

## INDUSTRIAL APPLICATION OF MICROBIAL PROTEASES

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## ABSTRACT

Proteases are the proteolytic enzymes that degrade the protein molecules. Proteases are isolate from animal, plants and microbial sources. Microbial proteases are favored over the others for manufacture at wide scale because of their growth that is very fast and easiness of life for new recombinant enzyme generation with properties that is preferred. This review describe the application of microbial proteases for the various industrial utilization. The main technical industrial utilization of microbial proteases are laundry detergents, degumming of silk, leather, food, pharmaceuticals and cosmetics. Developments in enzymology, microbial proteases are widely used to provide potentials advancement for a more biologically approachable in the different industries.

**KEYWORDS:** Endopeptidase, Exopeptidase, Protease, Industry, hydrolysis.

## INTRODUCTION

All enzymes are considered as catalyst in nature. Most enzymes are produced by the fermentation of microorganisms. Proteases occur in plants, animals and microorganism and have very important role in many pathological and physiological processes Fig-2. Proteases is proteolytic enzyme that catalyze hydrolyses of protein into amino acid and smaller peptides. Generally, microbial proteases are extracellular in nature and are straightforwardly discharged into the broth of fermentation by the manufacturer, in this way downstream processing of the enzyme are improving when contrasted with proteases acquired from animals and plants.<sup>[1]</sup> Protease which are intracellular in nature have great role in metabolic and cellular processes, for example protein turnover, maturation of hormones and enzymes and maintaining protein pool. Proteases are named peptide hydrolases and constitute a large group of proteins, separated into endopeptidases and exopeptidases. That are subsequently categorized into serine protease, aspartic protease, cysteine protease, aspartate and metalloprotease that base on their catalytic mechanism. They can also be characterized on the base of pH which they have a higher association: alkaline (pH 8 to 13), acidic (pH 2 to 6) and neutral (pH 6 to 8).

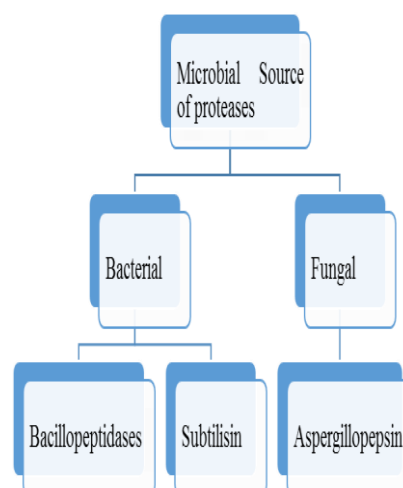
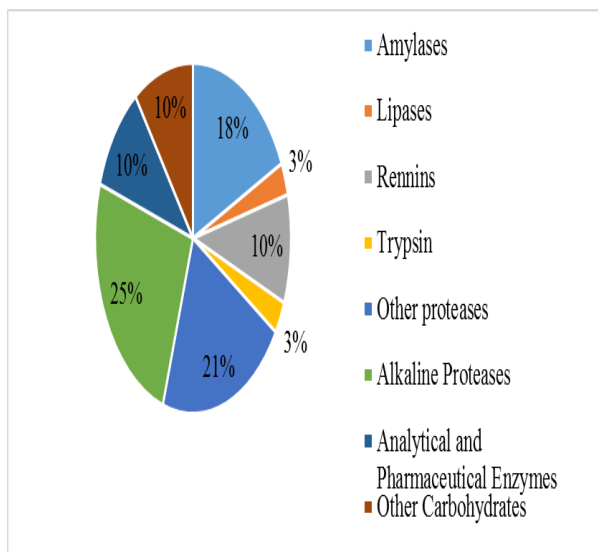


Figure-2 Schematic representation of microbial source of proteases.

A large number of alkaline protease of bacteria are industrially accessible, for example, subtilisin BPN, subtilisin Carlsberg and Savinase, with their significant application such as enzymes of detergents.<sup>[2]</sup> Progression in biotechnology deals a useful position for the advancement of the proteases and their application will continue to encouraging to give a supportable situation to improving the human life worth.<sup>[3]</sup> There are long history of protease enzymes that have been utilized in the detergent and food industries. 75% of industrially used enzymes are hydrolytic enzyme. One of the hydrolytic enzyme such as proteases symbolize third major group of enzymes for industrial use and are recorded around 60% of the aggregate overall offered proteins (Fig. 1).<sup>[4]</sup> One of the protease enzyme that is themostabilize in nature

such as alcalase are extracted by *Bacillus licheniformis*. Subtilisin is the significant element of their preparation, such as a serine endoprotease, showing stability at 60°C and pH 8.3. There are numerous applications of alcalase that are discovered in food industry, e.g., due to the reason of specificity that is low on the way to diverse proteins from different sources such as animals and plants.<sup>[5]</sup> Such catalysts can be likewise utilized for cleaning ultrafiltration layers at high temperatures, expanding the efficiency of this procedure.<sup>[6]</sup> An incredible number of fungal sources also been utilized to delivered proteases such fungus are *Penicillin*, *Mucor*, *Thermomyces*, *Aspergillus*, *Rhizopus*, *Humicola* and *Thermoascus* etc. A few of these excreted enzymes, created in a huge scale submerged fermentation, have been generally utilized in beverage and food industry for quite a long time.<sup>[7]</sup> *Penicillium* source of proteases have great potential in various industrialized developments such as textile, dairy, detergent, leather and pharmaceutical preparations. *Penicillium* such as *P. citrinum*, *P. restrictum*, *P. camemberti* etc. are the sources of protease production.<sup>[8]</sup> Alkaline proteases have vital role in detergent industry, leather processing, medical diagnosis, dairy and brewing industries, though *Bacillus* source of proteases have been exist satisfactory in detergent industry and that is the larger most application of protease enzymes.



**Fig. 1. Contribution of total sale distribution of different enzymes is indicated**

#### Source of Microbial Proteases

Proteases from animal and plant origin are produced by extraction. Proteases from bacterial and fungal origin are produced primarily by industrial scale fermentation, some are modified genetically. The familiar proteases from animal source are rennin, trypsin, pepsin and chymotrypsin. Renin (Chymosin) is found in the stomach of all nursing mammals. By the action of the pepsin, rennin converted to active renin. Rennin is widely used in the dairy industry to produce curd and cheese. Rennin cleaves C-terminal glycopeptide and in casein one

peptide bond that produce para-k casein that is insoluble form. Trypsin take parts in hydrolyses of the food protein. Trypsin is a serine endopeptidase produced by the pancreatic acinar cells. Trypsin activated after proteolysis of trypsinogen that cleaves peptide on the carboxyl terminal of positively charged side chains of lysine and arginine. Trypsin is utilized for the preparation of bacterial media and some specialized medical application. Pepsin is aspartic protease obtained from the glandular layer of the stomach. Pepsin activated by pepsinogen. Pepsin have two aspartic acid residues at active site, and show optimal activity at pH 1 and 2, as optimal pH of stomach is 2 – 4. Chymotrypsin is also a serine endopeptidase and produced by the pancreatic cells. Chymotrypsin activated by chymotrypsinogen by trypsin that cleaves peptide bonds on the carboxyl side of Tyrosine, Trptophane, Phenylalanine and Methionine. It can also catalyze the hydrolysis of ester bond. Chymotrypsin is used in preparation of the medicine. During cataract surgery, chymotrypsin is sometimes used to reduce damage to the eye. The proteases of plant origin are papain, bromalinm keratinases and ficin. Papain is extracted from latex of *Carica Papaya*.<sup>[9]</sup> Optimal pH of papain is 5-9 and are stable with substrate up to 80-90°C. Papain is widely used for the preparation of the protein hydrolysates that is highly soluble. Bromelain is cysteine protease with optimum pH 5-10. It is inactivate at temperature about 70°C. Bromelain is prepare from the juice of pineapple.<sup>[10]</sup> Keratinases is produce from the botanical group of plants which help in digestion of hair and wool that produce lysine. Microbial proteases are favored over the others for manufacture at wide scale because of their growth that is very fast, and easiness of life for new recombinant enzyme generation with properties that is preferred. Microorganisms represent a two-third share of business protease creation in the compound business sector over the world.<sup>[11]</sup> Proteases assume a pivotal role in leather, pharmaceutical, detergent, agriculture and food industry. Right now, the evaluated estimation of the worldwide offers of mechanical catalysts is more than 3 billion USD,<sup>[12]</sup> of which proteases represent around 60% of the aggregate deals. Some bacterial source of proteases that are commercially available are listed in the table-3 with their supplier name, products with trade name, sources and applications.<sup>[2]</sup>

Mostly neutral and alkaline proteases are used for commercial purpose are produced by bacteria belonging to the genus *Bacillus*. Bacterial neutral proteases are dynamic in pH range (pH 5 to 8) and have generally low thermostolerance. Neutral protease such as neutrase is insensitive to the regular plant protease inhibitors and is in this manner valuable in the brewery. The bacterial neutral proteases are described by their high affinity for hydrophobic pairs of amino acid. The metalloprotease, a neutral protease, need divalent metal ions to show their activity, while others are serine proteases, which are not influenced by chelating agents. Bacterial alkaline proteases are categorized by their activity that is high at

neutral pH, e.g., pH 10, and their specificity of broad substrate. Their ideal temperature is about 60°C.<sup>[13]</sup> These properties microbial alkaline proteases make them appropriate use in the detergent industry. Neutral and alkaline proteases have great potential for application in the leather tanning and detergent industries because of the development in creating environment-accommodating technologies. Intracellular proteases are key to maintain different metabolic and cellular procedures, for example, protein turn over, sporulation and cell separation, enzyme maturation and hormones furthermore in protoxin activation of Bt-based biopesticides.<sup>[1]</sup> Extracellular proteases perform protein hydrolysis of protein in fermented media and support to absorb the cell and use hydrolytic products. The most dominant group of proteases produced by bacteria, yeast, fungi and *actinomycetes* are alkaline serine proteases. Microbial proteases from fungal species are included in Table-1. Fungi elaborate a high diversity of enzymes than do bacteria. For example, *Aspergillus oryzae* produces alkaline, neutral and acid proteases. They are easily produced by the process of solid state fermentation. The pH of fungal proteases is active over a wide range (pH 4 to 11) and exhibit broad substrate specificity. Neutral proteases from fungal species are metalloproteases that are active at pH 7.0 and are inhibited by chelating agents. Microbial proteases from fungal species are included in Table-2. Viral proteases have gained great importance due to their functional involvement in the processing of proteins of viruses that cause certain fatal diseases such as AIDS and cancer. Serine, aspartic, and cysteine peptidases are found in various viruses.<sup>[14]</sup> Proteases backbone folds that show resemblance to those of serine protease like chymotrypsin, cysteine proteases like papain and aspartic proteases like pepsin. This is proceed by two viral proteases that have distinctive backbone folds i.e. Adenovirus proteases and herpesvirus proteases. Herpesvirus proteases is recognized recently that is very important for all herpesviruses replication and consists of novel Ser-His-His enzymatic characteristics.<sup>[15]</sup> The herpesvirus proteases are synthesized by the autoproteolytic processing through the assembly of virus. There are different types of virus proteases, such as XMRV protease, HIV protease, HIV Protease (HIV-PR), retropepsin and xenotropic-murine leukemia viral protease (XMRV-PR) that cleaved the polyproteins to produce the mature viral proteins.

### Classification of Proteases

A protease (also known as proteinase or peptidase) is a proteolytic enzyme that involved in the catabolism of the protein by hydrolysis of peptide bonds in protein or polypeptides. Protease classifications by organism source are animal, plant and microorganism such as bacteria, fungi. Some Animal, plant and bacterial source of proteases are listed in the Table-2. Protease classifications by proteolytic mechanism are serine proteases, threonine proteases, glutamic acid proteases, cysteine proteases, aspartic proteases and

metalloproteases (Table-1). Protease classifications on the base of optimum pH are acid proteases, neutral proteases and alkaline proteases as shown in table-1. Protease classifications by specificity of the peptide bond are Endopeptidases and Exopeptidases. Exopeptidases are cleaves off the substrate at the amino or carboxyl terminus that is N or C respectively,<sup>[16]</sup> i.e. Aminopeptidases, Dipeptidyl peptidases, Dipeptidases and Carboxypeptidases. On the other hand Endopeptidases are cleave off the internal peptide bond i.e. aspartic proteases, serine proteases, cysteine proteases and metalloproteases, shown in table-1. Serine protease enzyme are characterized by their serine residue on their active site. Serine proteases are widely found among the eukaryotes, viruses and bacterial that are very important to all the organisms. Serine protease enzymes are present in endopeptidases, exopeptidases, omega peptidases and oligopeptidase groups. Serine protease has ability to inhibit irreversibly by Tosyl-L-lysine chloromethyle ketone (TLCK), di-isopropyl fluoro phosphate (DFP) and phenyl methyl sulfonyl (PMSF). Particularly serine protease enzyme show their optimal activity at neutral and alkaline pH range from 7 to 11.<sup>[17]</sup> Alkaline serine proteases are isolated from bacteria, yeasts, molds. These proteases hydrolyzed the peptide bond which contain leucine, tyrosine and phenylalanine at their carboxyl terminus. They show their activity around pH 10 and isoelectric point is about pH 9. Subtilisins Novo is designed by BPN9 and are isolate from *Bacillus amyloliquefaciens*. Carlsberg is the subtilisins detergent that show their higher activity at alkaline pH as pH optima 10 and optimum temperature 60°C. Subtilisins and serine alkaline protease are show their activity at same temperature and pH, and their active site are made up with great substrate specificity as they have same amino acid sequencing such as Asp32, His64 and Ser221.<sup>[4]</sup> Although active site of subtilisins is similar with chymotrypsin and trypsin but having different molecular arrangement. Aspartic proteases an acidic endopeptidases, depends upon the residue of the aspartic acid for their enzymatic activity. These proteases are commonly found in the viruses. In bacteria and fungi they are not often exist, except the acidic nature of the bacteria with optimum pH 3 to 4 with isoelectric point (IP) range from pH 3 to 4.5. Cysteine or thiol Proteases are found in prokaryotes as well as eukaryotes. These enzymes are active in the presence of cysteine or reducing agents like HCN. Their activity are depends upon the cysteine and histidine residue that are ordered as Cys-His or His-Cys that are different among all families of this group. On the bases of side chain cysteine proteases are categorized into four group i.e. glutamic acid specific, trypsin with cleave off the arginine site, papain type and others. Cysteine proteases show their activity at neutral pH, though some show acidic pH for their optimum activity like lysosomes proteases. They are defenseless against sulfhydryl agent like PCMB and are not affected by DFP and chelating agents like EDTA. Metalloproteases have huge diversity in enzymatic activity such as particular requirement of

divalent metal ions, majority of which consist of zinc. Through the X-ray crystallography technique, the residues that helped in zinc binding have been easily recognized.<sup>[14]</sup> These type of proteases are originate from higher organisms. For the removal of environmental contamination, the most important composition is the protease enzyme. Alkaline protease enzyme is the most

important type among the protease enzyme that compose of approximately 35 percent of microbial enzyme. The *Bacillus* gram positive bacteria is the spore forming bacteria that resist to heat. These type of bacteria moves through flagella and produce protease as well as amylase.<sup>[18]</sup>

**Table-1 Classification of Proteases**

By proteolytic mechanism:	By pH optimum	By specificity of peptide bond:	
Serine proteases	Acid proteases	Endopeptidases	Exopeptidases
Threonine proteases	Neutral proteases	Serine proteases	Aminopeptidases
Cysteine proteases	Alkaline proteases	Cysteine proteases	Dipeptidyl dipeptidases
Aspartic proteases		Aspartic proteases	Carboxypeptidases
Metalloproteases		Metalloproteases	Dipeptidases
Glutamic acid proteases			

**Table-2 Organism source of protease Enzyme**

Organism	Enzyme
Animal	chymotrypsin, trypsin, pepsin
Plant	ficin, papain, bromelain
Bacterial	bacillopeptidases, subtilisin
Fungal	Aspergillopepsin

### Industrial Application of Microbial Proteases

For the most part microbial proteases have a vast variety of industrial application, such as detergent,<sup>[19]</sup> leather,<sup>[20]</sup> food, feed, dairy products,<sup>[21]</sup> pharmaceutical industries.<sup>[22]</sup> Filamentous fungi are economically important as it has ability to synthesize compounds such as enzymes, antibiotics, peptides, organic acids and peptides by metabolic pathway and these compounds are relevance to the biotechnology, food & feed, pharmaceutical and chemical industries.<sup>[23]</sup> Applications of proteases fluctuates remarkably. Proteases have very important role in detergent industry. The utilization of proteases in laundry household represents around 25% of the aggregate overall offers of enzymes.<sup>[24]</sup> Subtilisin an alkaline serine endopeptidases are produced by numerous types of species belongs to genera *Bacillus*, having fundamental application in detergents for household. Frequently, improvement in engineering techniques of the traditional detergent enzymes such as proteases and amylases, are technologically advanced. These enzymes are stabilized the detergent's performance and their composition is continuously developed. Specifically, the similarity of enzymes with detergents on base of the stability is addressed, yet their capacity to work at lower temperatures has been in the recent time reported enhancements. Proteases showing activity at low temperatures have been obtained from nature, yet have been developed in the lab by an advancement approach.<sup>[17]</sup> The accomplishment of detergent used for enzymes has develop the detergent protease series with their particular uses. Alkazym (Denmark, Copenhagen, Novodan) is an essential

commercial based enzyme for the membrane system for their cleaning and other types of enzymes utilize for the membrane cleaning are Ultrasil (Henkel, Dusseldorf, Germany), P3-pardigm (Henkel-Ecolab, Dusseldorf, Germany) and Terga-zyme (Alconox, New York, USA). A protease enzyme, Pronod 153L, based cleaner is utilized surgical instruments cleaning that are stained by blood proteins. The part of proteases in cleanser is to hydrolyze large number of protein molecule connected with hard pigments. Amid the procedure of hydrolysis, the peptide bonds that hold different amino acids together to frame a protein particle are separated, discharging littler polypeptides and individual amino corrosive units. They fill in as scissors to remove the stain physically piece by piece from the surface of the fabric.<sup>[25]</sup> The protease enzymes with specific and wide diversity have been utilized in developing operative therapeutic agents. As some proteases contain non proteolytic functions that play a major role in invasion of tissue, modulating immune response and in the adhesion of host epithelial cells, thus due to these extra functions they are associations with the therapeutic design.<sup>[26]</sup> In pharmaceutical and cosmetic industries protease enzymes are widely utilized for medicines arrangement for example treatments for debridement of wounds, and also used for the keratin elimination in acne, keratinases can remove scar and help in regeneration of epithelial cell, human callus elimination and keratinized skin degradation, vaccine preparation.<sup>[27]</sup> Protease enzyme in cosmetic products can hydrolyze the peptide bonds of elastin, collagen and keratin of the skin. Furthermore, the elastoterase preparation was connected for the treatment

of purulent injuries, burns, carbuncles and profound abscesses. Collagenolytic proteases have been straightforwardly utilized in clinical treatment, incorporates healing of wound, sciatica in herniated intervertebral plates treatment, retained placenta treatment, and as a pretreatment for improving adenovirus-mediated in gene therapy of cancer.<sup>[28]</sup> Combination of subtilisin or clostridial collagenase with broad-spectrum antibiotics is used in the treatment of wounds and burns. Isolation of asparaginase from *E. coli* is utilized to the asparagine elimination from bloodstream in different types of lymphocytic leukemia. Serrapeptase is a protease enzyme that is isolated from the nonpathogenic serratia species, that is the member of enterobacteriaceae and are found in the silkworm's gut. This enzyme is most effectively used in anti-inflammatory agent that hydrolyzed the fibrin that is insoluble, by result of coagulation of blood. Serrapeptase causes remarkably reduce the severity of pain, secretion and fever in patients with sinusitis and laryngitis, and serrapeptase also protect against stroke and more effective than ethylenediaminetetraacetic acid (EDTA) in replacement of arterial plaque. Serrazime, a protease enzyme isolated from *Aspergillus melleus* and *Aspergillus oryzae*, combination with serrapeptase have ability to clearance of sputum and viscoelasticity improvement. Lysostaphin, another microbial protease enzyme isolate from *Staphylococcus simulans*, an antimicrobial agent with high degree of anti-staphylococcal activity. Streptokinase-streptodornase (varidase) a protease commercially available as Varidase, is utilized specifically for cleaning wounds that contain necrotic tissue, fibrin, blood clots and purulent exudates.<sup>[4]</sup> Another industrial application of protease enzyme is helped in dehairing the skin and hide of animals in the leather industry.<sup>[29]</sup> There are some advantages of proteases as the stability of the enzyme at unlike environmental circumstances such as temperature, pH and duration dependable performance and their production cost and application, at commercial level, tanners are uncertain to utilizing such enzymes. In a tannery, a raw hide is subjected to a series of chemical treatments before tanning and finally converted to finished leather. Alkaline proteases play an important role in chemical treatment of leather by changing the harmful chemicals specifically involved in dehairing, soaking and bating. Protease enzymes are also utilized in a wide range of food processing application and helped in improvement of digestibility, high solubility and improvement in bitterness as well as good yield of protein. Protein hydrolysate, prepared from the alkaline proteases, have high nutritional value and vital role in regulation of blood pressure that utilized in food formulation of infants, fortification of fruit juices, soft drinks and therapeutic products, in cheese

manufacturing, cereal mashing.<sup>[2]</sup> The solid wastes are the major problem in environment that effect on human health. Spoilage of food also a wastes that is not fit for use. Protease enzymes are used for treatment of all these wastes.<sup>[30]</sup> In the meat tenderization,<sup>[31]</sup> protease enzyme hydrolyze the muscle protein and connective tissue protein.<sup>[8]</sup> The most important application of protease enzymes in dairy products is in the cheese making, where the principal role of enzymes is to breakdown the specific peptide bond to produce macro peptides and casein. Protease enzyme isolate for the *Endothia parasitica* (also called *Cryphonectria parasitica*) and *Mucor miehei* are particularly use in the manufacturing of the cheese to remove the rennin. To modify the gluten of wheat in baking processes, protease enzyme isolated from *Aspergillus oryzae* have been utilized. By the addition of protease enzyme in dough the mixing time is reduced which causes the volume of loaf increased. In the soy sauce processing fungal source of protease enzyme have played vital role. Extraction of the protein hydrolysate from soy protein, whey protein and casein have applications as important elements of the nutritional supplements, flavoring agents, health and dietetic products and those person who have allergy to milk protein. Protease enzymes are thermostable and have high pH activity and due to the improvement of pollution distinguishing have made this enzyme an ultimate applicant for laundry applications. Proteases are widely utilized in the laundry industry. Serine protease like subtilisin isolate from bacillus species are particularly used in the manufacturing of the detergents for dishwashing and laundry. As all the detergents composed of almost identical detergency mechanism that consist of one or more than one enzymes such enzymes are protease, cellulose, lipase and amylase. An Alkaline enzymes that are the strains of bacillus are consider as the good source to fulfill the enzymes requirement that are utilized for the detergents. The degumming of silk is made up of an alkaline and surfactant chemicals. This alkaline solution containing soap that is the harsh treatment and attack the structure of fibrin. There are different source of proteases that act as degumming agent, subsequently they can dissolve sericin but not effect on the protein of silk fiber.<sup>[32]</sup> Two fungal and two actinomycete proteases are exhibit the loss of weight that is similar to traditional method (19.58% - 21.78%). BOA-2 proteases and *Conidiobolus brefeldianus* proteases are exhibit the loss of weight that is traditional method with low concentration of enzyme within a short time.<sup>[33]</sup> Viral proteases are involved in the life cycle of different disease, thus they target the development of therapeutic agents against diseases as against inflammation, necrotic wounds, cancer, cardiovascular disorders.<sup>[34], [35]</sup>

**Table-3 Commercially available microbial proteases with their supplier, product trade name, source and applications. n. s. Non specific**

Suppliers Name	Product trade Name	Source	Applications
Nagase Biochemicals, Japan	Biopraxe SP-10	<i>B. subtilis</i>	Foods
	Biopraxe	<i>B. subtilis</i>	Cleanings and detergents
	Ps. elastase	<i>Pseudomonas aeruginosa</i>	Research
	Ps. protease	<i>Pseudomonas aeruginosa</i>	Research
	Biopraxe concentrate	<i>B. subtilis</i>	Pharmaceuticals and Cosmetic
	Cryst. protease	<i>B. subtilis</i> (bioteus)	Research
Amano Pharmaceuticals, Japan	Cryst. protease	<i>B. subtilis</i> (K2)	Research
	Amano protease S	<i>Bacillus species</i>	Foods
	Proleather	<i>Bacillus species</i>	Foods, silks degumming, detergents
Novo Nordisk, Denmark	Collagenase	<i>Clostridium species</i>	Technically used
	Nue	<i>Bacillus species</i>	Leathers
	Savinase	<i>Bacillus species</i>	Textiles and detergents
	Novozyme 243	<i>B. licheniformis</i>	Denture cleaners
	Novozyme 471 MP	n. s.	Hydrolysis of photographic gelatins
	Biofeed pro	<i>B. licheniformis</i>	Feeds
Genencor International, USA	Esperase	<i>B. lentus</i>	Foods, silks degumming, detergents
	Alcalase	<i>B. licheniformis</i>	Silks degumming and detergents
	Durazyme	<i>Bacillus species</i>	Detergents
	Prima Tan	Bacterial source	Leathers
	Purafect	<i>B. lentus</i>	Detergent
Solvay Enzymes, Germany	HT-proteolytic	<i>B. subtilis</i>	Leathers and foods
	Opticlean	<i>B. alcalophilus</i>	Detergents
	Maxapem	<i>Protein engineering in various Bacillus species</i>	Detergents
	Protease	<i>B. licheniformis</i>	Waste and Foods
Enzyme Development, USA	Optimase	<i>B. licheniformis</i>	Detergents
	Enzeco alkaline protease-L FG	<i>B. licheniformis</i>	Foods
	Enzeco high alkaline protease	<i>Bacillus species</i>	Industrially used
Wuxi Syder Bioproducts, China	Enzeco alkaline protease	<i>B. licheniformis</i>	Industrially used
	Wuxi	<i>Bacillus species</i>	Detergents
Godo Shusei, Japan	Godo-Bap	<i>B. licheniformis</i>	Foods and detergents
Gist-Brocades, The Netherlands	Maxatase	<i>Bacillus species</i>	Detergents
	Subtilisin	<i>B. alcalophilus</i>	Detergents
	Maxacal	<i>Bacillus species</i>	Detergents
Advance Biochemical, India	Protosol	<i>Bacillus species</i>	Detergents
Rohm, Germany	Corolase 7089	<i>B. subtilis</i>	Foods

**CONCLUSION**

This review conclude that by the utilization of advancement in biotechnology, microbial proteases applications are extensively used in different industries such as pharmaceutical, therapeutic, cosmetic, leather, degumming of silk, detergent, textile and food industry.

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