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PROTEASE: AN ENZYME WITH MUTIPLE INDUSTRIAL APPLICATIONS (REVIEW)

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ABSTRACT

Proteolytic enzymes are found in all kinds of organisms' i.e viruses to animals. These peptidases are included in vast group of the enzymes used in the bio industry with wide applications. They play vital part in the industrial biotechnology specifically in food, detergent and the pharmaceutical industries. Microbial proteases which are environmental friendly are interestingly used for their commercial importance. This review displays an overview on proteases chiefly from sources of micro-organisms i.e bacteria and fungi and their common properties are also discussed briefly. Proteases assume an essential part in detergent, pharmaceutical, leather, food industry, agricultural ventures. Recently, the estimate of the global sale of industrially important enzymes is over "3 billion US dollars" in which proteases constitute about 60% of the total sales. Microbial proteases assume an imperative part in various ventures, above all in the leather preparing, silver recuperation, medical purposes, food preparing, feeds, chemical enterprises, waste treatment.

KEYWORDS: Proteases, Commercial Importance, Proteolytic Enzymes, pharmaceutical, Food Industry.

INTRODUCTION

Versatility of protein molecules present in their diverse functioning including catalytic capabilities and structural organization. Enzymes are considered biocatalyst as they are involved in producing these molecules. Both fungal and bacterial species produce these molecules.[34] Protease also known as proteinase or peptidase is an enzyme group which is involved in proteolysis. Proteolysis is the hydrolysis of peptide bonds. Peptide bonds help in formation of polypeptide chain by linking amino acids together. Proteases have industrial uses and include more than 70% of the industrial enzyme. Fungi and bacteria are the major source of production of this enzyme. They show catalysis in broad range of pH and temperature.[22] Proteases differ in the properties including substrate specificity, catalytic mechanisms, active site, stability profile, pH, and temperature optimal.[29] Proteolytic enzymes are important for many physiological processes including cell division, apoptosis, signal transduction, protein turnover, polypeptide hormones processing, blood clotting cascade, and food protein digestion. Proteases have great pharmaceutical, academic and medical importance. Proteases have great industrial and commercial applications including food, detergent, leather and pharmaceutical industries. Alkaline proteases have great industrial applications. Proteases are eco-friendly as the sources of production are generally non-pathogenic and non-toxic.

Classification

The classification of proteases is based on targeted amino acid for the hydrolysis, substrate type and chemical environment.[6] On the basis of amino acid involved in the hydrolysis, proteases are classified in six large groups. Serine protease has great applications. Serine plays role as nucleophilic amino acid. These include subtilisin and chymotrypsin or trypsin. They have tremendous use in medicine and industry. Due to this reason many recombinant serine proteases are produced. Distinct proteases among super families or protease are like trypsin, elastate-like, chimotripsin-like, and subtilisin like protease. Threonine proteases are important as an industrial point of view. In, this threonine residue is present on catalytic site. They play significant role in physiology. Acyltransferase and proteasome are examples of this class. Till date, 5 families with 2 super families are identified from the different sources. The super families include DOM fold ornithine acyltransferases (Super family PE) and Ntn fold proteasomes (Super family PB).[17] Cysteine poteases are also called as thiol proteases have much industrial importance. Their catalytic activity is associated with the nucleophilic thiol which is in the form of catalytic dyad or triad. These are present in fruits such as papaya, fig, kiwi and pineapple. Cysteine proteases are used in poultry industry and are used to tenderize meat. They also have therapeutic applications such as control of viral infections. Cysteine protease has 14 super families.[15]

Aspartate proteases are different from the other proteases as they help in maintenance of physiological functions. The examples of this class are pepsins, renin and cathepsins. This class of enzyme is active at low pH and resemble with the acidic proteases. Till date, 4 super families of aspartate proteases are identified. These families are represented as Family A01 (pepsin family), Family A22, FamilyAx1 and Family A02.[19] Molecular weight of aspartate protease is 30-45kDa. Their catalysis involves 2 aspartic acid residues. There is a need of binding of water molecule to active site before the nucleophilic attack.[28] Glutamic Acid proteases are mostly present in fungal species. They are used in therapeutics as anticancer and antitumor. They are also used in food processing. Catalysis of metalloproteases is due to metal ions. They have use in drug development. Proteases have unique applications and are widely used in industries. Immobilization techniques have enhanced the efficiency of these enzymes. These are sensitive to EDTA. Most of the fungal and bacterial metalloproteases have zinc. Zinc is important for the catalysis. Calcium helps in stability of protein structure.[17]

Table: 1. Proteases general classification with enzyme commission code.

Protease	EC Code
Exopeptidases	3,4, 11-19
Aminopeptidases	3,4,11
Dipeptidyl peptidases	3,4,14
Dipeptidases	3,4,13
Tripeptidyl peptidaes	3,4,14
Peptidyl dipeptidases	3,4,15
Serine type protesases	3,4,16
Carboxypeptidases	3,4,16-18
Metalloprotease	3,4,17
Cysteine type protease	3,4,18
Omega Peptidases	3,4,19
Endo peptidases	3,4,21-24
Cysteine Protease	3,4,22
Serine proteases	3,4,21
Metelloproteases	3,4,24
Aspartate proteases	3,4,23
Endopeptidase of unknown mechanism	3,4,99

Proteases are also classified on the basis of reaction environment conditions. There are three sub classes based on pH range include Acid Proteases show catalytic activity at low pH of 2-6, Alkaline Proteases show catalysis at high pH of 8-10 and Neutral Proteases show catalysis near pH 7. Such type of classification helps in industrial use of enzymes and helps in designing of the reaction conditions to avoid loss of production.[7] EC code of proteases is described in table 1.

Sources of Proteases

Proteases that have animal source include pancreatic trypsin, rennin, pepsin and chymotrypsin. Trypsin is the intestinal enzyme used in digestion. Food proteins are hydrolyzed by this enzyme. Chymotrypsin is present pancreatic extract. It has analytical and diagnostic applications. Pepsin is acidic in nature. It is present in stomach of vertebrates. Pepsin had use in laundry industry. Pepsin converts rennet into active rennin. Rennin has applications in dairy industry. It helps in production of good flavor curd. Proteases that have plant origin include Papain, keratinases, ficin, and bromelin. However, the production of these proteases from plants is time consuming procedure. Papain has active pH range of 5-9. It is specially used in tonics.[33] The extract of papain is obtained from Carica papaya latex. Bromelin is obtained from juice and stem of pineapples. The problem in plant proteases extraction is associated with the suitable cultivation area selection. Plant tissues have low concentration of enzymes, so there is a need to process large quantity of plant material.[22] Sources of proteases are mentioned in table 2.

Microbial community is preferable in protease production at large scale. Microbes have fast growth rates and are easily used as for production of new recombinant enzymes. About two-third of commercial proteases are produced by microorganisms. These are used in detergent leather, pharmaceutical, agricultural and food industries. Currently, over 3 billion USD of industrial enzymes is estimated global[6] of which 60% is of proteases. Microorganisms degrade proteins and degradation products are utilized by these microorganisms for nutrition. Microorganisms secrete proteinases (endopeptidases) which initiate degradation. Peptidases (exopeptidases) do further hydrolysis. Alkaline and neutral proteases have great role in leather tanning and detergent industries. Alkaline proteases have applications in food industry, silver recovery and in bioremediation.

Table 2: Sources of proteases

Enzyme	Source
Endopeptidases 1. Serine Protease	
Chymotrypsin	Animal
Trypsin	Animal
Endoproteinase	Microbial
Enterokinase	Animal
Proteinase K	Microbial
Subtilisn	Microbial
Factor Xa	Animal
Thrombin	Animal

WNV Protease	Microbial
Elastate	Animal
2. Cysteine Protease	
Papain	Plant
Bromelain	Plant
Rhino Virus 3C	Microbial
Ficin	Plant
3. Metalloproteases	
Thermolysin	Microbial
Endoproteinase	Microbial
Dipase	Microbial
Collagenase	Microbial
4. Aspartic proteases	
Cathepsin D	Animal
Pepsin	Animal
Exopeptidases	
1. Serine Poteases	
Carboxypeptidases Y	Microbial
2. Cystein Proteases	

Secreted proteases have two types including extra-cellular and intra-cellular proteases. Intracellular proteases are important for many metabolic and cellular processes including sporulation, cell differentiation, enzyme maturation, hormones, and protein turn over and in protoxin activation of biopesticides which are Bt-based. [17] Alkaline proteases from bacterial sources are important in food, laundry, silk and leather industries as they have high catalytic activity and production capacity. Bacterial alkaline proteases have pH range of 8-12 and optimal temperature of 50˚C -70˚C. Due to this, these are used widely in detergent industry. Fungal proteases have high diversity, stability at extreme conditions and broader substrate specificity. Solid state fermentation process is used to produce fungal proteases. They are used in modification of food proteins. Some of the common examples include Saccharomyces cerevisiae, Candida parapsilosis, Debaryomyces castellii, Candida mogii, Saccharomyces pombe, and Aspergillus candidus.[23] Fungal and bacterial sources of proteases are described in table 3.

Table: 3. Fungal and bacterial sources of proteases

Fungal sources	Bacterial sources
Aspergillus candidus	*Pseudomonas aerogenosa*
A. flavus	*Microbacterium sp.*
A. fumigatus	*Lactobacillus helveticus*
A. niger	*Streptomyces microflavus*
A. oryzae	*Streptomyces vectus*
Conidioboluscoronatus	*Streptomyces sp YSA-130*
Cephalosporium. Sp. KSM 388	*Nocardiopsisdassonvillei*
Entomophthoracoronata	*Pseudomonas sp. SJ320*
Rhizopusoligosporus	*Pseudomonas maltophilia*

Commercial applications of proteases

These are important enzyme group having different commercial applications involved in the food processing, in detergent industry, leather making and for therapeutic applications. In food industry, beverages, and milk industry, bakery and grains processing use huge quantity of protease and additional enzymes from various sources containing fungi. Ethanol fermentation, making of detergent for the biological applications have enhanced in the last few decades. Large scales processing of the meat and the silk fabric require proteases, which bring with the climatic issues and worth of silk fabric and meat. Various applications of proteases are narrated in table 4 as below.

Table: 4. Applications of proteases

Proteases	Industry	Applications
Papain	Beverages	Haze removal and chill proofing
Neutral Protease	Baking	Conditioning of dough
Fungal Proteases, and chymosin	Dairy	Calf rennet replacement, EMC (Enzyme modified cheese) production, Processing of whey protein.
Subtilisn, alkaline proteases	Detergent	Detergents for the removal of stains of proteins
Trypsin and other proteases	Leather	Leather bating, dehairing from skins

Several proteases	Food processing	Protein rich material modification
Trypsin	Medicine	Removal of dead tissues, dissolution of the blood clot
Papain and other proteases		Tenderization of metal, protein recovery from the fish waste and bones
Thermolysin	Sweetener	Reverse hydrolysis during synthesis of aspartame
Several proteases	photography	Silver recovery

Protease in food processing

Different enzymes are required in food processing industries for making of nutritional and good quality food products. Whole bakery industry depends on single celled fungi called as yeast specifically named as Saccharomyces which brings various kinds of food items. Enzyme treatment plays vital role in the processing of the fruits and preservation of the juices for long term storage. Proteases particularly play important role for this purpose. Production of candies and milk products require full processing that is catalyzed by sequence of the enzymes comprising of proteases. Processing of substrate is essential for both hard and soft drinks in beverage industry for good flavor and enhancement of shelf life of the product. Accurate oxidation of the raw material (berry, ripen seeds and leaves) is necessary for processing of coffee, cocoa powder and tea to form new items. In this proteases play significant role. Ethanol which is the main component of the hard drinks is final product of the sugar fermentation that is carried by chain of different biochemical reactions catalyzed by special type of proteases. It offers catalysis from low temperature range to harsh high temperature conditions. Thus, proteases are proved as basic enzyme for food industries. [30]

Proteases in Detergent Industries

The industrial applications of proteases enzyme go back to about 1914 as detergent additives. The biological detergent are mainly utilized in washing of big boiler in the industries, hospitals are other vital consumer of the biological detergent along with poultry industry. Discovery of the thermostable protease improved effectiveness of the biological detergent. In current days, both the domestic and industrial detergents are using these enzymes highly. Use of proteases and amylases in laundry detergents is very common. Most of the powdered bleach additives contain enzymes which help in breakdown of the stains which are quite hard to remove by conventional surfactants alone. In detergents, protease help in hydrolyzing large sized protein molecules which are connected with the hard stains. [28] Meanwhile, the hydrolysis process, peptide bonds which hold several amino acids in one place to make a big protein molecule are broken and they release small polypeptides and single units of amino acids. [26] They work like scissors to cut stain piece by piece physically from surface of fabrics. The enzyme is found to be an efficient detergent additive and can remove variety of stains as grass, blood and beetle most effectively at 60 °C. Bacillus licheniformis RP1 protease exhibited excellent compatibility and stability with broad range of commercial solid detergents in temperature range from 40 to 50 °C, showing its more applications in the detergent industry. [21]

Microbial proteases in leather Industry

Leather and detergent industries need use of polluting and toxic chemicals to break down proteins. In leather industries, chemicals are utilized in dehairing, soaking, degreasing and bating to remove the proteins linked with collagen. [35] Processed leather is obtained by going through these steps: soaking, bating, liming, deliming, degreasing, dehairing, and pickling. [31]All these steps are carried out by toxic chemicals such as sodium sulphide, solvents, lime and salts etc. so. It also adds to the environment pollution. Collagen is major leather forming protein which is present in skins and hides in connection with several globular proteins, like mucoid, albumin, globulin and fibrous proteins as reticulin, elastin and keratin. Leather processing needs elimination of non-collagenous substances, partially or completely, the degree of exclusion of these substances decide the characteristics of final leather like its softness and durability. The only method to control pollution occurred by leather processing is alternation of used chemicals with the enzymes especially proteases.[28]

The enzyme manufacturing of the leather not only resolves environment problems but it yield good quality of leather also. Leather making that is chiefly carried at high temperature includes variety of thermostable proteases. Thermophilic fungi and bacterial proteases are probable sources for the leather processing. Moreover, cleaning of hair and its biological digestion had produced various important amino acids utilized as the dietary supplement. The utilization of the thermostable proteases have become an essential part of current leather making.[33]

Silver recovery

Alkaline proteases have also applications in bioprocessing of utilized X-ray films for the silver recovery. Utilized X-ray films consist of about 1.5to 2% silver. The conventional exercises of the silver recovery by burning the films cause chief pollution problems of environment. Thus, the enzyme hydrolysis of the gelatin layers on X-ray films enable not only the silver, but the polyester film base to recycle. The alkaline protease from Bacillus coagulans PB-77 and Bacillus sp. B21-2 decomposed gelatinous coating over utilized X-Ray films.[20]

Waste treatment

Alkaline protease offer great applications for organization of waste from different industries of food

processing and the domestic activities. These have ability to make proteins soluble into the wastes via several step process to recover the concentrates of liquids or the dry solids of great nutritional assessment for the fish or the livestock.[10] Enzymes may work on the precise unmanageable pollutants to exclude them via transformation or precipitation to the products. Alkaline proteinases from Bacillus subtilis are used in waste feather processing from the poultry slaughter houses. Feathers compose of about 5% body weight of poultry and they are regarded as great source of protein for feed and food provide their hard structure of keratin that is fully damaged. Total feathers solubilization is obtained after treating with the NaOH, enzyme hydrolysis and disintegration mechanically. The last product is bulky, gray color powder having more content of protein that can be utilized largely as feed components. Likewise, various further keratinolytic alkaline proteases are utilized in food technology for making of amino acids and peptides, for degradation of waste keratinous material in the household refuse and as the depilatory agent to eliminate hair in the bath tub drains that caused foul odors in public areas and houses.[12]

Textile Industry

Most significant commercial application of the proteases is textile industry in which enzyme treatment gives finishing elegant texture.[11] A thermostable protease is used in removing gum and impurities present in the core protein fiber specially in silk used world widely as most precious fiber. De-gumming of silk is a growing industry; it utilizes proteases in large amount and forms fine quality of silk. Synthetic fabric is also treated with protease for the elegant and smooth finished product. Fungal proteases are most important enzymes in textile industry since a decade and it is increasing greatly. Indian sericulture has grownup double in the last one decade and the consumption of protease also enhances in various folds. Use of these proteases not only impart good quality to fabric but also results in mechanical strength of fiber. Use of proteases minimizes the amount of chemical detergents that cause great pollution in environment.[27]

Chemical industry

Enzymes present in organic solvents have their applications of biocatalysts in chemistry. The disadvantage of this method is reduced activity of enzymes under anhydrous conditions. Thus, we can find ways to activate enzymes in organic solvents due to practical importance. We can possibly use proteases of alkaline nature to catalyze peptide synthesis in organic solvents.[20] Enzymatic synthesis of peptides is now being employed, sucrose polyester ewas synthesized by using anhydrous proleather, a commercial protease prepraton from bacillus species. A-lacase enzyme acts as a catalyst for resolution of N protected aminoacids esters and alkaline proteases replaced subtilisin.[20] Different applications of proteases are shown in Fig.i.

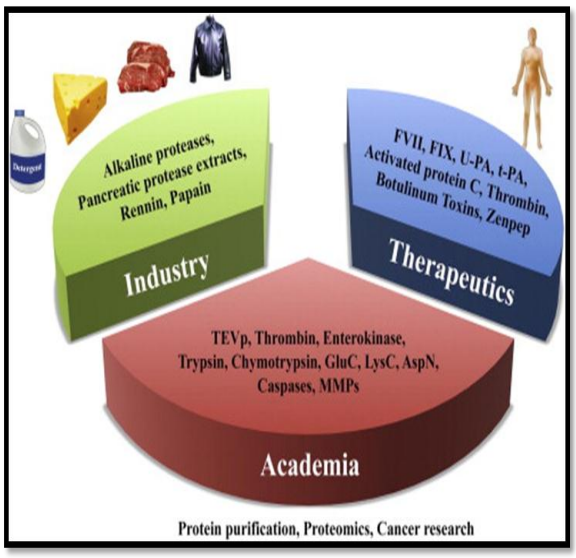

Fig. i: Applications of Proteases

Applications in Research

Other than the applications in industry and medicine proteases are very important in basic research. The peptide bond breakage is used in explaining the structural and functional relationship, in the sequencing and structure of proteins. The specificity of the hydrolytic action of proteases has extensive applications in the food industry and a lot of other commercial purposes such as detergent, leather, and pharmaceutical industries.[1] Gene cloning is an important method for manipulating genes. More than half of essential enzymes that are used in industry are extracted from microorganisms that are formed by genetic engineering. Proteases from natural origins are used extensively in molecular techniques. Their degenerative properties make them very important in protein degradation in general tissue degradation, isolation and culturing of cells. Proteins have wide applications that cause changes in the gene for the formation of a protein with slightly changed function. Recent advance research in recombinant DNA technology and the selective exchange of specific amino acids by "site-directed mutagenesis" (SDM) causes fast and major developments in the field of protein engineering.[22] Gene identification and knowing about the three-dimensional structure of protein in question are two important re-requisites for protein engineering. The X-ray crystallographic structures of a number of proteases are now being determined. Proteases from bacteria, fungi, and viruses are now also genetically engineered for improving their properties according to their particular applications.[5] The specific and predictable nature of cleavages caused by proteases enables their use for specific tasks e.g. antibody fragmentation production, the removal of affinity tags from recombinant proteins, specific protein digestion in the field of proteomics mainly for protein sequencing.

Therapeutic application of proteases

The diagnostic and therapeutic applications of protease for medicinal purposes are widely accepted and a lot of

enzymes are in from many years. Proteases are generally associated with development of anticancer, anti-inflammatory, anti-microbial and clot dissolving operators. Both fungal and bacterial strains are similarly required in characterizing helpful possibilities of protease. Inflammation is a physiological body reaction against infections caused by microbes and mechanical harms that are related with accumulation of plasma along with immune cells. The therapy for inflammation depends on the use of "non-steroidal" (NSAID) drugs in intense condition and steroidal medications in unending cases. Administration of inflammation relies on upon a few confusions that have certain reactions. These complications are combated by specific drugs (COX-II) are figured and are propelled in market commercially. These COX-II particular medications are exceptionally costly and are used for inflammation. Enzymes specially proteases are now emerged as better option for the treatment of acute and chronic inflammation and are not much expensive. Serratio-pepetidase is the best protease for its use in trating inflamation. This enzyme is not only produced by Serratia species of bacterium but also some other fungal species were explored for their anti-inflammatory proteases. A group of serine proteases from 'Indian Earthworm' has been used due to its anti-inflammatory potential and is studied for more molecular findings.[2]

Proteases play important part in upkeep of ordinary physiology and enzymes like 'caspase' is principally required in degradation of unusual cells. Caspase is a proteolytic enzyme that is produced by natural means and it serves as an important key part of our immune system. [7] A number of proteolytic enzymes are being tested for "anti-cancer" treatment by therapy. These proteases are cyto-toxic and they also induce cancer management (selective). Proteases obtained from many biological origins have become important key enzymes in formation of new-generation anti-cancer drug molecules. Advantages of using enzymes in cancer treatment over chemo-therapeutic agents are to reduce toxic effect imparted due to chemical based medicines. Later on these medicines made by enzymes for tumor treatment will serve a number of patients having cancer in an efficient way and more effectively. In 2014, a proteolytic enzyme from an earth-worm was characterized by its anti-tumor activity for the disease breast malignancy cell-lines results demonstrated a considerable measure of degree for protease in the improvement and advance of against tumor therapeutics.[3]

Worldwide cerebral and cardiac blood vascular disorders are the leading cause for death. Abnormal functioning of plasma proteins results in blood clot. This blood clot formation and thrombus formation blocks the blood supply and cause severe physiological consequences including tissue necrosis, embolism and ischemia. To overcome this blood clotting an external clot dissolving agent is needed. [20] The outside coagulation dissolving operators are thrombolytic that are fundamentally proteases. In last few decade several protease have been developed from microbial and animal origin and they are refined as external thrombolytic and are used clinically. External clot dissolving agent used for clinical application are Earthworm fibrinolytic Enzyme (EFE), Tissue plasminogen activators (t-PA), Staphylokinase (SAK), Streptokinase (SK), Urokinase (u-PA). They are efficient but complications associated with these agents include their stability, compatibility and specificity. Hence, for the development of external thrombolytic agents there is need of other protease. Proteases obtained from fungi are having a lot of scope in refining them into molecules important in therapy. Benefits of using fungal protease lies in substrate selectivity and stability for use in clot dissolving agents. In the future these sources will be used for life saving drug as key supplier.[37]

The antimicrobial capability of protein degrading enzymes is concentrated well in most recent couple of years and proteases has been risen as important key enzymes. The cyto-toxic nature of a few proteases acts as productive antimicrobial specialist and a lot of enzyme based anti-microbial components are used for clinical purposes [6]. A lot of proteases obtained from animals and also from microbial sources have been set apart as antimicrobial and it incorporates earthworm protease. Still numbers of antimicrobial peptides from bacterial and fungal origins accounted for their antimicrobial nature. There is a need to investigate parasitic biodiversity to disconnect these intense molecules.[41]

Future Scope

Proteases are unique class of the enzymes as they are of great physiological and commercial importance. They have both synthetic and degradative properties. Since, proteases are physiologically essential, they are found ubiquitously in microbes, animals and plants. However, microbes are goldmine of proteases because of their fast growth, limited area needed for cultivation and their accessibility to the genetic manipulation. Microbial proteases have been greatly used in leather, food, dairy and detergent industries from ancient times. There is new interest in proteases as targets for evolving therapeutic agents against the persistently spreading harmful and fatal diseases as AIDS, cancer and malaria. Analysis of series for acidic, alkaline and neutral proteases has provided new visions in evolutionary relationships of proteases.[5] The biodiversity shows invaluable resource for the biotechnological innovations and plays key role in search for improved strains of microbes used in industries. [23] The existing information relating structure-function relationship of the proteases, united with the gene-shuffling techniques, promise fair chances of success in near future, in progressing proteases that were not made in nature and which would be able to meet the requirements of mass of protease applications.

CONCLUSION

Proteases assume a part of driving catalysts having an incredible business esteem these are utilized broadly in industry and furthermore has a considerable measure of remedial applications. A lot of thermophilic-fungi contain a group of 23 species these belong to almost 9 genera. This is large number in reference to industrial demand and there is a need to re analyze to meet commercial demand. Proteases from fungi are not limited for applications in industry, their therapeutic potentials have been classified in last few years it constitutes one of the biggest groups of commercially important enzymes and modern techniques are needed to find these amazing and useful molecules for humans. Thermophilic fungi are the vital components of the micro-flora that are developed in stacked masses of materials including, plants, heaps of items that are forestery or rural, and other gathered natural matter in which the warm, muggy, aerobic condition gives the essential conditions to their advancement

REFERENCE

1. Argos, P.G., Kamer, M.J.H. Nicklin and E. Wimmer. Similarity in gene organization and homology between proteins of animal picornaviruses and a plant comovirus suggest common ancestry of these virus families. Nucleic Acids Res., 1984; 12; 7251–7267.
2. Bhagat, S. Serratiopeptidase: a systematic review of the existing evidence. Int J Surg., 2013; 11(3): 209-17.
3. Białas, A. and P. Kafarski. Proteases as anti-cancer targets molecular and biological basis for development of inhibitor-like drugs against cancer. Anticancer Agents Med Chem., 2009; 9(7): 728-62.
4. Browner, M.F., W. W. Smith and A. L. Castelhano. Matrilys ininhibitor complexes: common themes among metalloproteases. Biochemistry, 1995; 34(20): 6602–6610.
5. Browner, M.F., W. W. Smith and A. L. Castelhano. Matrilys ininhibitor complexes: common themes among metalloproteases. Biochemistry, 1995; 34(20); 6602–6610.
6. Bruinenberg, P.G., W.M. De Vos and R.J. Siezen. Prevention of C-terminal autoprocessing of Lactococcus lactis SK11 cell-envelope proteinase by engineering of an essential surface loop. Biochem. J., 1994; 302: 957–963.
7. Chiplonkar, J., M.S.V. Gangodkar, U.V. Wagh, G.D. Ghadge, M.V. Rele and M.C. Srinivasan. Applications of alkaline protease from Conidiobolus in animal cell culture. . Biotechnol. Lett, 1985; 7: 665–668.
8. Cunningham, E. L. Kinetic stability as a mechanism for protease longevity. Proc Natl Acad Sci USA., 1999; 96(20): 11008-11014.
9. Dalv, P.G. Utilization of waste feathers from as from poultry slaughter for production of a protein concentrate, Biosource Tecnol, 1994; 48: 265.
10. Deng, A.H., J.Wu, Y. Zhang, G.D. Zhang and T.Y. Wen. Purification and characterization of a surfactant- stable high-alkaline protease from Bacillus sp. B001. Biores Tech., 2010; 101: 7100-7106.
11. Dunaevsky, Y.E. Fungal inhibitors of proteolytic enzymes: classification, properties, possible biological roles, and perspectives for practical use. Biochimie, 2014; 101: 10-20.
12. Ellaiah, P., B. Srinivasulu and K. Adinaarayanta. A review on microbial alkaline proteases. Journal of Scientific and Industrial Research, 2002; 61: 690-704.
13. Freddi, G.R., Mossotti and R. Innocenti. Degumming of silk fabric with several proteases. J Biotechnol, 2003; 106(1): 101-112.
14. Friedman, H. A homolog of the proteasomerelated RING10 gene is essential for yeast cell growth. Gene, 1992; 122: 203–206.
15. Gajju, H., T.C. Bhalla, and H.O. Agarwal. Utilization of thermostable alkaline protease from Bacillus coagulans PB-77 for silver recovery from used x-ray films. Proc 37th Annu Conf Assoc of Microbial India Chennai, 1996; 79.
16. Godfrey, T. and S. West. Introduction to industrial enzymology. Ind enzy, Mac. Millan Press, London, 1996; 1-8.
17. Hasnain, S. Purification and characterization of an extracellular thiol-containing serine proteinase from Thermomyces lanuginosus. Biochem Cell Biol., 1992; 70: 117–122.
18. Henner, D. J. Proceedings of the 9th International Spore Conference. Expression of cloned protease genes in Bacillus subtilis, 1985; 95–103.
19. Hill, E.P. and A.S. Sussman. Development of trehalase and invertase activity in Neurospora. J Bacteriol, 1964; 88: 1556–1566.
20. Jean, K. and A. James. Potential Applications of Enzymes in Waste Treatment. Nicell Dep of Civil Eng & Applied Mechanics, McGill University, 817.
21. Johnsson, A.G. Protease production by species of Entomophthora. Appl Microbio, 1986; 16; 450-457.
22. Kinda, K. and S. Morimira. Enzymatic hydrolysis of horn and hoof of cow and buffalo, 1995; 80: 478.
23. Maurer, K.H. Detergent proteases. Curr Opin Biotechnol, 2004; 15(4): 330-334.
24. Murakami, K.. Isolation and characterization of the alkaline pro-tease gene of Aspergillus proteinase from Thermomyces lanuginosus. Biochem Cell Biol., 1992; 70: 117–122.
25. Qing L. Purification and Characterization of a Protease Produced by a Planomicrobium sp. L-2 from Gut of Octopus vulgaris. Prev Nutr Food Sci., 2013; 18(4): 273–279.
26. Sabotic, J. and J. Kos. Microbial and fungal protease inhibitors-current and potential applications. Appl Microbiol Biotechnol, 2012; 93(4): 1351-1375.

27. Saeki, K. Detergent alkaline proteases: enzymatic properties, genes, and crystal structures. J Biosci Bioeng, 2007; 103(6): 501508.
28. Schechler, I. and A. Berger. On the size of the active site in proteases I papain. Biochem and Biophy Res Comm, 1967; 27: 157-162.
29. Schroeder M. Restricting detergent protease action to surface of protein fibres by chemical modification. Appl Microbiol Biotechnol, 1967; 72(4): 738-744.
30. Srilakshmi, J., J. Madhavi, S. Lavanya, K. Ammani.. Commercial Potential of Fungal Protease: Past, Present and Future Prospects. Journal of Pharmaceutical, Chem and Bio Sci., 2014; 2(4): 218-234.
31. Sumantha, A.C., Sandhya, G. Szakacs, C.R. Soccol and A. Pandey. Production and partial purification of a neutral metalloprotease by fungal mixed substrate fermentation. Food Technology and Biotechnology, 2005; 43: 313-319.
32. Veloorvalappil, N., Jisha, B. Robinson, Smitha, P. Selvanesan, S. Sasidharan, N.U. Kizhakkepawothail, S. Sreedharan, P. Prakasan, S.J. Moolakkariyil, B. Sailas. Versatility of microbial proteases. Enzyme Tech Lab, Biotech Div, Dep of Bot, Uni of Calicut, Kerala, India, 2013; 1(3): 39-51.
33. Verma, A. 2014. Alkaline protease from Thermoactinomyces sp. RS1 mitigates industrial pollution. Protoplasma, 251(3): 711-810.
34. Vijayaraghavan, P. Dehairing protease production by an isolated Bacillus cereus strain AT under solid-state fermentation using cow dung: Biosynthesis and properties. Saudi J Biol Sci., 2014; 21(1): 27-34.
35. Wang, H.Y. Screening and mutagenesis of a novel Bacillus pumilus strain producing alkaline protease for dehairing. Lett Appl Microbiol, 2007; 44(1): 1-6.
36. Yogendra K.V. and K.V. Mahendra. Earthworm- A Potential Source For Stable And Potent Antimicrobial Compounds- Isolation & Purification Study. Int J Pharm Pharm Sci., 2012; 4(4): 540-543.
37. Zanphorlin, L.M., F.D. Facchini, F. Vasconcelos, R.C. Bonugli-Santos, A. Rodrigues, L.D. Sette, E. Gomes and G.O. Bonilla-Rodriguez. Production, partial characterization, and immobilization in alginate beads of an alkaline protease from a new thermophilic fungus Myceliophthora sp. J. Microbiol., 2010; 48: 331-336.