P. luteoviolacea* and *L. fusiformis* was assessed in goat skin obtained from the slaughter house at Nagercoil, Kanyakumari District, Tamilnadu. The de-hairing property of the enzyme was carried out at room temperature with crude enzyme preparation from an industrial point of view. Goat skin was cut into small pieces (2 × 2 cm) and incubated with 10.0 ml crude enzyme at a final concentration of 250 U/ml for up to 24 h. The control skin was incubated in solution devoid of enzyme. The skin pieces were then observed for dehairing activity (Huang *et al.*, 2003).

5.2.5. Effect of protease for its activity on chicken feather

To evaluate the keratinase activity of proteases from the three selected organisms (*B. subtilis*, *P. luteoviolacea* and *L. fusiformis*) chicken feathers were used as substrate. About 0.4 gm chicken feather was taken in a test tube and to this 10 ml crude enzyme (250 U/ml) from the selected all three isolates were
incubated at $31 \pm 1 \, ^\circ\text{C}$ for 24 h. After 24 h, feather degrading activity of enzyme was observed from the experimental tubes.

### 5.2.6. Effect of enzyme on silver recovery

X-ray film was used to study the effect of protease produced by the three potent bacterial isolates *B. subtilis*, *P. luteoviolacea* and *L. fusiformis* on silver recovery. Used X-ray film was washed with double-distilled water and impregnated with ethanol. The washed X-ray film was dried in an oven at 40°C for 30 min. About 0.5 g of X-ray film (2 cm × 2 cm) was incubated in 10 ml crude enzyme (250 U/ml) at room temperature (28.0 ± 1°C) for 24 h. Visual examination of films after complete hydrolysis of gelatin layer was done.

### 5.2.7. Evaluation of proteases on dirt removal

Clean cloth pieces (3 cm × 3 cm) were stained with three different stains like chicken egg, chocolate, and tomato sauce and then dried. To the stained cloth, 10 ml of crude protease produced by the three potent bacterial isolates *B. subtilis*, *P. luteoviolacea* and *L. fusiformis* (250 U/ml) were added in a Petri disc and incubated at $30 \pm 1 \, ^\circ\text{C}$ for 24 h. After incubation, cloth pieces were taken out, rinsed with tap water and dried at 50°C for 2 h. Then the wash performance was analyzed visually by observing the cloth pieces.

### 5.3. RESULTS

#### 5.3.1. Effect of surfactants on enzyme stability

Among the tested surfactants, protease from all the three tested microbes such as *B. subtilis*, *P. luteoviolacea* and *L. fusiformis* showed least enzyme
stability on SDS and it was registered as 142.00 ± 8.74%, 102.40 ± 6.82 and 120.40 ± 8.22%, respectively. The protease from *B. subtilis* was also stable towards the other non-ionic surfactants like tween-20, tween-40, tween-60, tween-80, PEG, and triton X-100 and the corresponding enzyme activity was determined as 101.50±5.76%, 113.60±7.20%, 107.30 ±6.98, 101.30 ±8.36, 136.30±7.46 and 118.80±5.49%, respectively (Table 4.1). However, the protease from *P. luteoviolacea* was stable only against SDS and the other surfactants expressed less enzyme stability. The protease from *L. fusiformis* was stable towards the non-ionic surfactants like tween-20, tween-40, tween-60, tween-80, PEG, and triton X-100 and the relative enzyme stability was found to be 111.60±5.98, 119.80±9.16, 105.10 ±6.95, 113.90 ±8.56, 114.70±7.66% and 128.50±8.34% respectively (Table 5.1).

### 5.3.2. Effect of detergents on enzyme stability

The stability of the enzymes produced by the tested microbes were assessed by incubating them in various commercial detergents. The protease produced by *B. subtilis* showed better stability and compatibility towards the tested detergents than that of proteases produced by the other two bacterial isolates such as *P. luteoviolacea* and *L. fusiformis*. The protease from *B. subtilis* retained 46.40 to 99.30% of its original activity at room temperature in the presence of detergents in the following order: Ariel (99.30 ± 4.36%) > Sunlight (89.40±6.52%) > Tide+ (85.60 ± 5.84%) >Mr. White (85.10 ± 5.35%) >Mr. White (85.10 ± 5.35%) >Mr. White (85.10 ± 5.35%) >Henko (78.30 ± 6.40%) >Surf excel (48.90± 2.23%) > and Ujala (46.40 ± 2.15%). The proteases from *P. luteoviolacea* and *B. subtilis* showed less stability against the selected detergents. Among all the tested detergents, the protease from
*P. luteoviolacea* was highly stable towards Henko (58.90 ± 3.44%), whereas the protease of *L. fusiformis* was highly stable towards Ariel (76.40 ± 3.90) (Table 5.2).

### 5.3.3. Effect of solvents on enzyme stability

The enzyme stability towards various solvents such as acetonitrile, benzene, chloroform, toluene, hexane and acetone was tested by incubating the crude proteases produced by the three organisms. The enzyme from *B. subtilis* and *L. fusiformis* was stable in the presence of chloroform and the relative enzyme activity recorded was 68.90±4.32%, 59.20±2.36%, respectively (Table 4.3). However, protease from *P. luteoviolacea* showed least stability against various solvents and the relative enzyme activity recorded was ranged from 1.90% to 33.90%. The enzyme from *L. fusiformis* was stable in the presence of toluene acetonitrile and acetone and the relative enzyme activity was 38.20 ± 1.92%, 53.40 ± 3.82 % and 39.60 ± 4.26%, respectively (Table 5.3).

### 5.3.4. Dehairing activity of protease

The dehairing property of the enzyme was evaluated by incubating crude enzyme of the selected bacterial isolates against goat skin for 24 h at room temperature. Among the tested enzymes, protease of *B. subtilis* had potent dehairing activity and this enzyme removed 100% of hair within 12 h at room temperature (Plate 5.1a and b). However, the protease from *P. luteoviolacea* dehaired approximately 20% of the goat hair (Plate 5.2a and b). Invariably, the protease from *L. fusiformis* did not show dehairing property (Plate 5.3a and b).
5.3.5. Feather degrading activity of proteases

The present study revealed that the crude proteases from *B. subtilis*, *P. luteoviolacea* and *L. fusiformis* were capable of degrading chicken feather after 24 h of incubation at room temperature. Among the three tested enzymes, the protease of *B. subtilis* digested chicken feather moderately after 24 h of incubation (Plate 4.4a and b); whereas, the protease of *P. luteoviolacea* showed more feather degrading activity than *B. subtilis* (Plate 5.5a and b). At the same time the protease from *L. fusiformis* also possessed feather degrading activity (Plate 5.6a and b).

5.3.6. Effect of proteases on silver recovery

In the present study to evaluate the effect of protease on silver recovery, pieces of used ‘X’ ray films were used. They were individually treated with 10 ml of crude protease of all three selected bacterial isolates. Among the three proteases, the protease produced by *B. subtilis* alone had gelatinase activity, which could digest the gelatine layer of the ‘X’ ray film simultaneously removed the silver ions coated on the X-ray film (Plate 5.7a and b). However, the protease from *P. luteoviolacea* and *L. fusiformis* did not express any gelatinase activity and it was confirmed by no change in colour on the ‘X’ ray film.

5.3.7. Analysis of stain removal ability by proteases

Stain removal ability of crude enzyme was assessed using cotton cloths stained with chicken egg, chocolate and tomato sauce individually. The proteases of *B. subtilis* and *L. fusiformis* removed stain from the cotton cloth completely;
whereas, the protease from *P. luteoviolacea* showed moderate activity on these stains (Plate 5.8a and b and Plate 5.9a and b).

**5.4. DISCUSSION**

Proteases are having innumerable industrial applications and hence it is appropriate to test its efficiency towards the ionic and non-ionic detergents to prove their effectiveness. Considering this in the present study, the proteases produced from the bacterial isolates *B. subtilis, P. luteoviolacea* and *L. fusiformis* were subjected for various industrial applications. The stability of proteases towards surfactants was determined by incubating the enzyme with different surfactants (1%) for 1 h at room temperature. The alkaline proteases produced by *B. subtilis, P. luteoviolacea* and *L. fusiformis* were stable towards almost all the tested ionic and non-ionic surfactants like SDS, tween-20, tween-80 and triton X-100. The protease produced by *B. subtilis* was found to be more stable than other tested organisms. This finding gains significance because modern bleach-based detergent formulations are mainly composed of SDS. The result of the present study is in consistence with the observation made by Joo *et al.* (2003) who have evaluated the compatibility of protease produced by *B. clausii* towards detergents and they further inferred that the protease had potent stability against various detergents including SDS. Subba Rao *et al.* (2009) tested the protease produced by *B. circulans* against various detergents and this enzyme was stable against the detergents such as SDS and Triton X100. The enzymatic properties of the protease suggested its suitability as an addition to detergent formulations. The results obtained in the present study are in agreement with previous report on alkaline proteases from *B. mojavensis*, and *Bacillus* sp., where these enzymes
were highly stable in the presence of detergents such as SDS, Tween 20, Tween 80 and Triton X100 (Gupta et al., 2002; Rai and Mukherjee, 2010). The stability towards SDS is important because SDS-stable enzymes are generally not available except for a few like *Bacillus clausii* I-52 (Joo et al., 2003) and *Bacillus* sp. RGR-14 (Oberoi et al., 2001).

The suitability of any protease for inclusion in detergent formulation is mainly depend on its stability and compatibility with detergent components (Kumar and Tagaki, 1999). Besides, the enzyme should be alkaline in nature and thermostable (Venugopal and Saramma, 2006). However, the stability and compatibility of any component should not be the only pre-requisite for its inclusion in detergent formulation. To save energy from heating, the water to be used for washing, the ability of the detergent components to perform wash function at lower temperature should also be addressed (Krik et al., 2002). There are only few reports published showing the activity of protease at room temperature (Venugopal and Saramma, 2006; Tamiya and Nakamura, 1996). In the present study, the protease by *B. subtilis* was highly stable towards commercial detergents than the proteases of *P. luteoviolacea* and *L. fusiformis*. The proteases produced by these isolates were remained active at room temperature and also at neutral pH and thus favoured their inclusion for making laundry detergents. The present study revealed that the proteases produced from the bacterial isolates were stable against almost all the tested detergents. The proteases belonging to *Bacillus* genus are known to be unstable against the oxidants and bleaching agents (Anwar and Saleemuddin, 2000). Detergent stable proteases were reported from bacteria such as *B. clausii* and *B. mojavensis* (Joo et
al., 2003; Rai and Mukherjee, 2010). Arulmani et al. (2007) applied crude protease from *Bacillus* sp. on various detergents and found its stability towards these tested detergents and this result was comparable with the present study.

There is a greater industrial demand for the organic solvent tolerant proteases for application in the synthesis of useful products with the presence of organic solvents (Gupta and Khare, 2007). Hydrophobic solvents are usually superior to hydrophilic solvents, because the latter have a greater tendency to strip tightly bound water from the enzyme molecules essential for catalytic activity. If organic solvents are used as media in the synthetic reaction, the reaction equilibrium of hydrolytic enzymes can be shifted towards completion of the reverse of hydrolysis. Therefore, proteases, which are naturally stable in the presence of organic solvents, could be very useful for synthetic reactions. In the present study, the enzymes under investigation showed stability after 1 h incubation with the tested organic solvents (acetonitrile, chloroform, benzene, toluene, hexane and acetone). Among the proteases, protease from *B. subtilis* showed more stability towards selected organic solvents than the proteases of *P. luteoviolacea* and *L. fusiformis*. There are very few reports available on organic solvent tolerant alkaline proteases from bacterial species (Rahman et al., 2006; Thumar and Singh, 2009). The solvents such as acetonitrile and toluene which were also been reported to be quite harmful to other solvent-stable proteases (Tang et al., 2010). In the present study, the protease of *B. subtilis* showed more stability towards solvents such as acetonitrile, chloroform and acetone. However, the protease of *P. luteoviolacea* was not stable towards these said solvents. Akolkar et al. (2008) stated that the protease from *Halobacterium*
SP1(1) has high stability towards various solvents such as toluene, xylene and n-decane, the majority of which are frequently used in paints. In the present study, the proteases from the selected three bacterial isolates showed stability towards toluene. The protease may be stable because of the replacement of some water molecules in an enzyme with organic molecules, sometimes stabilizing the structure of the enzyme (Frikha *et al*., 2003).

Also bacterial proteases are used in leather industry. It is gaining importance as an alternative chemical process and is significant in the reduction of toxicity in addition to the improvement of the texture of leather (Sivsubramanian *et al*., 2008). Although microbial protease may be an alternative eco-friendly strategy to replace the use of eco-hazardous chemicals for the dehairing purpose (Huang *et al*., 2003); however, most of them were unsuitable for dehairing purpose because of the associated collagen-degrading activity (Huang *et al*., 2003). In the present study, *B. subtilis* protease effectively removed hair from the goat skin after 24 h of incubation at room temperature. Several microbial proteases were evaluated for their dehairing property. Sivasubramanian *et al*. (2008) reported that the protease produced by *B. subtilis* had potent dehairing activity, and it removed hair within 24 h of incubation at room temperature. Aravindan *et al*. (2007) have also reported the dehairing property of protease from *B. cereus* and this protease dehaired goat skin within 24 h of incubation. Similarly, in this study, the protease from *P. luteoviolacea* dehaired goat skin; however, this enzyme digested other layer of skin also. Hence, proteolytic enzyme from *P. luteoviolacea* is not suitable in leather processing.
However, this protease may be useful in leather waste effluent treatment because of this activity. Alexandre et al. (2005) reported the dehairing nature of protease from *B. subtilis* S14 with elastase, keratinase and collagenase activities.

Feathers are largely produced as a waste by-product at poultry industries (Williams et al., 1991). They are insoluble structural proteins cross-linked by disulfide, hydrogen and hydrophobic bonds, but could represent a rich protein resource, because they contain over 90% (w/w) keratin. Keratins cannot be degraded by the usual proteolytic enzymes such as pepsin, trypsin and papain. Nevertheless, feathers do not accumulate in nature, because keratins could be degraded only by keratinase produced by selected microorganisms (Onifade et al., 1998). Many keratinases from species of *Bacillus* (Williams et al., 1990; Riffel et al., 2003; Lucas et al., 2003), fungi (El-Naghy et al., 1998; Gradišar et al., 2000; Friedrich et al., 2005) and *Actinomycetes* (Ignatova et al., 1999; Gushterova et al., 2005) had been reported and some of them were purified and characterized (Lin et al., 1992; Böckle et al., 1995; Nam et al., 2002). Keratinases from microorganisms have many applications in the feed, fertilizer, detergent, leather and pharmaceutical industries (Gupta and Ramnani, 2006). In the present study, proteases from *B. subtilis*, *P. luteoviolacea* and *L. fusiformis* showed keratinase activity. This degradation suggested that this enzyme possessed both keratin disulfite reductase and keratinase activity. *Bacillus* species have been known to produce a number of hydrolytic enzymes including keratinase, which is able to degrade feathers, wool and hair (Gupta et al., 2002b). Williams et al. (1990) stated that protease from *B. licheniformis* PWD-1 had potent activity against chicken feather. Atalo and Gashe (1993) described a
thermophilic *Bacillus* species that could produce protease to degrade various fibrous proteins such as feather, hair, sheep skin or horn.

In the present study, among the protease produced by the three tested bacterial isolates such as *B. subtilis*, *P. luteoviolacea* and *L. fusiformis*, only *B. subtilis* protease digested the gelatin layer of X ray film and released silver ions. Masui *et al.* (1999) studied the decomposition of gelatin by proteases from alkalophilic *Bacillus* sp. and its three mutants. They reported 0-17% gelatin degradation in 30 min, depending on the enzyme from wild or mutant strains. Most of the proteases used so far to recover silver are from bacteria such as *B. sphaericus* (Singh *et al.*, 1999), *B. subtilis* (Nakiboglu *et al.*, 2001; Ramakrishnan *et al.*, 2010), and fungi such as genetically-engineered *Aspergillus oryzae* (Samarntan and Tanticharoen, 1999) and *Conidiobolus coronatus* (Sankar *et al.*, 2010). Fujiwara *et al.* (1989) have used alkaline protease of *Bacillus* sp. to recover silver ions from the used ‘X’ ray film. Ishikawa *et al.* (1993) carried out fundamental studies on the kinetics and mechanism of the enzymatic hydrolysis of gelatin layers on film and release of silver particles.

Similarly in this study proteases from *B. subtilis* and *L. fusiformis* showed potent activity on stain removal. Stain removal ability of crude enzyme was assessed using cotton cloths stained with chicken egg, chocolate and tomato sauce. In case of removing stain from cloths, it was seen that the protease enable to remove stain very easily without addition of any detergents. Adinarayana *et al.* (2003) have studied the effect of alkaline protease from *B. subtilis* PE11 in removal of blood stains. The compatibility of protease from *B. subtilis* on stain
removal suggested the use of this protease along with detergent solution too. Its ability to act in the presence of solvents and detergents can be exploited for this purpose. Anwar and Saleemuddin (1997) also reported usefulness of protease from *Spilosoma obliqua* for removal of blood stains from cloth in the presence and absence of detergents. This present study revealed that the proteases produced by *B. subtilis*, *P. luteoviolacea* and *L. fusiformis* were stable against almost all tested surfactants, detergents and solvents. These enzymes removed hair from goat skin, which may have great application in leather industry. These enzymes also have potential applications such as feather degradation, silver recovery and stain removal.