Journal of Pharmaceutical, Chemical and Biological Sciences
ISSN: 2348-7658
Impact Factor (GIF): 0.701
Impact Factor (SJIF): 2.092
September-November 2016; 4(3): 365-374
Published on: 31st October 2016

Review Article

Microbial Proteases in Commercial Applications

Rajendra Singh[1], Anshumali Mittal[2], Manoj Kumar[1], Praveen Kumar Mehta[3]*

[1]Department of Biochemistry, VP Chest Institute, University of Delhi, Delhi-110007, India
[2]Division of Structural Biology and Biophysics, Mill Hill laboratory, The Francis Crick Institute, London, UK
[3]Centre for Molecular Biology, Central University of Jammu, Jammu-181143, India

***Corresponding Author:** Praveen Kumar Mehta, Centre for Molecular Biology, Central University of Jammu, Jammu-181143, India

Received: 06 October 2016 Revised: 18 October 2016 Accepted: 21 October 2016

ABSTRACT

Proteases catalyze hydrolysis of peptide bonds in proteins and are one of the most widely used industrial enzymes. Though they are ubiquitously found in a wide diversity of sources such as plants, animals, and microorganisms but microbial sources are preferred for the production of proteases due to technical and economic advantages. Microbial proteases have potential for application in different industries including detergent, leather, silver recovery, dairy, baking, beverages and pharmaceutical industries. These hydrolytic enzymes are efficiently involved in food industry for enhancing nutritional value, digestibility, palatability, flavour and reducing allergenic compounds as well as in management of domestic and industrial wastes. Furthermore, they are also involved in synthesis and structural elucidation of proteins. The present communication is an overview of the proteases produced from bacterial and fungal sources and their role in various industrial applications.

Keyword: Protease; microbial; alkaline; application

INTRODUCTION

Proteases, one of the most valuable industrial enzymes, have potential applications in a wide number of industrial processes such as food, feed, leather, textile, pharmaceutical industries. Proteases can be obtained from animals, plants and microorganisms. However, proteases from microbial sources have dominated their presence in industrial applications due to their easy availability, and fast growth rate [1]. In recent decades, proteases have been recognized for their considerable applications in industry and therapeutics worldwide. Proteases constitute a large group of enzymes that catalyze the cleavage of peptide bonds in other proteins as well as within proteins [2].

Proteases, hydrolytic enzymes, are the most commercially applicable enzymes occur in a wide diversity of plants, animals, and microorganisms. They have vital role in both physiological processes, e.g. zymogen activation by proteolysis, blood coagulation, transport of secretory protein across membranes, tumor growth, protein catabolism, inflammation, cell growth, tissue arrangement and morphogenesis in development [3, 4].

Microbial origin proteases are preferred over other proteases for industrial application due to the technical as well as economic advantages [4]. Microbial proteases have been studied extensively due to easy cultivation, high productivity and ease with genetic manipulation to improve the catalytic properties [3, 5]. In general, proteases production from microorganisms is constitutive or partially inducible in nature. Proteases of microbial sources have find commercial applications in detergents industry, tannery, leather industry, peptide synthesis, dairy processing, brewing, tenderization of meat, baking and pharmaceutical industry [6-9].

Proteases, particularly alkaline proteases, hold a great potential for application in the detergent and leather industries due to the increasing trend to develop environmentally friendly technologies. There is a great deal of interest in using proteolytic enzymes as targets for developing therapeutic agents. The current estimated value of the worldwide sales of industrial enzymes is $4.2 billion [1]. Proteases represent one of the three largest groups of industrial enzymes and their global market is projected to reach approximately $ 2.21 billion in terms of value by 2021 at a CAGR of 6% from 2016 to 2021. Proteases from microbial origin are accounted for the largest share in the market in terms of value, followed by the animal source [10].

Sources

Proteases have physiological role in all living organisms and therefore, they are present in a wide range of sources such as animals, plants and microorganisms [3].

Plant Proteases

Plant proteases are widely used in food and pharmaceutical industry. Most extensively explored plant proteases are bromelain, ficin and papin extracted from *Ananas comosus, Ficus carica* and *Carica papaya*, respectively. These proteases are utilized for different application such as brewing, tenderization of meat, milk coagulation, digestion, viral and cancer treatment. Keratinases, another important plant protease, hydrolyze hair and wool to produce essential amino acids and to prevent clogging of waste water system. In recent time plant proteases have gained increasing attention, though the proteases production from plants is a time consuming process. In addition to it, other factors such as land area for cultivation and climatic conditions also regulate production of proteases by plants [3, 11].

Animal Proteases

The most widely used animals derived proteases are pancreatic trypsin, chymotrypsin, pepsin and rennin [3]. Trypsin, an intestinal digestive enzyme, is utilized for biocontrol of insect pests, microbial growth media and few medical applications. Chymotrypsin and rennin are extensively used in deallergenizing of milk protein hydrolysate and preparation of curd, respectively [3].

Microbial Proteases

To meet continuously growing demand of proteases for various applications, microorganisms are preferred over plant and animal sources for the production of proteases due to their wide substrate specificity and ease of genetic manipulation. Proteases from

microorganisms are the largest group of industrial enzymes and account for greater than 60% of total global sale of enzymes [4, 12]. Fungal source that produce these hydrolytic enzymes belong to the genus *Aspergillus, Humicola, Mucor, Penicillum, Rhizopus, Thermomyces* etc. [13]. Though fungal proteases have lower reaction rate and heat stability than bacterial enzymes but they represent wider variety of enzymes and broad substrate specificity. A great number of bacterial and fungal species used as a source of acid, neutral and alkaline proteases are mentioned in Table 1.

Table 1: Microorganisms having protease activity [4, 6, 14-16]

Bacteria	*Bacillus clausii, B. cereus, B. licheniformis, B. sphaericus, B. subtilis, B. sterothermophilus, B. mojavensis, B. megaterium, B. brevis, B. anthracis, B. thuringiensis B. circulans B. coagulans, B. marmarensis, B. firmus, B. stratosphericus, B. polymyxa, B. Lentus, B. alcalophilus, B. amyloliquifaciens, B. subtilis, B.intermedius, B. thermoruber, Bacillus pumilus B. cohnii, B. fastidiosus, B. pseudofirmus, B. pantotheneticus, B. aquimaris, B. proteolyticus, B. laterosporus, B. coagulans, B. amovivorus, B. flexus, B. horikoshii, Pseudomonas aeruginosa, P. fluorescens, P. putida, Aromonas hydrophila, Serratia liquefaciens, Flavobacterium balustinum, Exiguobacterium sp.*
Fungi	*Aspergillus awamori, A. clavatus, A. flavus, A. fumigates, A. niger, A. oryzae, A. parasiticus, A. ustus, Beauveria bassiana, B. feline, Botrytis cinérea, Clonostachys rosea, Conidiobolus coronatus, Cordyceps militaris, C. sinensis, Fusurium oxysporum, Graphium putredinis, Mucor circinelloides, Penicillium camemberti, P. citrinum, P. restrictum, P. roqueforti, Phanerochaete chrysosporium, Rhizomucor sp., Rhizopus SMC, R. oryzae, Thermoascus aurantiacus, T. aurantiacus, Thermomyces lanuginosus, T. lanuginosus, T. lanuginosus, Trichoderma harzianum, T. reesei*

Classification of Microbial Proteases

The classification of proteases is based on either their origin, catalytic mechanism, specificity or the nature of reactive group in the catalytic site. Based on the site of action on polypeptide chains, proteases are divided in to two groups, i.e. exopeptidases and endopeptidases. Exopeptidases act only near the ends of polypeptide chains and are further sub-classified as amino- and carboxy-peptidases based on the site of action at the amino- or carboxyl terminus, respectively.

The endopeptidases cleave peptide bonds in the inner regions of the polypeptide chains away from the both end terminus. These are further sub-classified into six groups i.e., serine, aspartic, cysteine, metallo, glutamic acid and threonine proteases based on the essential catalytic residue present in the active site[5].

Serine Proteases (EC 3.24.21)

Serine proteases, most widely distributed among microorganisms and eukaryotes, are characterized by the presence of a reactive serine residue in the active site [3]. These proteases are optimally active over a wide pH range 7 – 11 and have broad substrate specificities including amidase and esterolytic activity. Serine alkaline proteases have largest commercial application owing to their high activity and stability in extreme reaction conditions [8]. This group of enzymes are generally inhibited by di-isopropyl fluorophosphates, 3,4-dichloroisocoumarin (3,4-DCI), l-3-carboxytrans 2,3-epoxypropyl-leucylamido (4-guanidine) butane (E.64), tosyl-l-lysine chloromethyl ketone (TLCK) and phenylmethylsulfonyl fluoride (PMSF).

Cysteine proteases (EC 3.4.22)

Cystein proteases are proteins that constitute a catalytic dyad consisting of cysteine and

histidine for their activity. Though reducing agents such as HCN or cysteine, DTT, EDTA are required for stimulation of the catalytic activity of cysteine proteases but inhibited by sulfhydryl (SH) reagents such as 4-hydroxy mercuri benzoic acid (p-CMB), iodoacetic acid, iodoacetamide, etc [3]. They have high potential in food and pharmaceutical applications due to their activity over a wide range of temperature and pH [11]. They are involved in metabolic degradation of proteins and peptides such as scrapie protein degradation dendritic and neuronal cells [17]. Papain is one the best known microbial cysteine protease, which is widely used in food industry [4, 18, 19].

Aspartate proteases (EC 3.4.23)

These endopeptidases are acid proteases and contain two aspartic residues for their catalytic activity [20]. Most aspartic proteases are optimally active at acidic pH (pH 3 to 4) and have isoelectric points in the range of pH 3 to 4.5. These proteases preferentially cleave peptide bonds between non-polar amino acids residues [20]. These proteases are inhibited by pepstatin and, in the presence of copper ions by diazoketone compounds [3].

Metallo Proteases (EC 3.4.24)

This group of enzymes require divalent metal ion, such as zinc, cobalt or manganese for their catalytic activity [21]. These proteases are sensitive to chelating agents, such as EDTA, due to sequestering effect of chelating agents on the metal ions involved in the catalytic mechanism. They have a wide range of substrate specificity and find wide range of applications in different industries including drug development [22].

In addition, proteases are also classified into three other important groups, namely threonine proteases, glutamic acid proteases and asparagine proteases. Threonine proteases (EC 3.4.25) are represented by the presence of a threonine residue at catalytic site. The classic examples of this group are acyltransferases and proteasome [3, 22]. Glutamic acid (EC 3.4.19) and asparagine proteases are characterized by the presence of glutamic acid and asparagine residue at their active site, respectively. Glutamic acid proteases have potential applications in food industry and therapeutic management [22].

Commercial Applications

The global demand of enzymes, for a wide variety of applications, is significant. Proteases have extensive application in food, detergent, leather and pharmaceutical industries. In addition, they are also involved in management of waste from domestic and industrial activities [15, 16].

Table 2: Applications of proteases in different industries [3, 11, 14, 15, 22]

Industry	Applications
Food	Improved digestibility, solubility flavor, palatability and viscoelsatic properties; enhanced oil recovery from seafood, meat tenderization, reduced allergenicity
Detergent	Improved washing
Peptide synthesis	Enantioselective peptide synthesis
Textile	Degumming, texture development
Leather	Leather processing: Dehairing, bating, tanning
Bioremediation	Waste treatment
Pharmaceuticals	Anticancer, anti-inflammatory, clot-buster agents
Others	Silver recovery, silk degumming

Detergent industry

In 1913, pancreatic extract was reported to be used for the first time in the enzyme-detergent preparation [3]. Then, after four decades, a microbial enzyme was used commercially in the detergents under the trade name of BIO-40 [3, 23]. Detergent industry represent the largest industrial application of enzymes amounting to 25–30 % of the total sales of enzymes and expected to grow faster at a CAGR of about 11.5 % from 2015 to 2020 [1]. Protease digests on stains due to food, blood and other body secretions. Proteases are used as one of key constituent in detergents formulations to improve washing performance for use in domestic laundering to solution for cleaning contact lenses or dentures [3, 24, 25]. The application of enzymes in detergents has the advantages of removing spots in eco-friendly manner with shorter period of soaking and agitation [26]. The enzymes used as detergent additives should be effective in very small amount over a broad range of pH and temperature with longer shelf life [27].

Most often, the proteases used in detergent formulations are serine proteases produced by *Bacillus* strains [28]. Alkaline proteases from fungal sources are also gaining interest due to ease in downstream processesing. In many formulations, cocktail of different enzymes including protease, amylase, cellulase and lipase are also used for improved washing effect for household purposes [27].

Peptide synthesis

Peptide synthesis through chemical methods has disadvantages, such as, low yield, racemization issues and health and environmental concern due to toxic nature of solvents and reagents used in the processes[29, 30]. Whereas the enzyme mediated peptide synthesis offers several advantages like enantioselectivity, racemization free, environmental friendly reaction conditions etc [31]. Besides, no or minimal requirement of pricey protective groups, solvents, reagents in enzyme based synthesis are cost effective in comparison to chemical synthesis [32].

Enzymatic synthesis of peptides has attracted a great deal of attention in recent years. Proteases from bacterial, fungal, plant and animal sources have been successfully applied to the synthesis of several small peptides, mainly dipeptides and tripeptides. Peptide bonds can be synthesized using proteases in either a thermodynamically controlled or a kinetically controlled manner.

Proteases from microbial sources have been used satisfactorily for synthesis of peptide bonds as well as hydrolysis of peptide bonds [14, 33-36]. Organic solvent tolerant alkaline proteases from the species of *Aspergillus, Bacillus, Pseudomonas* have shown promising potential in the synthesis of peptide. Proteases from microbial sources have also established their potential for synthesis of peptide in minimal water system. Small peptides such as di or tripeptide synthesized through enzyme mediated processes are used for nutrition and in pharmaceuticals [37, 38].

Leather Industry

The conventional methods for leather processing involve toxic and hazardous chemicals that generate environmental pollution and consequently a detrimental effect on living organisms. The enzyme mediated leather processing has proved, successfully, to overcome the issues generated by chemical methods. The application of enzymes in leather processing has improved leather quality and reduction of environmental pollution [1, 39, 40]. Proteases are used to degrade noncollagenous constituents of the skin and elimination of nonfibrillar proteins. Microbial alkaline proteases are used to ensure faster absorption of water, which reduce the soaking time [40]. Application of alkaline proteases coupled with hydrated lime and sodium chloride during dehairing and dewooling reduce

waste disposal. The protease mediated leather processing is an efficient alternative in an environmental friendly manner to improve the quality of leather, help to shrink waste and, save time and energy [3, 12, 41].

Food Industry

Proteases are used in food industry for a wide range of applications. These enzymes are efficiently involved in the modification of properties of food proteins to improve nutritional value, solubility, digestibility, flavour, palatability and minimizing allergenic compounds [14, 42]. Besides, their basic function, they are also used to modify functional properties, such as coagulation, emulsification, foaming, gel strength, fat binding etc. of food proteins [43]. The catalytic function of proteases is used in the preparation of protein hydrolysate of high nutritional value, which is used in infant food products, medicinal dietary products, fortification of fruit juice and soft drinks [14, 44, 45].

In dairy industry, proteases are primarily used in cheese manufacturing to hydrolyze specific peptide bonds to produce casein and macropeptides [3]. The ability of proteases to hydrolyze connective tissues and muscle fibre proteins is used for tenderization of meat [6]. The alkaline proteases play an important role in the production of soy sauce and other soy products.

In baking industry, they are added to ensure dough uniformity, reduce dough consistency, maintain gluten strength in bread and, improve flavor and texture in bread [46]. These hydrolytic enzymes are utilized for degradation of the turbidity complex resulting from protein in fruit juices & alcohol based liquors; in gelatin hydrolysis and recovery of meat proteins [4].

Therapeutic application

The wide diversity and specificity of microbial proteases are extensively used in diagnostic and therapeutic purposes. Bacterial and fungal proteases have contributed equally in developing effective therapeutic agents, such as anticancer, clot dissolving, antimicrobial, anti-inflammatory etc [22, 47].

Protease mediated treatment of acute and chronic inflammation is cost effective without any reported side effect. Serratiopeptidase, a protease produced by *Serratia* species, is the most effective protease used against inflammation [48]. It is also used to inhibit the release of pain inducing peptide i.e. bradykinin to alleviate pain [49, 50].

Protease from *Aspergillus oryzae* is used as digestive aid to cure lytic enzymes deficiency [3]. Asparaginase from *Escherichia coli* and clostridial collagenase are used in treatment of lymphocytic leukemia and burns & wounds, respectively. Nattokinase from *Bacillus subtilis* is used as cardiovascular disease nutraceutical and helps to lower risk of the disease [51]. It prevents blood coagulation and dissolves thrombus [52]. Several proteases from bacterial and fungal sources have also been reported for anti-microbial properties [22]. Furthermore, proteases are also exploited in degradation of keratinized skin and preparation of vaccine for dermatophytosis therapy [53-55]. As trauma medicine, these hydrolytic enzymes are applied to remove scar, regenerate epithelia, and faster healing processes [4, 56].

Other Applications

Since ancient time proteases have been included in the preparation of sauce and other products from soy that help in the degradation of high protein content grains. Proteolytic modification by fungal alkaline and neutral proteases in soy processing improve their functional properties [3, 57].

Protein hydrolysates are used in the synthesis of many food and healthcare products and the bitter taste of protein hydrolysates, due to presence of hydrophobic amino acids and proline, is a major hurdle for their commercial applications. The peptidases have great

potential for debittering of protein hydrolysates as they have high specificity for hydrophobic amino acids and proline[3, 58]. Proteases are also required for their key role in basic research. Their specific peptide bond hydrolytic potential is used in the structural elucidation and synthesis of proteins.

The silver recovery from X-ray and photographic films also involved proteases. A large amount of this precious and noble metal is used in photographic industry and the recovery of silver through conventional methods poses serious environmental issues. Alkaline proteases from *Bacillus subtilis, Conidiobolus coronatus* and *Streptomyces avermectinus* genus have been successfully used in the recovery of silver [14, 59]. Proteases have also proved their significant role in silk degumming and final elegant texture finishing in textile industry [22].

Proteases from microbial sources have also been used in the management of industrial as well as household wastes. Alkaline protease from *Bacillus subtilis* have been reported for the processing of waste feathers from poultry abattoir [15]. A combination of proteases from *Bacillus subtitlis , B. amyloliquefacines* and *Streptomyces* sp. with thioglycolate is used commercially to clean pipes clogged with hair containing deposits [60, 61].

CONCLUSION

Proteases have shown their potential role in different industries and a number of microbial sources exist for the efficient production of these enzymes to meet continuously increasing demand. Their immense diversity, precise range of action and property of being active over a very wide range of temperature and pH have attracted the attention of biotechnologists worldwide. They are ubiquitous in all living organisms and, microorganisms are the preferred source due to easy cultivation, faster generation time and ease with the genetic manipulation to generate new enzymes with desirable properties or simply for enzyme overproduction. Biotech industries invest a significant amount of their revenues in search of new microorganisms that can be used for novel protease production. Proteases have various applications in major areas of food processing, beverage production, leather, textiles, detergents, etc. and with the advent of new frontiers in biotechnology; the spectrum of protease applications has expanded into many new fields such as clinical, medicinal and analytical chemistry.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

REFERENCES

1. Singh R et al. Microbial enzymes: industrial progress in 21st century. 3 Biotech 2016; 6: 174.
2. Chang HY and Yang X. Proteases for cell suicide: functions and regulation of caspases. Microbiol Mol Biol Rev 2000; 64: 821-846.
3. Rao MB et al. Molecular and biotechnological aspects of microbial proteases. Microbiol Mol Biol Rev 1998; 62: 597-635.
4. Souza PM et al. A biotechnology perspective of fungal proteases. Brazilian J Microbiol 2015; 46: 337-346.
5. Li et al. Commercial proteases: present and future. FEBS letters 2013; 587: 1155-1163.
6. Kumar CG and Takagi H. Microbial alkaline proteases: from a bioindustrial viewpoint. Biotechnol Adv 1999; 17: 561-594.
7. Pandey A et al. Solid state fermentation for the production of industrial enzymes. Current Sci 1999; 77: 149-162.
8. Nigam PS. Microbial enzymes with special characteristics for biotechnological applications. Biomolecules 2013; 3: 597-611.

9. Benmrad MO et al. A novel organic solvent- and detergent-stable serine alkaline protease from *Trametes cingulata* strain CTM10101. Int J Biol Macromol 2016; 91: 961-72.
10. Proteases Market by Source (2016). Available from: http://www.marketsandmarkets.com/ Market-Reports/proteases-market-184780427.html
11. González-Rábade N et al. Production of plant proteases in vivo and in vitro—a review. Biotechnol Adv 2011; 29: 983-996.
12. Zambare V et al. A novel extracellular protease from *Pseudomonas aeruginosa* MCM B-327: enzyme production and its partial characterization. New Biotechnol 2011; 28: 173-181.
13. Wu T et al. Investigations on protease production by a wild-type *Aspergillus terreus* strain using diluted retentate of pre-filtered palm oil mill effluent (POME) as substrate. Enzyme Microbial Technol 2006; 39: 1223-1229.
14. Gupta R et al. Bacterial alkaline proteases: molecular approaches and industrial applications. Appl Microbiol Biotechnol 2002; 59: 15-32.
15. Jisha VN et al. Versatility of microbial proteases. J Adv Enzyme Res 2013; 1(3): 39-51.
16. Aruna K et al. Production and partial characterization of alkaline protease from *Bacillus tequilensis* strains csgab0139 isolated from spoilt cottage cheese. Int J Appl Biol Pharmaceut Technol 2014; 5(3): 201-221.
17. Luhr KM et al. Scrapie protein degradation by cysteine proteases in CD11c+ dendritic cells and GT1-1 neuronal cells. J Virology 2004; 78: 4776-4782.
18. Otto HH and Schirmeister T. Cysteine proteases and their inhibitors. Chem Rev 1997; 97: 133-172.
19. Rawlings ND et al. MEROPS: the peptidase database. Nucleic Acids Res 2006; 34: D270-D272.
20. Domingos A et al. Production of clotting enzymes by in vivo plants and cell suspension cultures of Centaurea calcitrapa (Compositae). Kluwer, Netherlands: NATOASI series; 1992.
21. Vranova V et al. Proteolytic activity in soil: A review. Appl Soil Ecol 2013;70:23-32.
22. Srilakshmi J et al. Commercial potential of fungal protease: past, present and future prospects. J Pharmaceut Chem Biol Sci 2014; 2: 218-234.
23. Kumar D et al. Microbial proteases and application as laundry detergent additive. Res J Microbiol 2008; 3: 661-672.
24. Grbavčić S et al. Production of lipase and protease from an indigenous *Pseudomonas aeruginosa* strain and their evaluation as detergent additives: compatibility study with detergent ingredients and washing performance. Bioresour Technol 2011; 102: 11226-11233.
25. Baweja M et al. An Alkaline Protease fromBacillus pumilus MP 27: Functional Analysis of Its Binding Model toward Its Applications As Detergent Additive. Frontiers in Microbiology 2016; 7: 1195.
26. Saba I et al. Purification and characterization of a cold active alkaline protease from *Stenotrophomonas* sp., isolated from Kashmir, India. World J Microbiol Biotechnol 2012; 28: 1071-1079
27. Cherif S et al. A newly high alkaline lipase: an ideal choice for application in detergent formulations. Lipids in Health and Disease 2011; 10: 221.
28. Gaur S and Wadhwa N. Alkaline protease from senesced leaves of invasive weed Lantana camara. Afr J Biotechnol 2008; 24: 4602–4608.
29. Gill I et al. Biologically active peptides and enzymatic approaches to their production.

Enzyme and Microbial Technol 1996; 18: 162-183.
30. Kumar D and Bhalla TC. Microbial proteases in peptide synthesis: approaches and applications. Appl Microbiol Biotechnol 2005; 68: 726-736.
31. Cheetham P. Case studies in applied biocatalysis–from ideas to products. Appl Biocat 1994; 87-89.
32. Morihara K. Using proteases in peptide synthesis. Trends Biotechnol 1987; 5: 164-170.
33. Riva S et al. Protease-catalyzed regioselective esterification of sugars and related compounds in anhydrous dimethylformamide. J Am Chem Soc 1988; 110: 584-589.
34. Chen ST et al. Diastereoselective hydrolysis of peptide esters by alkaline protease Preparation of racemization-free peptides. Int J Pept Prot Res 1991; 37: 347-350.
35. Barros RJ et al. Enhancement of immobilized protease catalyzed dipeptide synthesis by the presence of insoluble protonated nucleophile. Enzyme Microb Technol 1999; 24: 480-488.
36. So JE et al. Protease-catalyzed tripeptide (RGD) synthesis. Enzyme Microb Technol 2000; 26: 108-114.
37. Guzman F et al. Peptide synthesis: chemical or enzymatic. Electron J Biotechnol 2007; 10: 279-314.
38. Singhal P et al. Studies on production, characterization and applications of microbial alkaline proteases. Int J Adv Biotechnol Res 2012; 3: 653-669.
39. Choudhary R et al. Enzyme technology applications in leather processing. Indian J Chem Technol 2004; 11: 659-671.
40. Jaouadi NZ et al. Biochemical and molecular characterization of a serine keratinase from *Brevibacillus brevis* US575 with promising keratin-biodegradation and hide-dehairing activities. PloS one 2013; 8:e76722.
41. Adrio JL and Demain AL. Microbial enzymes: tools for biotechnological processes. Biomolecules 2014; 4: 117-139.
42. Tavano OL (2013) Protein hydrolysis using proteases: An important tool for food biotechnology. J Mol Catal B Enzym 90: 1-11.
43. Pardo MF et al. Purification of balansain I, an endopeptidase from unripe fruits of Bromelia balansae Mez (Bromeliaceae). J Agric Food Chem 2000; 48: 3795–800
44. Neklyudov AD et al. Properties and uses of protein hydrolysates (review). Appl Biochem Microbiol 2000; 36: 452–459
45. Ward O. Proteases. In: Moo-Young, M. (Ed.), Comprehensive Biotechnology, 2nd edr ed, Canada: Waterloo; 2011, p 571.
46. Heredia-Sandoval NG et al. Microbial Proteases in Baked Goods: Modification of Gluten and Effects on Immunogenicity and Product Quality. Foods 2016; 5: 59.
47. Banik RM and Prakash M. Laundry detergent compatibility of the alkaline protease from *Bacillus cereus*. Microbiol Res 2004; 159: 135-140.
48. Vaisar T et al. Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of HDL. J ClinInvest 2007; 117: 746-756.
49. Rothschild J. Clinical use of serrapeptase: An alternative To non-steroidal anti-inflammatory agents. American Chiropract 1991; 58:17.
50. Esch P et al. Reduction of postoperative swelling. Objective measurement of swelling of the upper ankle joint in treatment with serrapeptase--a prospective study. Fortschritte der Medizin 1989; 107: 67-68, 71-62.
51. Hsia CH et al. Nattokinase decreases plasma levels of fibrinogen, factor VII, and factor VIII in human subjects. Nutri Res 2009; 29: 190-196.

52. Milner M. Nattokinase: Clinical updates-Doctors support its safety and efficacy. FOCUS Allergy Research Group News. Letter 2008; 2-6.
53. Vignardet C et al. Comparison of two hard keratinous substrates submitted to the action of a keratinase using an experimental design. Int J Pharmaceut 2001; 224: 115-122.
54. Brandelli A et al. Biochemical features of microbial keratinases and their production and applications. Appl Microbiol Biotechnol 2010; 85:1735-1750.
55. Mane P and Tale V. Overview of Microbial Therapeutic Enzymes. Int J Curr Microbiol App Sci 2015; 4: 17-26.
56. Chao YP et al. Screening for a new Streptomyces strain capable of efficient keratin degradation. J Environ Sci 2007; 19: 1125-1128.
57. Inacio FD et al. Proteases of wood rot fungi with emphasis on the genus Pleurotus. BioMed Res Int 2015;290161.
58. Tchorbanov B et al. Debittering of protein hydrolysates by Lactobacillus LBL-4 aminopeptidase. Enzyme Research 2011; 538676.
59. Al-Abdalall AH and Al-Khaldi EM. Recovery of silver from used X-ray film using alkaline protease from *Bacillus subtilis* sub sp. subtilis. African J Biotechnol 2016; 15: 1413-1416.
60. Rani K et al. Review on latest overview of proteases. Int J Current Life Sci 2012; 2: 12-18.
61. Ray A. Protease enzyme-potential industrial scope. Int J Tech 2012; 2: 1-4.

Cite this article as:
Rajendra Singh, Anshumali Mittal, Manoj Kumar, Praveen Kumar Mehta. Microbial Proteases in Commercial Applications. J Pharm Chem Biol Sci 2016; 4(3):365-374