

Versatility of microbial proteases

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ABSTRACT

Proteases or peptidases constitute the largest group of enzymes in bio-industry with a long array of uses. They play an invincible role in industrial biotechnology, especially in detergent, food and pharmaceutical arena. This focused review encompasses an overview on alkaline proteases, mainly of microbial sources in a handy module. Following an introduction and general classification with evolutionary insight, major sources of proteases (animal, plant and microbial including fungal, bacterial), their general properties with mechanism of action and molecular masses are discussed. Proteases from *Bacillus* spp. have been given special attention. In addition to this, an overview on the applications of proteases in detergent, tannery, food, metal recovery and waste treatment industries is also addressed briefly.

Keywords: Review; Proteases; Classification; Sources; *Bacillus*; Industrial Uses

1. INTRODUCTION

Recent years have witnessed a phenomenal increase in the use of enzymes as industrial catalysts. Proteases (EC 3:4, 11-19, 20-24, 99) (synonymous as peptidase or proteinase) constitute a very large and complex group of enzymes, widely utilized in a host of industries. They differ in properties such as substrate specificity, active site and catalytic mechanism, pH and temperature optima, and stability profiles. Studies relating to such properties are imperative for the successful application of these enzymes in their respective industry [1]. The main sources of the enzymes were from animals (e.g. calf stomach), plants (e.g. pineapple, fig, and papaya), microbes (e.g. *Bacillus* spp., *Pseudomonas* spp.) [2,3], etc. But the

production of enzymes from plant and animal sources is limited due to climatic reasons and ethical issues, respectively [2,3]. Microbial sources have occupied an invincible domain in the production of all the three—acidic, neutral, and alkaline—major types of proteases. The alkaline proteases, an important group of industrial enzymes are produced by a wide range of organisms including animals, fungi and bacteria. *Aeromonas*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Halomonas*, *Pseudomonas* and *Serratia* are the major bacterial genera which contribute to proteases [2]. *Bacillus*-derived alkaline proteases are of immense utility in other industrial sectors too, viz., leather, food, textile, organic synthesis, and waste water treatment. *Bacillus*-derived alkaline proteases are stable at elevated temperatures and pH, but majority of them are incompatible with detergent matrices [4]. Therefore, alkaline proteases with superior performance for commercial exploitations, especially for detergents, are being focused.

2. GENERAL CLASSIFICATION OF PROTEASES

According to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology, proteases are classified under the subgroup 4 of Group 3 (hydrolases) (Table 1). However, proteases do not comply easily with the general system of enzyme nomenclature due to their huge diversity of action and structure. On the basis of their site of action on protein substrates, proteases are broadly classified as endo- or exo-enzymes [3]. They are further categorized as serine proteases, aspartic proteases, cysteine proteases or metallo proteases—depending on their catalytic mechanism (Table 1). Proteases are also classified into different clans and families depending on their amino acid sequences and evolutionary relationships. Based on the pH optima, they are referred to as acidic, neutral, or alkaline proteases [3].

Table 1. General classification of proteases with their enzyme commission (EC) code, coupled with specific mechanism of action of each subgroup.

Protease	EC code	Mechanism
Exopeptidases	3, 4, 11-19	cleave the peptide bond proximal to the amino or carboxy termini of the substrate
Aminopeptidases	3, 4, 11	Those acting at a free N-terminus liberate a single amino acid residue
Dipeptidases	3, 4, 13	Exopeptidases specific for dipeptides
Dipeptidyl peptidase	3, 4, 14	Release of an N-terminal dipeptide from a polypeptide
Tripeptidyl peptidase	3, 4, 14	Release of an N-terminal tripeptide from a polypeptide
Peptidyl dipeptidase	3, 4, 15	Release of free C-terminus liberate a dipeptide
Carboxypeptidase	3, 4, 16-18	Release of a single residue C-terminal from a polypeptide
Serine type protease	3, 4, 16	Carboxypeptidase have an active centre serine involved in the catalytic process
Metalloprotease	3, 4, 17	Carboxypeptidase use a metal ion in the catalytic mechanism
Cysteine type protease	3, 4, 18	Carboxypeptidase have a cysteine in the active centre
Omega peptidases	3, 4, 19	Remove terminal residues that are linked by isopeptide bonds
Endopeptidases	3, 4, 21-24	Cleave internal bonds in polypeptide chains
Serine protease	3, 4, 21	Endopeptidases have an active centre serine involved in the catalytic process
Cysteine protease	3, 4, 22	Possesses a cysteine in the active centre
Aspartic protease	3, 4, 23	An aspartic acid residue for their catalytic activity
Metalloprotease	3, 4, 24	Use a metal ion (often, but not always, Zn ²⁺) in the catalytic mechanism
Endopeptidases of unknown catalytic mechanism	3, 4, 99	Acting on peptide bonds

3. PHYLOGENETIC TREE

Based on their amino acid sequences, proteases (peptidases) are classified into different clans and families, which have diverged from a common ancestor [5]. Each peptidase has been assigned a code letter denoting the type of catalysis, *i.e.*, S, C, A, M, or U for serine, cysteine, aspartic, metallo-, or unknown type, respectively (Figure 1).

4. SOURCES OF MAJOR PROTEASE

Animal Proteases: The most familiar proteases of animal origin are pancreatic trypsin, chymotrypsin, pepsin and rennin. Trypsin is the main intestinal digestive enzyme responsible for the hydrolysis of food proteins. Chymotrypsin is found prepared from the pancreatic extracts of animals. Pure chymotrypsin is an expensive enzyme, which is used only in diagnostic and analytical applications. Pepsin is an acidic protease that is found in the stomach of almost all vertebrates [3,6]. Pepsin had been used in laundry detergents as early as 1913, which is now being replaced by a mixture of serine and metal microbial proteases, which appears to be less degradable

by detergents, alkaline conditions and high temperatures [7]. Rennet is a pepsin-like protease that is produced as an inactive precursor in the stomach of all nursing mammals. It is converted to active rennin by the action of pepsin. It is being used extensively in the dairy industry to produce stable curd with good flavor [3].

Plant Proteases: Papain, bromelain, keratinases, and ficin are some of the well-known proteases of plant origin, however, their production from plant sources is a time consuming process. Papain is a traditional plant protease with a long history of use especially in tonics, which is active between pH 5 and 9 [8]. It is extracted from the latex of *Carica papaya* fruits. Bromelain is prepared from the stem and juice of pineapples [9]. But the problem associated with the production of plant proteases lies in the selection of suitable climatic areas for cultivation. As the concentration of enzyme in plant tissue is generally low, processing of large amounts of plant material is necessary.

Microbial proteases: Although protease-producing microorganisms, plants and animals have cosmopolitan distribution in nature; microbial community is preferred over the others for the large scale production of proteases

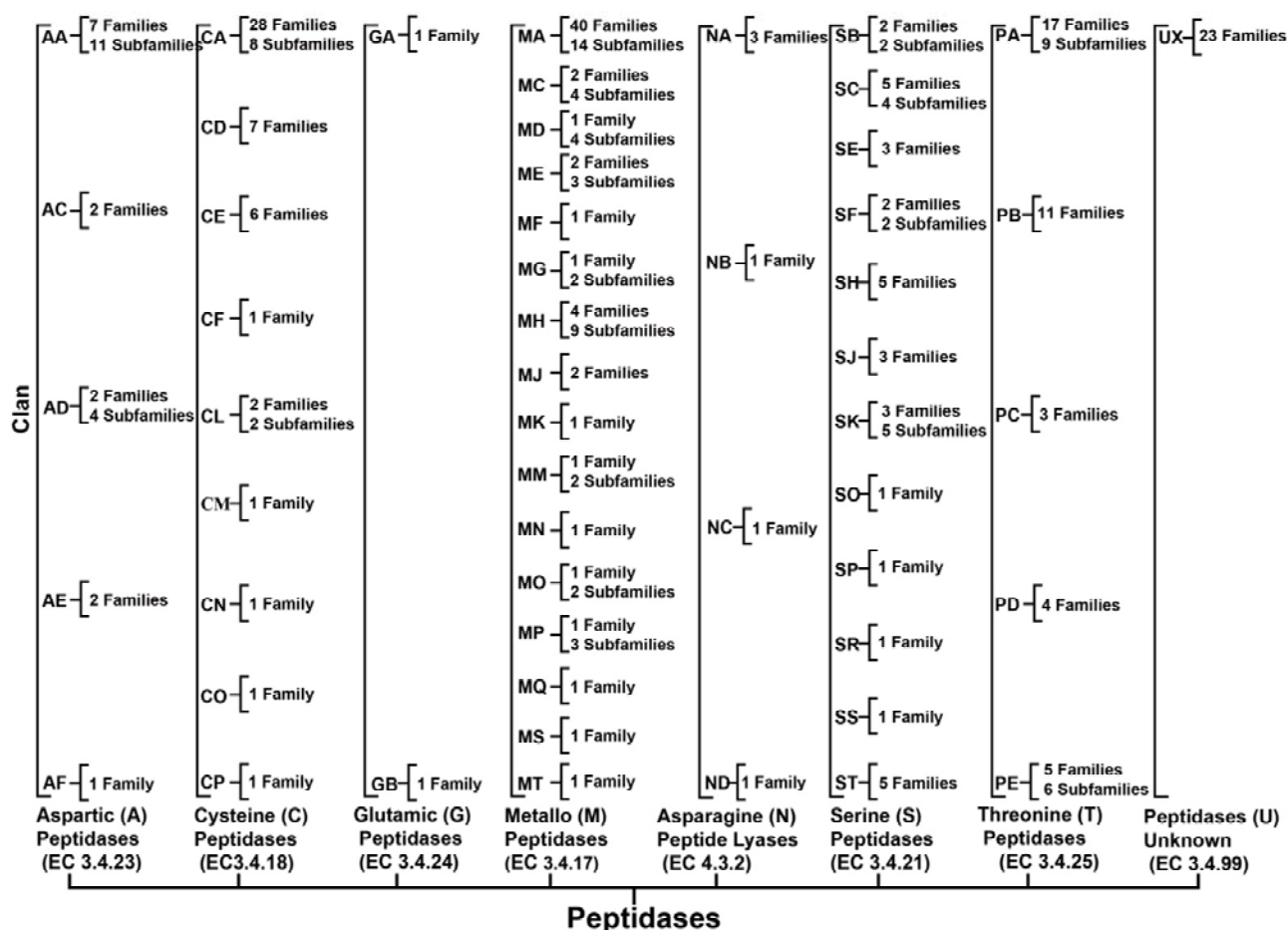


Figure 1. Classification of proteases (peptidases), depending on their amino acid sequences and evolutionary relationships.

due to their fast growth and simplicity of life for the generation of new recombinant enzymes with desired properties. Microorganisms account for a two-third share of commercial protease production in the enzyme market across the world [10]. 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 [4], of which proteases account for about 60% of the total sales [3,11].

Proteins are degraded by microorganisms, and they utilize the degradation products as nutrients for their subsistence. Degradation is initiated by proteinases (endopeptidases) secreted by microorganisms followed by further hydrolysis by peptidases (exopeptidases) at the extra- or intra-cellular locations. A variety of proteases are produced by microorganisms depending on the species of the producer or the strains, even belonging to the same species. Neutral and alkaline proteases hold great potential for application in the detergent and leather tanning industries due to the increasing trend in developing environment-friendly technologies [3]. Alkaline proteases have numerous applications in the food industries,

silver recovery from X-ray films and several bioremediation processes. There are two types of secreted proteases-intracellular and extra cellular. Intracellular proteases are vital to sustain various cellular and metabolic processes, such as, sporulation and cell differentiation, protein turn over, enzyme maturation and hormones and also in protoxin activation of *Bt*-based biopesticides. Extracellular proteases carry out protein hydrolysis in fermented media and enable the cell to absorb and utilize hydrolytic products [12]. Alkaline serine proteases are the most dominant group of proteases produced by bacteria, fungi, yeast and actinomycetes.

Fungal proteases: Fungal proteases magnetized the interest of researches due to high diversity, broad substrate specificity, and stability under extreme conditions; it offers an advantage of separation of mycelium by simple filtration. Fungal proteases can conveniently be produced in solid-state fermentation process. Fungal proteases are also used in for modifying food proteins. The different alkaline proteases producing fungal species are included in **Table 2**.

Bacterial proteases: Bacterial alkaline proteases have more commercial importance in laundry, food, leather

and silk industries due to their high production capacity and catalytic activity. Bacterial alkaline proteases are characterized by their high activity at alkaline pH (8 - 12), with optimal temperature between 50°C and 70°C. These properties of bacterial alkaline proteases make them suitable for use in the detergent industry. Prominent bacteria producing proteases are displayed in the **Table 3**.

Alkaline proteases from *Bacillus* spp.: Alkaline proteases are of considerable interest in view of their activity and stability at alkaline pH. Of all the alkalophilic microorganisms, members of the genus *Bacillus* were found to be predominant and a prolific source of alkaline proteases (**Table 4**). Alkaline proteases are a physiologically and commercially important group of enzymes used primarily as detergent additives. They play a specific catalytic role in the hydrolysis of proteins. Alkaline protease from *Bacillus* species RGR-14 shows silk degumming efficiency [76]. *B. firmus* MTCC7728 produces extracellular alkaline protease, with great potential in various industries, and several processes like silver recovery, bioremediation and protein hydrolysate production [77]. Three intracellular proteases were identified from sporulated culture of *Bacillus thuringiensis*-subsp. *tenebrionis* by fractionation with ammonium sulfate; of these, one with 81 kDa was identified as metalloprotease hav

Table 2. Major fungi producing alkaline proteases.

Fungus	References
<i>Aspergillus candidus</i>	[13]
<i>A. flavus</i>	[14]
<i>A. fumigatus</i>	[15,16]
<i>A. melleus</i>	[17]
<i>A. niger</i>	[18,19]
<i>A. oryzae</i>	[20-22]
<i>A. sojae</i>	[23]
<i>A. sydowi</i>	[24]
<i>Cephalosporium</i> sp. KSM 388	[25]
<i>Chrysosporiumkeratinophilum</i>	[26]
<i>Conidioboluscoronatus</i>	[27]
<i>Entomophthoracoronata</i>	[28]
<i>Fusariumemartii</i>	[29]
<i>Paecilomyces lilacinus</i>	[30]
<i>Scedosporium apiospermum</i>	[31]
<i>Tritirachium album</i> Limber	[32-34]
<i>Rhizopusoligosporus</i>	[35]

Table 3. Major bacteria producing proteases.

Organism	References
<i>Alteromonas</i> sp.	[36]
<i>Arthrobacterprotophormiae</i>	[37]
<i>Brevibacterium linens</i>	[38,39]
<i>Hyphomonasjannaschiana</i> VP 3	[40]
<i>Lactobacillus helveticus</i>	[41]
<i>Malbrancheapulchella</i> var. <i>sulfurea</i>	[42]
<i>Microbacterium</i> sp.	[43]
<i>Nocardiopepsidassonvillei</i>	[44,45]
<i>Oerskoviaxanthineolytica</i> TK-1	[46]
<i>Pimelobacter</i> sp. 2483	[47]
<i>Pseudomonas aeruginosa</i>	[48]
<i>Pseudomonas maltophilia</i>	[49]
<i>Pseudomonas</i> sp. SJ320	[50]
<i>Salinivibrio</i> sp. Strain AF-2004	[51]
<i>Staphylothermusmarinus</i>	[52]
<i>Streptomyces</i> isolate EGS-5	[53]
<i>Streptomyces microflavus</i>	[54]
<i>Streptomyces moderatus</i>	[55]
<i>Streptomyces rectus</i>	[56]
<i>Streptomyces rectus</i> var. <i>proteolyticus</i>	[57]
<i>Streptomyces rimosus</i>	[58]
<i>Streptomyces</i> sp. YSA-130	[59,60]
<i>Thermoactinomyces</i> sp.	[61,62]
<i>Thermoactinomyces</i> sp. THM1	[63]
<i>Thermobacteroidesproteolyticus</i>	[64]
<i>Thermococcusceler</i> , <i>T. stetteri</i> , <i>T. litoralis</i>	[65]
<i>Thermomonosporafusca</i>	[66,67]
<i>Thermusaquaticus</i> YT-1	[68]
<i>Thermussp.</i> strain Rt41A	[69]
<i>Torulathermophila</i>	[70]
<i>Vibrio alginolyticus</i>	[71-73]
<i>Vibrio metschnikovii</i> RH 530	[74]
<i>Xanthomonasmaltophila</i>	[75]

ing major proteolytic activity at 60°C. *B. thuringiensis* H14 in aqueous two phase system—composing of PEG X (X = 9000, 6000, 4000) and potassium phosphate—was able to produce an alkaline protease [78]. The beha-

Table 4. Alkaline protease-producing *Bacillus* species.

<i>Bacillus</i> spp. and their strains	References
<i>Bacillus alcalophilus</i> ATCC 21522	[88]
<i>B. alcalophilus</i> subsp. <i>halodurans</i> KP1239	[89]
<i>B. amyloliquefaciens</i>	[90,91]
<i>B. amyloliquefaciens</i> S94	[92]
<i>B. cereus</i> strain CA15	[93]
<i>B. circulans</i>	[94,77]
<i>B. coagulans</i> PB-77	[95]
<i>B. firmus</i>	[96,97]
<i>B. intermedius</i>	[98]
<i>B. lentus</i>	[99]
<i>B. licheniformis</i>	[100-102]
<i>B. licheniformis</i> UV-9 Mutant	[103]
<i>B. megaterium</i>	[104]
<i>B. proteolyticus</i>	[105]
<i>B. pumilus</i>	[106,107]
<i>B. pumilus</i> CBS	[108]
<i>B. sphaericus</i>	[109]
<i>B. subtilis</i>	[85]
<i>B. subtilis</i> var. <i>amylosacchariticus</i>	[110]
<i>B. subtilis</i> DKMNR	[111]
<i>Bacillus</i> sp. Ya-B	[112]
<i>Bacillus</i> sp. NKS-21	[113]
<i>Bacillus</i> sp. B21-2 [42]	[114]
<i>Bacillus</i> sp. Y	[115]
<i>Bacillus</i> sp. CW-1121	[116]
<i>Bacillus</i> sp. KSM-K16	[117]
<i>B. thermoruber</i> BT2T	[118]
<i>B. stearothermophilus</i>	[119,13]
<i>Bacillus</i> sp. B001	[4]

viour of the synthesis of intracellular protease was studied by gelatin zymography in *B. thuringiensis* (*Btk*) strains HD1, *Btk* HD73 [79]. Alkaline protease was purified and characterized from a mutant of *B. polymyxa* [80]. Several proteases may be produced by the same strain under various culture conditions.

Alkaline proteases are generally produced by submerged fermentation (SmF). In addition, solid-state fermentation (SSF) processes have been exploited to a

lesser extent for the production of these enzymes [81,82]. Research efforts have been directed mainly toward the evaluation of the effects of various carbon and nitrogenous nutrients as cost-effective substrates on the yield of enzymes, requirement of divalent metal ions in the fermentation medium and optimization of environmental and fermentation parameters such as pH, temperature, aeration and agitation. In addition, no defined medium has been established for the best production of alkaline proteases from different microbial sources. Each organism or strain has its own special conditions for maximum enzyme yield. Production of an enzyme exhibits a characteristic relationship with regard to the growth phase of that organism. The synthesis of protease in *Bacillus* species is controlled by numerous complex mechanisms operative during the transition state between exponential growth and the stationary phases [83]. The extracellular enzyme production pattern is varied with the *Bacillus* strains. There is a little or no enzyme production occurs during the exponential growth phase [84]. However, in the case of *B. subtilis* ATCC strain 14416 [85] and *B. sphaericus* strain BSE 18 [86], enzyme production was growth-associated and it occurs at the mid-exponential phase, and often a rapid auto deactivation process was observed after the culture reached the maximum enzyme activity. During alkaline protease production, it was also observed that the pH of the fermented medium dropped from alkaline to acidic; for instance, from pH 10.1 to 8.5 in the case of an alkalophilic *Bacillus* strain Ya-B [87].

***Bacillus thuringiensis* proteases:** *Bacillus* is a gram +ve bacterium and is widely distributed in nature. *Bacillus* spp., are important industrial tools for a variety of reasons, including their capacity to secrete proteins in to the extra-cellular media and their GRAS (generally regarded as safe) status with the food and drug administration [120]. This genus includes a variety of commercially important species, responsible for the production of a range of products including enzymes, fine biochemical like antibodies and insecticides. Most species are harmless to humans and animals and only a few pathogens are known. *B. thuringiensis* (*Bt*), one of the most widely studied bacterium produces a potent insecticidal protein, which makes it a successful biopesticide. *Bt* is also an excellent source of proteases; *israelensis*, *kurstaki* and *tenebrionis* are the major sub-species of *Bt* (with many strains) capable of producing different proteases [78, 121-127].

5. GENERAL PROPERTIES OF ALKALINE PROTEASES

Alkaline proteases useful for detergent applications were mostly active in the pH range 8 - 12 and at temperatures between 50°C - 70°C [128]. The optimum pH range of alkaline proteases is generally between pH 9

and 11, with a few exceptions exhibiting higher pH optima, up to a pH range 12 - 13. The optimum temperature of alkaline proteases ranges from 50°C to 70°C. Interestingly, the enzyme from an alkalophilic *Bacillus* sp. B189 showed an exceptionally high optimum temperature of 85°C. Alkaline proteases from *Bacillus* sp., *Streptomyces* sp. and *Thermus* sp. are quite stable at high temperatures, and the addition of CaCl₂ further enhances enzyme thermostability [129]. In general, alkaline proteases require metal ions for their maximum activity. The most commonly used metal ions are Ca²⁺, Mg²⁺ and Mn²⁺. Ca²⁺ ion is also known to play a major role in enzyme stabilization by increasing the activity and thermal stability of alkaline proteases at higher temperatures [130, 62]. Other metal ions such as Ba²⁺, Mn²⁺, Mg²⁺, Co²⁺, Fe³⁺ and Zn²⁺ are also used for stabilizing proteases [39]. These metal ions protect the enzyme against thermal denaturation and play a vital role in maintaining the active confirmation of the enzyme at higher temperatures. Presence of Ca²⁺ is known to activate proteases by increasing thermostability [131,132]. Metal ions like Hg²⁺; Cu²⁺, Ag²⁺, Fe²⁺ and Zn were found inhibitory to majority of proteases [133,134].

6. MOLECULAR MASSES OF PROTEASES FROM *BACILLUS* SPP

Alkaline proteases have different ranges of molecular masses such as 45 kDa, 36 kDa for the proteases from the wild strains and 40 kDa for the standard *B. subtilis*

ATCC 6633 strain [135; 30 - 33 kDa [29], 40 kDa [136] are other predominant proteases from *Bacillus*. In general, molecular mass of protease is ranged between 15 and 45 kDa [10,137]. In some *Bacillus* sp., multiple electrophoretic forms of alkaline proteases were observed. The multiple forms of these enzymes may be due to the non-enzymatic, irreversible deamination of glutamine or asparagine residues in the protein molecules, or of autoproteolysis. **Table 5** gives a summary of the molecular masses characterized from various species of *Bacillus*.

7. OVERVIEW ON THE INDUSTRIAL APPLICATIONS OF PROTEASES

Proteases have a large variety of applications, mainly in the detergents, leather processing, metal recovery, medical purposes, food processing, feeds, and chemical industries, as well as in waste treatment (**Table 6**).

Detergent additives: The history of detergent enzymes dates back to 1914, when two German scientists, Rohm and Haas used pancreatic proteases and sodium carbonate in washing detergents. The product was named Burnus. The first detergent containing the bacterial enzyme was introduced into the market in 1956 under the trade name Bio-40. An alkaline protease, alcalase, was

Table 5. Molecular masses of proteases characterized from *Bacillus* spp.

Source	Molecular Weight (kDa)	Reference
<i>Bacillus</i> sp. No. AH-101	30	[138]
<i>B. pumilus</i> MK6-5	28	[133]
<i>B. pumilus</i> UN-31-C-42	32	[139]
<i>B. stearothermophilus</i> F1	33.5	[119]
<i>Bacillus licheniformis</i> MIR29	25/40	[140]
<i>Bacillus</i> sp. NKS-21	30	[141]
<i>Bacillus</i> sp. SSR1	29, 35	[142]
<i>Bacillus</i> sp. GX6638	36	[143]
<i>Bacillus pseudofirmus</i> AL-89	24	[144]
<i>Bacillus</i> sp. B18	28, 30	[145]

effectively incorporated in detergent powder and was marketed by Novo Industry, Denmark under the trade name Biotex in 1963. Today, detergent enzymes account for 89% of the total protease sales in the world; and a significant share of the market is captured by subtilisins and alkaline proteases from many *Bacillus* species [17, 32,74].

Leather tanning: Leather processing involves several steps such as soaking, dehairing, bating, and tanning. The conventional methods of leather processing involve hazardous chemicals such as sodium sulfide, which create problems of pollution and effluent disposal. The use of enzymes as alternatives to chemicals has proved successful in improving leather quality and in reducing environmental pollution. Proteases are used for selective hydrolysis of non-collagenous constituents of the skin and for removal of non-fibrillar proteins such as albumins and globulins; at present, alkaline proteases with hydrated lime and sodium chloride are used for de-hairing, which resulted in a significant reduction in the amount of waste water generated. In addition, studies carried out by different workers have demonstrated the successful use of alkaline proteases in leather tanning from *Aspergillus flavus*, *Streptomyces* sp., *B. amyloliquifaciens* and *B. subtilis* [8].

Silver recovery: Alkaline proteases are used in silver recovery from used X-ray films. Used X-ray film contains approximately 1.5% to 2.0% (by weight) silver in its gelatin layers. The silver recovery by burning film causes a major environmental pollution problem; hence the enzymatic hydrolysis of the gelatin layers on the X-ray film enables the recycling of both silver and polyester film base [75].

Food industry: Alkaline proteases have been routinely used for various purposes such as cheese making,

Table 6. Common protease products from *Bacillus* spp. available in the market.

Organism	Trade names	Manufacturer
<i>Bacillus licheniformis</i>	Alcalase	Novo Nordisk, Denmark
Alkalophilic <i>Bacillus</i> sp.	Savinase, Esperase	Novo, Nordisk, Denmark
Alkalophilic <i>Bacillus</i> sp.	Maxacal, Maxatase	Gist-brocades, The Netherlands
Alkalophilic <i>Bacillus</i> sp.	Opticlean, Optimase	Solvay Enzymes GmbH, Germany
Alkalophilic <i>Bacillus</i> sp.	Proleather	Amano Pharmaceuticals Ltd., Japan
<i>Aspergillus</i> sp.	Protease P	Amano Pharmaceuticals Ltd., Japan
<i>B. amyloliquefaciens</i> (savinase)	Durazym	Novo Nordisk, Denmark
Alkalophilic <i>Bacillus</i> sp.	Maxapem	Solvay Enzymes GmbH, Germany
Variant of <i>B. lentus</i>	Purafect	Genencor International, Inc

baking, preparation of soya hydrolysates, and meat tenderization [140]. Proteases are invariably used in tonics, especially for indigestion.

Waste treatment: Dalev in 1994 [146] reported an enzymatic process using a *B. subtilis* alkaline protease in the processing of waste feathers from poultry slaughterhouses. Feathers constitute approximately 5% of the body weight of poultry and can be considered as a high protein source for food and feed, provided their rigid keratin structure is completely destroyed. Pretreatment with NaOH, mechanical disintegration, and enzymatic hydrolysis resulted in total solubilization of the feathers. The ended product was a heavy, grayish powder with a very high protein content which could be used as a feed additive. Similarly, many other keratinolytic alkaline proteases were used in feed technology for the production of amino acids.

Other uses: Besides their industrial and medicinal applications, proteases play an important role in basic research. Their selective peptide bond cleavage is used in the elucidation of structure-function relationship, in the synthesis of peptides, and in the sequencing of proteins. *B. thuringiensis* is used for the integrated pest management in forestry. The derivatives of *Bt* strain HD1 subsp. *kurstaki* have widely been used to control the forest pests such as the gypsy moth (*Lymantria dispar*), spruce budworm (*Choristoneura fumiferana*), the pine processionary moth (*Thaumetopoea pityocampa*), the European pine shoot moth (*Rhyacionia buoliana*) and the nun moth (*Lymantria monacha*) [147].

8. CONCLUSIONS

Though this review gives a glimpse into the proteases, it mainly focused on the general aspects of proteases giving special emphasis on to the proteases from *Bacillus* spp., especially of alkaline proteases. 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. 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.

9. PROSPECTS

Heterogeneity of proteases is its uniqueness, which odds it out from its counterparts; of course, which makes them versatile biocatalyst too. Nevertheless, its full potential has not yet been tapped. In fact, its prospects in waste management are underutilized, especially in urban settings. The engineering of proteases for novel or combined catalytic abilities with long half-life seems to be a less addressed area. Protease-based industry looks forward to receiving engineered fusion proteases with multiple activities combined in one. Thus, the ever-growing protease market demands efficient and fast-acting proteases at cheaper price.

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