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Applications, Challenges and Future Prospects of Proteases: An Overview

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Abstract: *Enzymes have greatly contributed to environmentally adapted clean and green technology due to their biodegradable nature and therefore have replaced harsh chemicals to a great extent. Proteases are a unique class of enzymes as they possess both degradative and synthetic properties. Their applications in industry and therapeutics have grown rapidly in the last two decades. Proteases comprise a vast group of enzymes with applications in various industries viz; pulp and paper, textiles, detergent, leathering, baking and bioremediation processes. In the present review, classification, major sources and physiological properties of proteases have been discussed. In addition to this, an overview on the applications of proteases in agriculture, tannery, food processing, silver recovery and pharmaceutical industry is also addressed briefly. This review mainly focuses on the challenges and the future scope of protease enzymes.*

1. INTRODUCTION

Proteases (also termed as proteolytic enzymes or proteinases) refer to a group of enzymes whose catalytic function is to hydrolyze peptide bonds of proteins. These enzymes are widely distributed in all plants, animals and microorganisms. Proteases account for approximately 2% of the human genome and 1 to 5% of genomes of infectious organisms [1]. Regulating most physiological processes by controlling the activation, synthesis, and turnover of proteins, proteases play pivotal regulatory roles in conception, birth, digestion, growth, maturation, aging, and even death of all organisms. Proteases are also essential in viruses, bacteria, and parasites for their replication and the spread of infectious diseases; in all insects, organisms, and animals for effective transmission of disease; and in human and animal hosts for the mediation and sustenance of diseases Their use in medicine is gaining more and more attention because several clinical studies are indicating their applications in oncology, inflammatory conditions, blood rheology control and immune regulation. They are extensively used in the food industry (baking, brewing, cheese manufacturing, meat tenderizing), tanning industry and in the manufacture of biological detergents.

The actions of proteases can be extremely selective, with each protease being responsible for splitting very specific sequences of amino acids under a preferred set of environmental conditions. Different types of proteases have different action mechanisms and biological processes. The extracellular proteases are commercial value and find multiple applications in various industrial sectors. Proteases represent one of the most significant groups of industrial enzymes and account for at least a quarter of the total global enzyme production.

2. CLASSIFICATION OF PROTEASES

The physiological function of proteases is essential for all living organism, from viruses to humans and the enzymes can be classified based on their origin: microbial (bacterial, fungal and viral), plant, animal and human enzymes [2]. Proteases are broadly classified as endopeptidases or exopeptidases enzymes on the basis of the site of action on substrate. Exopeptidases cleave the peptide bond proximal to the amino or carboxy termini of the substrate further they are classified as aminopeptidases and cabroxypetidases based on the site of action at the N or C terminus. Endopeptidases cleave peptide bonds distant from the termini of the substrate. Based on the functional group present at the active site, endo-peptidases are further classified into four major groups, i.e., serine proteases, aspartic proteases, cysteine proteases and metallo-proteases.

3. PROTEASE SOURCES

As known fact that proteases are physiologically essential for living organisms, constituted in a wide diversity of sources such as plants, animals and microorganism [3]. Papain, bromelain, keratinases are some of the well-known proteases of plant origin [4, 5]. The most familiar proteases of animal origin are pancreatic trypsin, chymotrypsin, pepsin and rennins. These are prepared in pure form in bulk quantities. However, their production depends on the availability of livestock for slaughter, which in turn is governed by political and agricultural policies [5, 6]. The lack of ability of the plant and animal proteases to meet current world demands has led to an increased interest in microbial proteases. Microorganisms represent an excellent source of enzymes owing to their broad biochemical diversity and their susceptibility to genetic manipulation. Proteases from microbial sources are preferred to the enzymes from plant and animal sources since they possess almost all the characteristics desired for their biotechnological applications [7]. The advantages comprise lower production costs, possibility of large-scale production in industrial fermentors, wide range of physical and chemical characteristics, possibility of genetic manipulation, absence of effects brought about by seasonality, rapid culture development and the use of non-burdensome methods which make microbial enzymes suitable biocatalysts for various industrial applications. The development of new enzymatic systems which cannot be obtained from plants or animals is made possible and important progress in the food industry may be achieved through microbial enzymes [8].

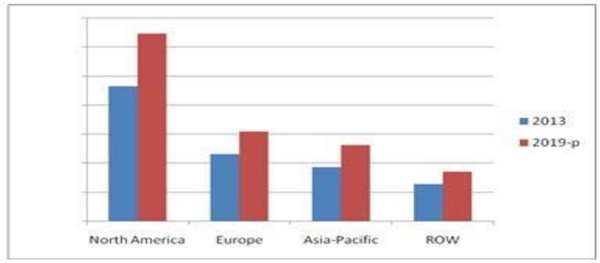
4. PHYSIOLOGICAL FUNCTIONS OF PROTEASES

Proteases play role in various physiological functions and pathological processes such as protein catabolism, blood coagulation, cell growth and migration, tissue arrangement, morphogenesis in development, inflammation, tumor growth and metastasis, activation of zymogens, releasr of hormones and pharmacologically active peptides from precursor proteins and transport of secretory proteins across membranes.

Proteases assist the hydrolysis of large polypeptides into smaller peptides and amino acids, thus facilitating their absorption by the cell. The dormant spores lack the amino acids required for germination. Extracellular acid proteases are believed to be involved in the breakage of cell wall polypeptide linkages during germination of *Dictyostelium discoideum* spores [9]. Proteases mediate the degradation of variety of regulatory proteins that control the heat shock response, the SOS response to DNA damage and the life cycle of bacteriophage [10].

5. PROTEASE INDUSTRIAL APPLICATIONS

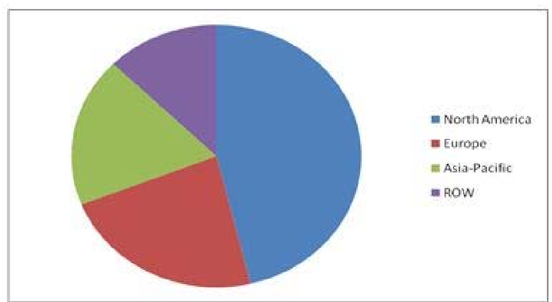
Protease is used in the industry for its key function of hydrolysis of protein peptides into amino acids. Proteolytic enzymes account for nearly 60% of the industrial market in the world. They find application in a number of biotechnological processes, viz. in food processing, and pharmaceuticals, leather industry, silk, bakery, soy processing, meat tendering and brewery industries. Proteases are highly demanded group of enzymes in various industries with a market share of 60% of the total enzyme market [11].



(Source: Markets and Markets Analysis)

Fig. 1: Protease Enzymes Market Size (Revenue), by Geography

Geographically, the report is segmented into North America, Asia-Pacific, Europe, and Rest of the World (ROW). The sources of protease enzymes included in this report are microorganisms, animals, and plants. This report highlights the major functions of protease enzymes in different industries and also estimates the market size of protease enzymes, based on sources and applications. The global protein hydrolysis enzymes market revenue is estimated to be valued at $2,767 million by 2019. The global protein hydrolysis enzymes market is expected to grow at a rate of 5.3% from 2014 to 2019. Protease enzymes find application in diverse end-use markets, such as detergents, pharmaceuticals, food, and other industries such as textile, animal feed, chemical, and photography.



(Source: Industry Journals, Company Presentations, and Markets and Markets Analysis)

Fig. 2: Protein Hydrolysis Enzymes Market Share, by Region, 2013

The Fig. above highlights the shares of North America, Europe, Asia-Pacific, and Rest of the World in the global protein hydrolysis enzymes market. Developing countries in Asia-Pacific and Rest of the world (ROW) show a lot of untapped potential for the growth of the protease enzymes market. As the detergent and cleaning industry is modernizing and developing in these countries, the consumers are exploring new products which are convenient to use. China is the world's second-largest market for protease enzymes. The Chinese market is growing at a faster rate on the accounts of one-tenth of the global market.

Table 1: Protease in industry

Industry	Enzymes	Application
Leather	Trypsin Alkaline protease	Bating of leathers Dehairing
Food processing	Several proteases	Modification of protein rich material i.e. soy protein
Baking	Neutral protease	Dough conditioners
Dairy	Calf rennet and other ficin, trypsin, chymortypsin	Coagulation of milk protein, production of enzyme modified cheese, chey processing
Detergent	Alkaline protease	Extensive use in laundry detergents for protein stain removal
Meat	Papain, Ginger protease, alkaline elastase and thermophillic alkaline protease	Meat tenderization
Beverages	Papain	Removal of turbidity
Confectionery	Thermolysin	Reverse hydrolysis in aspartame synthesis
Pharamaceutical	Trypsin	Treatment of certain types of hemia, Production of human insulin

Detergent Industry

Proteases are one of the standard ingredients of all kinds of detergents ranging from those used for household laundering to reagents used for cleaning contact lenses or dentures. The use of proteases in laundry detergents accounts for approximately 25% of the total worldwide sales of enzymes. Rohm Company in Germany isolated the first enzyme for industrial use in 1914 [12]. The first detergent containing the bacterial enzyme was introduced in 1956 under the trade name BIO-40. In 1962, Novo Industry A/S introduced alcalase, produced by *Bacillus licheniformis*; its commercial name was BIOTEX. Maxatase, a detergent made by Gist-Brocades, followed this. All detergent proteases currently used in the market are serine proteases produced by *Bacillus* strains. An alkaline protease from *Conidiobolus coronatus* was found to be compatible with commercial detergents used in India [13]. Alkaline proteases showed high capability for removing proteins and stains from cloth so it is used in detergent powder or solutions. Protease from *Spilosoma oblique* was used for removal of blood [14].

Wool Industry

Wool fibres are covered in overlapping scales pointing towards the fiber tip. A successful method involved the partial hydrolysis of scale tips with the protease, papain.

Protease in Silver Recovery

Alkaline proteases find potential application in the bioprocessing of used x-ray films for sliver recovery. The enzymatic hydrolysis of the gelatin layers on the x-ray film enables not only the silver but also the polyester films base, to be recycled. The alkaline protease from Bacillus sp. B21-2 decomposed the gelatinous coating on the used X-ray films from which the silver was recovered.

Food Industry

Certain proteases have been used in food processing dates back to antiquity. Papain from the Kaves and unripe fruit of carica papaya has been used to tenderize meat. Proteases play a prominent role in meat tenderization, especially of beef. An alkaline elastase and thermophillic alkaline protease have proved to be successful and promising meat tenderizing enzymes, as they possess the ability to hydrolyze connective tissue proteins as well as muscle fiber proteins. Current trend in similar research shows yet another alkaline protease from *B.amyloliquefaciens* resulted in the production of a methionine rich protein hydrolysate form chickpea protein [15].

Pharmaceutical industry

The wide diversity and specificity of proteases are used to great advantage in developing effective therapeutic agents. Oral administration of proteases from *Aspergillus oryzae* has been used as a digestive aid to correct certain lytic enzyme deficiency syndromes. Clostridial collagenase or subtilisin is used in combination with broad-spectrum antibiotics in the treatment of burns and wounds. An asparginase isolated from *E. coli* is used to eliminate aspargine from the bloodstream in the various forms of lymphocytic leukemia. Alkaline protease from *Conidiobolus coronatus* was found to be able to replace trypsin in animal cell cultures [16].

Medical applications

It also regulates various metabolic processes such as blood coagulation, fibrinolysis, complement activation, phagocytosis and blood pressure control [17]. Collagenases with alkaline protease activity are increasingly used for therapeutic applications in the preparation of slow-release dosages forms. Elastosterase, a preparation with high electrolytic activity from *B.subtilis* 316M was immobilized on a bandage for the therapeutic application in the treatment of burns and purulent wounds, carbuncles, furanches and deep abscess and alkaline proteases having fibrinolytic activity have been used as a thrombolytic agent [18].

6. CHALLENGES

Historically, proteases were regarded as non-selective enzymes responsible for indiscriminately degrading dietary proteins. Proteases have been implicated in almost all aspects of life and death, including fertilization, development, differentiation, immunity, cell migration, cell activation, wound healing, and cell death. From a therapeutic standpoint, the modulation of proteolytic activity offers considerable promise for the treatment of a number of human diseases

ranging from cardiovascular, metabolic, lung, and infectious disease to osteoporosis, cancer, stroke, neurodegeneration, and inflammation.
The complete sequencing of multiple genomes has identified the full repertoire of proteases; however, large gaps still remain in our understanding of the biological role of most proteases. Exploiting the multiple technologies developed and used at GNF, the protease group is addressing some of the major challenges in protease biology to aid in the development of protease-targeted therapeutics. Among the capabilities of the GNF protease platform are broadly enabling technologies that aid in both optimal and native substrate identification, as well as a suite of assays that assess selectivity within the protease family.

Environmental pollution has been a major irritant to industrial development. The limitation of conventional protease are render by chemically and thermostable protease. Leather industry contributes to one of the major industrial pollution problems facing the worldwide. The effluent form these industry release many toxic chemicals like lime, sodium sulphide, salt, solvents, etc. which extensively used in the pre-tanning steps of leather processing. The use of conventional protease has minimized application of chemical significantly but not completely. Chemical and chemical-based industries are the prime targets of the environmentalists for their crusade against pollution, and leather industry has also not been left out of the reckoning. The generation of pollution is significantly high in the pre-tanning operations compared to the post-tanning operations [19].

7. FUTURE SCOPE

Proteases which constitute a broad class of industrially most significant enzymes are involved in diverse physiological and cellular processes. Since proteases are physiologically necessary, they occur ubiquitously in animals, plants, and microbes. However, microbes are a goldmine of proteases and represent the preferred source of enzymes in view of their rapid growth, limited space required for cultivation, and ready accessibility to genetic manipulation. Microbial proteases have been extensively used in the food, dairy and detergent industries since ancient times. There is a renewed interest in proteases as targets for developing therapeutic agents against relentlessly spreading fatal diseases such as cancer, malaria, and AIDS.

The applications of proteases in industry and therapeutics have grown rapidly in the last two decades. Novel protein engineering strategies and techniques will continue to expand the commercial protease markets. Encouragingly, the recent success of apoptotic caspase activation with engineered small-molecule-activated proteases [20] represents a new way to specifically control human protease activity for clinical applications. In addition, taking advantage of the proteolytic activities of proteases in diseased tissues may also offer a new strategy for site-specific drug targeting [21] and tumor imaging [22].

The advent of techniques for rapid sequencing of cloned DNA has yielded an explosive increase in protease sequence information. Analysis of sequences for acidic, alkaline, and neutral proteases has provided new insights into the evolutionary relationships of proteases. Despite the systematic application of recombinant technology and protein engineering to alter the properties of proteases, it has not been possible to obtain microbial proteases that are ideal for their biotechnological applications. Industrial applications of proteases have posed several problems and challenges for their further improvements. The biodiversity represents an invaluable resource for biotechnological innovations and plays an important role in the search for improved strains of microorganisms used in the industry.

A recent trend has involved conducting industrial reactions with enzymes reaped from exotic microorganisms that inhabit hot waters, freezing Arctic waters, saline waters, or extremely acidic or alkaline habitats. The proteases isolated from extremophilic organisms are likely to mimic some of the unnatural properties of the enzymes that are desirable for their commercial applications. Exploitation of biodiversity to provide microorganisms that produce proteases well suited for their diverse applications is considered to be one of the most promising future alternatives. Introduction of extremophilic proteases into industrial processes is hampered by the difficulties encountered in growing the extremophiles as laboratory cultures. Revolutionary robotic approaches such as DNA shuffling are being developed to rationalize the use of enzymes from extremophiles. The existing knowledge about the structure function relationship of proteases, coupled with gene-shuffling techniques, promises a fair chance of success, in the near future, in evolving proteases that were never made in nature and that would meet the requirements of the multitude of protease applications.

A century after the pioneering work of Louis Pasteur, the science of microbiology has reached its pinnacle. In a relatively short time, modern biotechnology has grown dramatically from a laboratory curiosity to a commercial activity. Advances in microbiology and biotechnology have created a favorable niche for the development of proteases and will continue to facilitate their applications to provide a sustainable environment for mankind and to improve the quality of human life. With the development of synthetic biology, computational design, crystallography, and screening technologies, we can anticipate that the future of protease will be a multi-disciplinary task with many dramatic successes to come.

8. CONCLUSIONS

This review is mainly focused on the general aspects of proteases giving special emphasis on the industrial applications of the proteases, challenges and future scope. Proteases play a decisive role in detergent, pharmaceutical, leather, food and agricultural industries. Currently, the estimated value of the global sales of industrial enzymes is over 3 billion USD, of which proteases account for about 60% of the total sales. Microbial alkaline proteases already play a pivotal role in several industries, mainly in the detergents, leather processing, silver recovery, medical purposes, food processing, feeds, and chemical industries, as well as in waste treatment their potential is much greater and their applications in novel processes are likely to increase in the near future. The potential for use of microbial enzymes in leather processing lies mainly in areas in which pollution-causing chemicals, such as sodium sulphide, lime and solvents are being used and conversion of waste products into potentially saleable by-products is possible. The limitation of conventional protease are render by chemically and thermostable protease. An extensive research is going on worldwide in order to hunt a common stable enzyme which will be more effective in various classes of hides. The potential commercial applications of proteases are rapidly growing as recent technological advances are producing proteases with novel properties and substrate specificities. Advancement in biotechnology offers a constructive position for the development of proteases and will continue to facilitate their applications to provide a sustainable environment for improving the quality of human life.

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REFERENCES

[1] Puente, X. S., Sanchez, L. M., Overall, C. M. and Lopez, O. C., Human and mouse proteases: A comparative genomic approach. *Nauture Reviews Genetics*, 4, July 2003, pp. 544– 548.

[2] Motyan, J. A., Toth, F. and Tozser, J., "Research Application of proteolytic enzymes in molecular biology", *Biomolecules*,3, 8, November 2013, pp. 923-942.

[3] Rao, M. B., Tanskale, A. M, Ghatger, M. S., Deshpande, V. V., "Molecular and Biotechnological aspects of Microbial proteases", *Microbiology and Molecular Biology Reviews*, 63, September 1998, pp. 596-635.

[4] Shankar, S., More, S., V. and Seeta, L. R., "Recovery of silver from waste x-ray film by alkaline protease from *Conidiobolus Coronatus*", *Indian journal of Biotechnology"*, 6, 1, June 2010, pp. 60-69.

[5] Kalpana, D. M, Rasheedha, B. A, Gnanaprabhal, G. R., Pradeep, B. V. and Palaniswamy, M., "Purification, characterization of alkaline protease enzyme from native isolate *Aspergillus niger* and its compatibility with commercial detergents", *Indian journal of Science and Technology*, 1, December 2008, pp. 1-6.

[6] Divakar, G. and Ellaiah, P., "Optimization of process parameters for alkaline protease production under solid-state fermentation by Thermoactinomyces thalpophilis", *Indian Journal of Biotechnology*, 5, 1, January 2006, pp. 80-83.

[7] Vishalakshi, N. and Dayanand, A., "Production of alkaline protease from *Streptomyces gulbargensis* and its application in removal of blood stain", *Indian Journal of Biotechnology*, 8l , 2009 pp. 280-285.

[8] Usharani, B. and Muthuraj, M., "Production and Characterization of protease enzyme from Bacillus laterosporus", *African Journal of Microbiology Research*, 4, 2010, pp. 1057-1063.

[9] Jackson, D. P. and Cotter, D. A., "Expression of proteolytic enzymes during *Dictyostelium discoideum* spore germination" *Archives of Microbiology*, 137, 1, March, 1984, pp. 205-208.

[10] Gottesman, S. And Maurizi, M. R.,"Regulation by protolysis: energy dependent proteases and their targets", Microbiological. Reviews. 56, December 1992, pp. 592-621.

[11] Gaur, S., Agrahari, S. and Wadhwa, N., "Purification of protease from Pseudomonas thermaerum GW1 isolated from poultry waste site", *The Open Microbiology Journal*, 4, 13 August 2010, pp. 67-74.

[12] Mark, S. L., "For more and more industrial applications, enzymes, natural and engineered are replacing traditional chemistry" *Today's Chemist at Work*. December 2003, 20-23.

[13] Phadatare, S. U., Srinivasan, M. C., Deshpande, V. V., "High activity alkaline protease from *Conidiobolus coronatus* (NCL 86.8.20): enzyme production and compatibility with commercial detergents", *Enzyme and Microbial Technology*, 15, January 1993, pp. 72–76.

[14] Anwar, A. and Saleemuddin, M., "Alkaline-pH-acting digestive enzymes of the polyphagous insect pest *Spilosoma oblique* : stability and potential as detergent additives", *Biotechnology and Applied Biochemistry*, 25, February 1997, pp. 43-46.

[15] George, S., Sivasankar, B., Jayaraman, K. and Vijayalakshmi, M. A., "Production and separation of the methioninerich fraction from chick pea protein hydrolysate generated by proteases of *Bacillus amyloliquefaciens*", *Process Biochemistry*, 32, 1997, pp. 401-404.

[16] Chiplonkar, J., M., Gangodkar, S., V., Wagh, U., V., Ghadge, G., D., Rele, M., V., Srinivasan, M., C., "Applications of alkaline protease from *Conidiobolus* in animal cell culture", *Biotechnol Lett*, 7, 1, September 1985, pp. 665–668.

[17] Adinarayana, K., Bapiraju, K. V. V. S. N. and Ellaiah, P., " Investigations on alkaline protease production with *B.substilis* PE-11 immobilized in calcium alginate gel beads", *Process Biochemistry*, 39, 30, July 2004, pp 1331–1339.

[18] Kim, S., Kim, S. J., Choi, J. H., Sapkota, K., Park, S. E., "Thrombolytic, anticoagulant and antiplatelet activities of codiase, a bi-functional fibrinolytic, enzyme from Codium fragile", *Biochimie*, 95, June 2013, pp. 1266-1277.

[19] Li, K., Chen, H., Wang, Y., Shan, Z., Yang, J. and Brutto, P., "A salt-free pickling regime for hides and skins using oxazolidine", *Journal of Cleaner Production*,17, 4, June 2009, pp. 1603–1606.

[20] Gray, D., Mahrus, S. and Wells, J., "Activation of specific apoptotic caspases with an engineered small- molecule-activated protease". *Cell* 142, 20 August 2010, pp. 637–646.

[21] Erster, O., Thomas, J., Hamzah, J., Jabaiah, A., Getz, J., Schoep, T., Hall, S., Ruoslahti, E. and Daugherty, P., "Site-specific targeting of antibody activity in vivo mediated by disease-associated proteases", *Journal of controlled release*, 161, 23, May 2012, pp. 804–812.

[22] Jiang, T., Olson, E., Nguyen, Q., Roy, M., Jennings, P. and Tsien, R., "Tumor imaging by means of proteolytic activation of cell-penetrating peptides", *Proceedings of National. Academy of. Science*, 101, 21, December 2004, pp. 17867–17872.