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Title: Microbial protease: a hallmark enzyme for industrial and commercial applications

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LIST OF ABBREVIATIONS

GMO.....	Genetically modified organism
rDNA.....	Recombinant DNA
GE.....	Genetic engineering
JECFA.....	Joint Expert Committee on Food Additives
FAO.....	Food and Agricultural Organization
WHO.....	World Health Organization
FDA.....	Food and Drug Administration
GRAS.....	Generally recognized as safe

ABSTRACT

Proteases are associated with a wide microbial community and relevant for biocatalysts that constitute the largest group of enzymes acting as proteinases, peptidases, and amidases with a dozen range of industrial applications. The template of these reviews, specific attention has been given to comprehensive profoundly the proteases. In aspect of industries the use of chemical around the globe have had increased tremendously and affecting the health of people. Besides to the impact the intention of modern world to replace these noxious chemicals bring to environmental friendly products for the betterment of life on the planet. Hence, improving enzymatic processes in spite of chemical processes has been initial objective of scientists. A dozen enzymes, particularly microbial proteases, are the most crucially used in different corporate sectors, like textile, detergent, leather, feed, and waste. These enzymes with respect to physiological and commercial roles hold a pivotal position. From the different producers of proteases, Bacillus sp. is predominating commercially available of microbial proteases. Proteases are safe and good considered as an alternative to chemicals and an eco-friendly indicator for nature or the surrounding in the globe. Due to the reasoned, the researchers are looking for microbial proteases, it can tolerate harsh conditions, ways to prevent autoproteolytic activity, stability in optimum pH, and substrate specificity. These review profoundly on the current issues faced during production and application at the industrial level. Besides from the issues would enable us to promote microbial proteases source bring to economically and commercially around the world.

Key words: screening of strain, cloning, silk degumming, immobilization

1. INTRODUCTION

Proteases or peptidases are crucial and essential constituents of all form of life on earth including prokaryotes, fungi, plants and animals. Proteases are degradative enzymes and show specificity and selectivity in protein modification (Rao *et al.*, 1998). It is a powerful tool in cause of modification of the properties of proteins and proteic systems applying proteolysis process ideal in food processes, including changes insolubility, gelation, emulsifying and foaming characteristics in a specific product (Singh *al et al.*, 2012) In the industrial sector, *Bacillus* sp. are the most active and dynamic extracellular alkaline protease producer. By a process of fermentation, proteases can be isolated and purified in a relatively shorter period of time, exhibiting high substrate specificity and catalytic activity (Kumar and Takagi, 1999; Rifaat *et al.*, 2007; Singhal *et al.*, 2012). However, proteolytic enzymes from the sources of microbial are safe and good us much as preferred over the enzymes derived from plant and animals since they possess almost all characteristics desired for their biotechnological applications (Balakrishnan, P. *et al.*, Rajendran, N. and Flanet, R. 2012).

Currently the world energy demand, industrialization for considering ever-growing, rapidly depleting fossil-fuel reserves, environmental health issues, there is emphasis on development of sustainable industrial technologies that are environmentally safe and involve reduce emissions (Jegannathan and Nielsen., 2013; Vaid and Bajaj., 2017). As a revealed result there is a paradigm shift from traditional concepts of chemical-based production and manufacturing to eco-benign processes that are equally efficient and economic (Sarrouh *et al.* 2012; Nargotra *et al.* 2016). According to the scientific evidence, proteases control the activation, synthesis, and turnover of proteins to regulate physiological processes (Rawlings *et al.*, 2004). Different physiological processes, such as formation, birth, aging, and even death are regulated by proteases (Chou *et al.*, 1997, 2000, 2003; Chou and Howe, 2002; Chou, 2004, 2006). The constitute of Proteases 60% of the global industrial market is based on sustainable industrial for due to their dozen application and positive impacts in diverse industries, like; used in pharmaceutical industry (Kumar *et al.* 2015), food industry for peptide synthesis (Kumar and Bhalla., 2005), leather industry for dehairing (Pillai *et al.* 2011; Singh and Bajaj., 2017), photographic industry for silver recovery (Joshi and Satyanarayana., 2013), detergent industry as an additive for detergent formulation (Giri *et al.*, 2011) and in processing of keratin residues (Harde *et al.*, 2011).

2. Microbial Proteases

The microbial proteases have been successfully produced by researchers from different microbial sources. Two-thirds of microbes account share of commercial protease around the globe (Beg and Gupta., 2003). Since the advent of enzymology, microbial proteolytic proteases have been the most widely studied enzyme. These enzymes have gained interest not only due to their vital role in biological pathway but the revealed research result showed that huge utilization in industries (Rao., *et al.*, 1998; Sandhya *et al.*, 2005; Younes and Rinaudo., 2015). The available of protease enzyme from the market of microbial origin are predominant, due to the reason of their high yield, less time consumption, less space requirement, lofty genetic manipulation, and cost-effectiveness.

According to (Nisha and Divakaran., 2014; Ali *et al.*, 2016,)proteases have made them suitable for biotechnological application in the market, and preferred to plant and animal proteases because of the presence of all desired characteristics for industrial applications (Palsaniya *et al.*,2012; Sathishkumar *et al.*, 2015). It is isolated from these microbial sources have a large number of dilutions in various industrial sectors (Pastor *et al.*,2001; Beg and Gupta., 2003; Das and Prasad., 2010). in contrast, proteases produced by plants and animals are more labor-intensive than microbially produced proteases (Gupta *et al.*, 2002; Kalaiarasi and Sunitha., 2009).

3. Classifications of protease

According to the kinetic characteristics, proteases are classified in Class 3 in the international system for classification and nomenclature of enzymes (EC number) (Webb, 1993)as hydrolases, subclass 3.4 (hydrolysis of peptide bonds) divide proteases between13 sub-classes on the basis of the catalytic reaction. Moreover, MEROPS databases(a peptidase database), based on the principal its tertiary structure and catalytic sites, classified proteases in clans, The group names take into account the iconic amino acid or metal present in the active site: Aspartic peptidases(A), Cysteine peptidases (C), Metallo peptidases (M), Serine peptidases (S), Mixed catalytic type (P) and Unknown type (U) (Rawlings and Barrett, 1993; Rawlings *et al.*, 2008; Rawlings *et al.*, 2012; Polgár, 2005). The specificity of a protease determines the position at which the enzyme will catalyze the peptide bond hydrolysis from the following table.

Table 1.Examples of protease activities

Protease	Microorganism	Preferential Cleavage Site
Subtilisin(EC3.4.21.62)	<i>Bacillus amyloliquefaciens</i>	Preference for a large uncharged residue in P1
Subtilisin(EC3.4.21.62)	<i>Bacillus thermoproteolyticus</i>	Preferably -/-Leu> -/-Phe
Protease from <i>Aspergillusoryzae</i>	Non-toxicogenic and nonpathogenic strains of <i>Aspergillus oryzae</i>	Hydrolysis at the N-terminal, serine and aspartic acid bonds

3.1. Alkaline Proteas

The genus *Bacillus* is vital for commercially important alkaline protease (EC.3.4.21-24.99), which is active at alkaline pH ranging between 9 and 11 (Varela *et al.*, 1997; Kocher and Mishra, 2009; Singhal *et al.*, 2012). Its producers are distributed in water, soil, and highly alkaline conditions. From a variety of sources, such as detergent contamination (Hsiao *et al.*, 1994; Singh *et al.*, 1999), dried fish (Centeno *et al.*, 1996), sand soil, and slaughterhouses, segregation of alkaline proteases has been stated (Adinarayana *et al.*, 2003). The detergent industry consumes alkaline proteases most abundantly, which are serine proteases with an alkaline pH range (Gupta *et al.*, 2002).

Table 2.Summary of paper the availability of commercial microbial alkaline proteases (subtilisins) used in the detergent industry

Industrial manufacturer	Product trade name	Enzyme properties (pH; temperature)	Microbial source
Novozymes (previously Novo Nordisk), Denmark	Alcalase	8–9; 60 °C	<i>Bacillus licheniformis</i>
	Savinase	9–11; 55 °C	<i>Bacillus clausii</i>
	Esperase	9–11; 60 °C	<i>Bacillus halodurans</i>
	Durazyma	10–10.5; 55 °C	<i>Bacillus</i> sp.
	Polarzym	9–11, 20–40 °C	<i>Bacillus</i> sp.

3.2. Acidic Protease

The enzyme are exploited for use in clearing beer and fruit juice, enhancing texture of flour paste, and tenderizing the fibril muscle (Zhang *et al.*, 2010). In contrast with alkaline proteases, these extracellular acid proteases are mostly produced by fungal species, such as *Aspergillus Niger* (Sieleckiet *et al.*, 1991), *Aspergillusoryzae* (Yongquan., 2001), *Aspergillus awamori* (Ottesen and Rickert., 1970), *Aspergillusfumigatus* (Shinmyo *et al.*, 1972), and *Aspergillussaitoi* (Sodek and Hofmann., 1970).

3.3. Neutral Proteases

Weakly acidic or weakly alkaline pH of neutral proteases belongs to the genus *Bacillus* and with a relatively low thermos tolerance ranging from pH 5 to 8. Neutrased is associated in the brewing industry due to its insensitivity to plant proteinase inhibitors. On the basis of high affinity toward hydrophobic amino acids, neutral proteases are identified and characterized. A divalent metal ion is required for the activity of neutral proteases belonging to the metalloprotease type (Barrett., 1995;Woessneret *al.*, 2000;Chavan and Patil., 2007).

4. Sources of Proteases

Due to proteolytic processes are intimacy skill to associate with vital biological aspects, is not difficult to predict the importance and enormous range of structures and specificities of proteases with a very wide repertory of functions. Therefore, it can usually recover from animal, plant or microbial sources and even the most primitive organism. So, Microorganism proteases sources are especially interesting for many reasons. The path to culture on large scale over a short time-process of fermentation(Devasena.,2010).However, production of plant proteases, such as bromelain, keratinases, and ficin, is time-consuming (Rani *et al.*, 2012). The production of proteases from animal sources is insufficient to fulfill the industrial demand worldwide; therefore, scientists have extended their research of producing protease from bacterial sources. Owing to the broad-spectrum biochemical variety and easy genetic manipulation, microbes produce an exceptionally promising number of proteases (Godfrey and West, 1996a; *Kuhad et al.*, 2011).

Table 3. Microbial protease preparations used in food processing following general specifications of Joint FAO/WHO Expert Committee on Food Additives (JECFA)

Enzyme Preparations	Action	Sources	Applications in Food Processing
Acid protease from <i>Bacillus cereus</i>(Rennet from <i>Bacillus cereus</i>	Aspartic proteinase preparation (EC 3.4.23.6), which hydrolyze polypeptides such as casein.	Produced by controlled fermentation of <i>Bacillus cereus</i> .	Used in cheese making.
Aspartyl protease* (Chymosin A from <i>Escherichia coli</i> K-12 containing the Prochymosin A gene)	Chymosin (EC 3.4.23.4) which cleaves a single bond in kappa-casein	Produced intracellularly by the controlled fermentation of <i>Escherichia coli</i> K-12 containing the bovine Prochymosin A gene.	Used in clotting of milk cheese production.

Source :Adapted from online database for food additives evaluated by JECFA, accessible free at <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa-additives/en/>

5. Microbial Proteases and Industry

The recent research interested for studying the proteases has increased not only due to the regulation of different biological and biological pathway but also due to the significant use in industrial community. The microbes producing substantial numbers of extracellular proteases are of great importance for the industry, and few products of alkaline protease are successfully marketed (Gupta *et al.*, 2002; Gupta and Ramnani., 2006; Vijayaraghavan *et al.*, 2014).

5.1 As Enzyme for food application

The importance of the enzyme is to consider that different processes of food modification can use the isolation and identification of enzyme or even the cell itself, which does not always control the effect, but has the advantage to rely on the effect of the mixture of enzymes released by the microorganism. For example, in cheese proteolysis is not limited to the action of added enzymes, but also to the enzymes present from microorganisms (Souza *et al.*, 2001; Seratlic *et al.*, 2011).

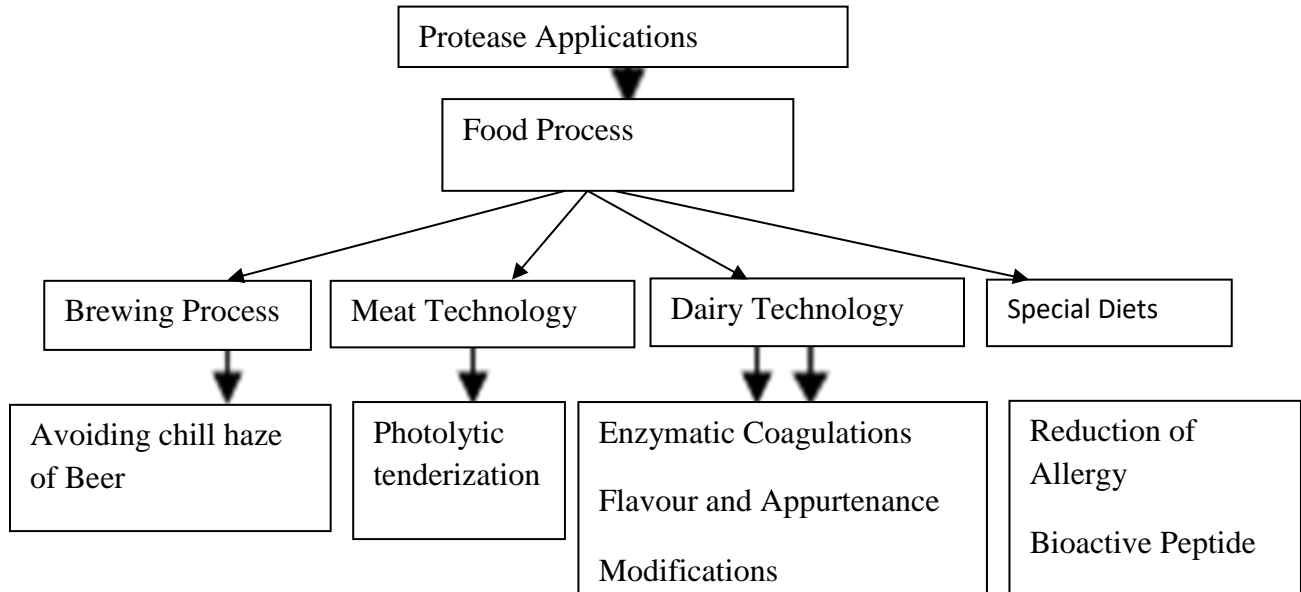


Figure 1.Some protease applications in food processes.

5.2. As food additives

Due to FAO/WHO Expert Committee on food Additives (JECFA) is administered jointly by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). The major item the identification of enzyme to evaluation and preparations applied to food processes is the safety assessment of the production strain do not point of view of their specificity and/or stability, but in the case of food processing it has to be safe for health. The Joint It has been meeting since 1956, initially to evaluate the good of food additives. As mention the general specifications and Considerations for enzyme Preparations used in food Processing.

5.3. Waste Management

The development and sustainable of environmental are good alternative ecofriendly way for treatment of waste management. However, the use of chemicals in industries is detrimental to the environment and the surroundings. Poultry waste can be degraded into feed and food by the keratinolytic process (Neklyudov *et al.*, 2000;Lasekanet *al.*, 2013). For depilation and cleaning of hairs from drains and clogged pipes, a formulation containing hydrolytic enzymes isolated from *B. subtilis*, *B. amyloliquefaciens*, and *Streptomyces sp.* has been prepared and patented as Genex (Blanch and Moo-Young., 1985; Drew *et al.*, 1985; Ichida *et al.*, 2001; Lasekan *et al.*, 2013).

5.4. Leather Industry

The application of alkaline protease is essential for leather industries is due to the elasto lytic and keratinolytic activity. These influential properties of alkaline protease are very effective in leather processing industries. The effective of these is relevant in soaking, bating, and dehairing phase of preparing skin and hides. Microbial alkaline proteases have become very popular in leather industries (Takami *et al.*, 1992; Brandelli *et al.*, 2010).

5.5. Detergent Industry

Proteases have been widely used at commercial scale in the detergent industry. There are a few parameters, such as compatibility with surfactants, bleaches and perfumes (De Virgilio *et al.*, 1993; Bayouhd *et al.*, 2000), good activity, optimum pH, and temperature (Aehle *et al.*, 1993; Bechet *al.*, 1993; Kumar *et al.*, 1998; Gupta *et al.*, 1999), ionic strength, stability and removal potential of stain, which have also been considered for the choice of detergent proteases (*Beg et al.*, 2002; Baş and Boyaci., 2007). Traditionally, detergents work at high temperature but the interest has been increased to search and identify alkaline proteases working in a wide range of temperature (Csuk and Glaenzer., 1991; Breuer *et al.*, 2004). Generally, in the presence of bleaching or oxidizing agent, commercially available proteases are not stable. Recently, rDNA technology has been incorporated to produce bioengineered detergent proteases with greater stability and shelf life. By the use of protein engineering, the replacement of few specific amino acid residues has been studied for bleach and oxidation stability of proteases (Oberoi *et al.*, 2001; Sellami-Kamoun *et al.*, 2008; Haddar *et al.*, 2009b).

Table 4. New proteases (subtilisins) from different microbes with potential for use in the detergent industry

Microbial source	Enzyme properties (pH; temperature)	References
<i>Virgibacillus pantothenicus</i> (MTCC 6729)	10; 40–50 °C	Gupta <i>et al.</i> (2008)
<i>Bacillus licheniformis</i> RP1	10–11; 65–70 °C	Kamoun <i>et al.</i> (2008)
<i>Brevibacillus</i> sp. strain AS-S10-II	12.5; 45 °C	Rai and Mukherjee (2011)
<i>Pseudomonas aeruginosa</i> MCMB 327	8; 35 °C	Zambare <i>et al.</i> (2011)
<i>Termitomyces albuminosus</i>	10.6; 60 °C	Zhenget <i>al.</i> (2011)
<i>Streptomyces fungicidus</i> MML1614	11; 60 °C	Ramesh <i>et al.</i> (2009)
<i>Vibrio metschnikovii</i>	11; 60 °C	Jellouli <i>et al.</i> (2009)
<i>Stentrophomonas maltophilia</i>	10; 20 °C	Kuddus and Ramteke (2009)
<i>Pseudoaltermonas</i> sp. NJ 276	8; 30 °C	Wang <i>et al.</i> (2008)
<i>Alkaliphilustransvaalensis</i>	12.6; 40 °C	Kobayashi <i>et al.</i> (2006)

5.6. Photographic Industry

The revealed research result of Alkaline proteases produced by *B. subtilis*, *Streptomyces avermectinus*, and *Conidiobolus coronatus* have been successfully reported to recover silver from X-ray films, ensuring that the process is eco-friendlier over the use of chemicals (Godfrey and West., 1996b; Wolff *et al.*, 1996; Yang Y. *et al.*, 2000).

5.7. Silk Degumming

Alkaline protease is the best choice to remove sericin while not attacking the fiber. A proteinaceous substance, “sericin or silk gum,” must be removed by the process of degumming from raw silk in an alkaline solution of soap conventionally (Yadav *et al.*, 2011; da Silva *et al.*, 2017; Radha *et al.*, 2017).

5.8. Medical Field

The process of fibrinolytic enzyme recommends for its future application as an anticancer drug and in thrombolytic therapy (Jaouadi *et al.*, 2011, 2012). The hydrolysis of collagen by the enzyme liberates low-molecular-weight peptides without any amino acid release for therapeutic use (Romsomsa *et al.*, 2010; Suwannaphan *et al.*, 2017). For the treatment of various diseases, such as burns, carbuncles, furuncles, and wounds, a preparation of elastoterase immobilized on bandage is used (Davidenko, 1999; Palanivel *et al.*, 2013).

6. Protease and Yield Improvement

The template of protease production from microbial sources to make them high quality and cost-effective, genetic manipulation provides researchers a new opportunity to make changes in bacterial genome using various biotechnological tools to enhance the yield of proteases with desired characteristics. The diversity of the microbial community and tools opens a new path for strain improvement for industrial use as well (Rathakrishnan and Nagarajan., 2012; Aruna *et al.*, 2014). Under extreme conditions, the expression of gene encoding for proteases through using different vector systems including pHY300PLK, pKL9610, pFX1, and plasmid may be maintained and expressed in *Bacillus stearothermophilus*, *B. stearothermophilus*, *E. coli*, and *B. subtilis* (Roja Rani *et al.*, 2012; Kostyleva *et al.*, 2016).

7. Conclusions

Proteases associated with the microbial community and also constitute the dozen groups of enzymes with a broad range of uses in food processes. Be considering the reviews proteolysis as a powerful tool in food protein modifications, including changes in solubility, gelation, emulsifying and foaming characteristics, and also nutritional and bioactive properties. The microorganism associated protease sources are especially interesting for many reasons, such as relatively easy culture on a large scale, enormous range of variety of microorganisms producing many species of enzymes and the possibility of engineering by site-directed mutagenesis. Fungal cultures are particularly involved to protease production. Thus, the study of protease applications to food processes is still a large open Field, asking for more innovative studies and directed at actual application possibilities.

8. Recommendation

In the current study showed profoundly huge amount of protease will be produced from microbial community and researchers will produce proteases with new properties. Among microbial protease source like, alkaline bacterial proteases play a vital role in different industries due to their potential, and their future use is likely to be increased. Researchers will achieve microbial proteases source with essential characteristics, such as yield improvement, changing substrate specificity, enhancement of thermal stability, altering optimum pH, and prevention of auto-proteolytic inactivation.

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