© 2017 IJSRST | Volume 3 | Issue 7 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section: Science and Technology

Biotechnological Perspectives of Microbial Proteases

Mimpal Singh, Beenish Muhammad Maalik, Sehrish Muhammad Maalik, Shumaila Arshad

Institute of Industrial Biotechnology, GC University Lahore, Pakistan

ABSTRACT

The review briefly elucidates the importance of proteases in living organisms and their wide range of potential applications in the vast areas of research and biotechnology. The important features of the proteases are also exploited in a number of ways and can be used to serve various applications in different industries. Apart from their natural potential of performing various important operations in living tissues, various microbial proteases have potential applications in a number of industries. In cellular environment, proteases are involved in the breakdown of the proteins' peptide bonds and transform them into smaller fragments of amino acids and peptides which are prerequisite for the differentiation and cellular growth. Proteases have also vast applications in a range of industrial procedures such as food, pharmaceutical, dairy and detergent. Microbial proteases have dominated roles in the industrial sectors. Microbial proteases are exploited for their characteristic feature of hydrolyzing the protein and the rest of the components of wheat and soy beans in the production of soy sauce. The production of proteases can be enhanced via substantiated fermentation methods. The variation in the composition of growth media such as changes in carbon and nitrogen ratio and some other features affecting microbial growth are significant in the evaluating the fermentation procedures. The production of microbial proteases is advantageous because they can be generated rapidly, their production is cost effective and the manipulation of microbial enzymes is quite easy. Proteolytic enzymes can be produced by either submerged fermentation (SmF) or solid state fermentation (SSF). But the latter is far more advantageous because it direct towards many potential benefits for the protease production. The review mainly focuses on the microbial protease production, their functional and structural aspects and the application of these proteolytic enzymes in different industries.

Keywords: Proteases, Enzyme Production, Microbial Proteases, Industrial Level.

I. INTRODUCTION

Proteases are among the most significant industrial enzymes. These can be synthesized by a variety of microorganisms such as yeasts, bacteria and molds. Some proteases are naturally produced in a number of animals and plant tissues (Walsh & Wilcox, 1970). Proteases are one of the main classes of enzymes that are involved in the hydrolysis of the peptide bonds present in proteins and convert them to smaller molecules of amino acids and peptides. These enzymes are present in almost all organisms; this is the reason why proteases are essential for an organism's growth and cell differentiation. Apart from their important roles in living organisms, the important features of proteolytic enzymes can be exploited and they can be used in a number of other research and biotechnological areas. They have a wide range of potential applications in a number of industrial sectors including pharmaceutical, dairy, food and detergent (De Sauza *et al*., 2015).

On the base of characteristic mechanistic features, these enzymes can be grouped into six classes. These groups are serine, aspartate, glutamate, cysteine, metallo, and threonine (Lopez Otin & Bond, 2008). These characteristics are consistently present in every member of a group. A range of proteases are produced in different microorganisms which can either be extracellular or intracellular. Extracellular proteases are required for hydrolyzing the proteins molecules in extracellular environments. They also enable the cell to absorb or take in and consume hydrolytic products. On the other hand, intracellular proteases are essential for a number of metabolic and cellular mechanisms, like protein turnover, differentiation, and sporulation, maintenance of the cellular protein pool, maturation of enzymes and hormones (Kalisz, 1988).

Proteases of microbial sources are of special filed of interest for a number of reasons. Recently a number of researches are being conducted to study theses microbial proteases extensively. This group of proteases is one of the widest groups that are exploited for their magical properties in a large number of industries and constitutes almost 60%of the overall enzyme sales in the present world (Zambare *et al.*, 2011). Now proteases constitute almost 40% of the overall enzyme purchase in many industries, like laundry detergent, biomedical, food, leather, waste management, diagnostics, and silver recovery. According to an estimate, this percentage of proteases is further ascending by 2005 in different industrial sectors (Godfrey &West, 1996). Proteases also have many applications in biotechnology. Coagulation and ripening are fundamental steps in the cheese making which originate basic organoleptic features and textures, flavor and aroma in the final product as presented Siota *et al.* (2014) as shown in Figure 1.

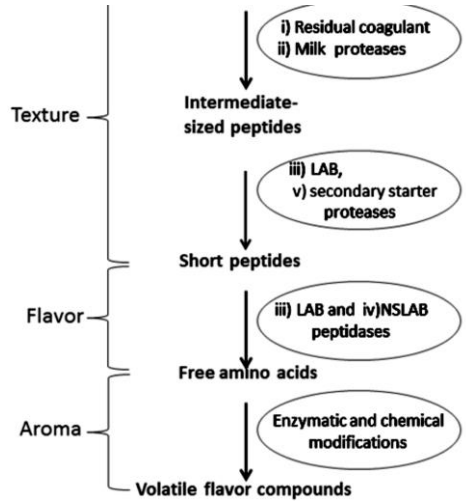

Figure 1. Proteolytic agents in cheese during ripening.

Proteases and peptidases have an important role in both processes (Soita *et al*., 2014). For the formulation of laundry detergents many microbes are being in use including *Bacillus brevis* (Banerjee *et al*., 1999), *Bacillus stearothermophilus* (Dhandapani & Vijayaragvan, 1994), and *Bacillus* sp. SSR1 (Singh *et al.*, 2001). From these strains alkaline proteases can be extracted and used for making detergents. Fungal proteases have attained the attention of environmental biotechnologists due to the unique feature of the fungi that it can be fermented using cheap substrates and releases bulk amount of proteases into the growth medium which could facilitate in down streaming (Anitha & Palanivelu, 2013).

Proteases

Proteases also known as proteolytic enzymes or peptidases, occupies a large class of enzymes. These proteolytic enzymes function in the catalysis of hydrolysis of proteins' peptide bonds. Breaking of peptide bonds direct towards the degradation of protein substrates and transforming them into their component monomers i.e., amino acids. Proteases are classified as peptide hydrolases or peptidases. They constitute a large family of enzymes which are further divided into two groups; endopeptidases and exopeptidases. This division is based on the location of peptide bond to be cleaved. Proteases can also be classified on the basis of their pH ranges. Acidic proteases work efficiently in an acidic environment with the pH range of 2-6, neutral proteases being active at pH range of about 6-8 and alkaline proteases being potent at pH range of 8-13 as described by Sabotic & Kos (2012), Rao *et al.* (1998) and Gupta *et al.* (2002).

The exopeptidase class of proteases is further sub grouped into amino and carboxyl peptidases on the basis of their site of proteolysis at C or N terminus. Amino peptidases break the peptide bond that is present at a free N terminal of the polypeptide and result in the production of either free single monomers or two amino acids joined together forming a dipeptide, or three married to form a tripeptide while the carboxy peptidases function to lyse the peptide bond at C terminus of the chain and produces a free single residue or a dipeptide as described by Rao *et al.* (1998) and shown in Figure 2. Another way of classifying the enzymes is on the basis of the nature of the amino acid residues that is present at the active site of the protease. In this way, the carboxy peptidases can be further be grouped into three chief classes. These groups are metallo peptidases, serine peptidases and cysteine peptidases. The endopeptidases are also divided on the basis of their catalytic mechanism into four subgroups. These subgroups are cysteine proteases, metallo proteases, aspartic proteases and serine proteases (Godfrey &West, 1996).

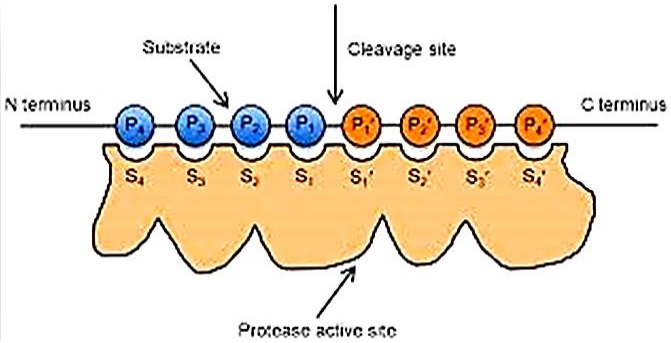

Figure 2. Different exopeptidases acting on N and C terminus.

Proteases are produced in animals, plants and microorganisms. They have important pathological and physiological roles in different processes such as blood coagulation, protein catabolism, cell growth and inflammation, tissue arrangement, tumor growth and metastasis, release of hormones, morphogenesis and transfer of some secretary proteins across cellular membranes (Rao *et al.*, 1998). A very important function of peptidases is the conversion of protein molecules into their constituent monomers for successive absorption into cell membranes to be used in nitrogen metabolism is catalyzed by extracellular proteases (Sabotic & Kos, 2012).

Protease Production

Proteases can be produced in bulk amounts in a limited time by employing a range of fermentation techniques. The proteases, on the other hand, can produce a large continued flux of the product of interest in growth media (Gupta *et al.*, 2002). A wide range of proteases are produced by microorganisms that can either be intracellular and extracellular as mentioned earlier (Gupta *et al.*, 2002). Exoproteases are produced in the complex medium on an industrial level which contains glucose and other expensive substrates. For the good enzyme production important cultivation conditions like temperature, media composition and pH must be controlled in the process development (Abidi *et al.*, 2011). Different organisms like filamentous fungi are used in many industrial procedures for the cultivation of metabolites and enzymes. Use of fungal species for the protease cultivation is very beneficial because material cost is low and production is high and faster. In addition these enzymes can be modified easily and can be removed easily from the media because these enzymes are extracellular (Vishwanatha *et al.*, 2010). Proteases cultivation from fungal sources has more advantages as compared to bacterial proteases because of the ease with which fungal mycelium can be removed by filtration. Further, the use of fungi as a producer is better than that of bacteria because fungi are normally recognized as generally regarded as safe or GRAS (Germano *et al.*, 2003).

Many mechanisms have been explained to elaborate the synthesis and secretion of the extracellular proteolytic enzymes. The protease secretion can be induced by the presence of a substrate. Protease production is normally repressed by the end products like NH^{+4}, amino acids and easily metabolizable origins of the carbon which are produced in large amount. The protease cultivation can be increased in the presence of insufficient levels of nitrogen and sulfur, carbon. That is why the extracellular enzymes can be secreted at low levels in spite of the presence of a substrate (Geisseler & Horwath, 2008).The protease synthesis via microorganisms is usually effected by the quality of nitrogen source. The sources of nitrogen commonly used for the cultivation of proteases are complex nitrogen sources. Different organisms require different supplements of the specific nitrogen (Kucera, 1981). Efficiently usable nitrogen sources like amino acid or ammonium ion concentrations present in the growth media are known to be the main cause of repression in the synthesis of enzyme (Kumar &Takagi, 1999).

Proteases can be produced either by SmF (submerged fermentations) or SSF (solid state fermentations). Either of these techniques have specific advantages. SSF has some benefits over conventional SmF, such as low production cost, requires less energy, space and employing raw substances as substrates. Little problems are associated in the downstream process by using these techniques and the product is more stable due to less dilution in the medium, and it is manufactured with higher productive rate (Sun & Xu, 2009). SmF has benefits in the easy recovery of the fungal mycelia, extracellular enzymes, or spores and process control. Although, the products are dilute and the extracts of the enzymes may be less stable as compared to those from SSF. Major problems in the large scale SSF for microbial growth are the limited amount of water and the heat removal. In SmF, water is present in abundant and variations on oxygen concentration, nutrients and

temperature are very small (Biesebeke *et al*., 2002). Less water used during SmF allows the synthesis of metabolites in a concentrated form and hence making the downstream processing less expensive and time consuming.

Although, the requirements in SSF, especially limited moisture content in growth media, direct towards many potential benefits for the cultivation of enzymes. First of all these conditions help in the production of filamentous fungi, that in nature grow on the solid substrates, like the roots of plants, pieces of wood , leaves and naturally occurring materials. Secondly, the problems with the bacterial contamination during fermentation can be minimized by low moisture content. Finally, the conditions of the solid state fermentation environment can stimulate the microbes to cultivate enzymes with different properties rather than those of the enzymes produced by the similar microbe under the conditions present in the SmF (Germano *et al.,* 2003).

Microbial Proteases

Proteases can be obtained from different organisms e.g. microbes (bacteria and fungi), algae, plants and animals. Particularly, the microbial proteolytic enzymes of the *Aspergillus* species have been studied extensively because of their capacity of secreting high levels of the protease enzymes in their growth environment. Many of these secreted enzymes are cultivated in large scale submerged fermentations and have been used rapidly for decades in the food and beverage industry. Some examples of these species are *Aspergillus flavus* (Macchione *et al.,* 2008; Kranthi *et al.,* 2012), *Aspergillus oryzae* (Yang & Lin, 1998) and *Aspergillus niger* (Ogawa *et al.,* 1995; Vishwanatha *et al.,* 2009; Vishwanatha *et al.,* 2010).

Aspergillus oryzae has been recognized as the non toxigenic strain because of either due to its evolution or its long history of industrial use. It is also considered safe due to more than a thousand years of its use in food fermentation. *Aspergillus oryzae* grows on the solid material surfaces like ground soybean, steamed rice and agricultural byproducts e.g., rice bran, wheat bran and several other substrates. These substrates are initially deficient in the amino acids and sugars (Vishwanatha *et al.,* 2009). Many species of *Aspergillus* have been studied extensively for the cultivation of proteases in the presence of solid state fermentations (SSF) conditions. Alkaline proteases were found to be produced by *A. flavus* and *A. oryzae* in SSF (solid state fermentations) system. *Penicillium* species also have a very good biotechnological potential for the cultivation of proteases and some other enzymes. These contain *Penicillium* species such as *P. citrinum*, *P.camemberti*, *P. restrictum*, *P. griseoroseum* and *P. roqueforti*. Species of *Penicillium* gained the attention for the cultivation of alkaline proteases.

Some microbial species like, *Mucorpusillus*and *Mucormiehei* have been reported to secrete aspartate proteases into the medium. These aspartate proteases are also recognized as *Mucor rennins*. These enzymes contain higher activity of the milk clotting and a low activity of the proteolytic origin, which enable them to be utilized as the alternatives of the rennin in the industry of cheese (Andrade *et al.,* 2002). The higher thermal stability of the *Mucor rennins* have been found an unwanted quality, till a residual enzyme activity which after cooking, can easily spoil the flavor of the cheese during the long maturation procedure (Maheshwari *et al.,* 2000). Nowadays the cultivation of extracellular proteases by *Mucor circinelloides* that use glucose as the substrate was found. This *M. circinelloides* enzyme was found stable in pH 5.2 and it gave maximum activity at 25 °C (Andrade *et al.,* 2002).
The hydrolases are cultivated by the thermophilic fungi with the essential characteristics of optimum activity at high temperature ranges, higher rates of hydrolysis and higher thermostability. Thermostable proteases which are efficient in the temperature spectrum of 65-85 °C for proteins bioconversions into peptides and amino acids, have found potential applications in the leather industry, baking, brewing and detergents (Haki & Rakshit, 2003; Merheb *et al.,* 2007). Some species of *Thermoascus aurantiacus* and *Thermomyces lanuginosus* were identified as the origin of thermostable acid proteases. The *Thermoascus aurantiacus* enzyme was cultivated at 60 °C in SSF (solid state fermentations) system which contains wheat bran. Protease cultivation is the inherent capability of all the microbes. A vast range of bacterial species are recognized to produce the alkaline proteases of serine type but very less species of bacteria are selected as commercial producers. Only those microorganisms which cultivate substantial quantities of the extracellular enzymes are industrially important

(Merheb *et al.*, 2007). Many products on the basis of bacterial alkaline proteolytic enzymes are being successfully introduced in the market in the past some years as described by Siota *et al.* (2014) as shown in Table 1.

Table 1. Commercial proteases, sources, applications and their industrial suppliers

Supplier Product	Trade Name	Microbial Source	Application
Novo Nordisk	Denmark Alcalase Savinase	*Bacillus Licheniformis* *Bacillus* sp.	Detergent, silk Degumming Detergent
Genencor International, USA	Purafact Primatan	*B. lentus* Bacterial source	Detergent Leather
Gist-Brocades, TheNetherlands	Subtilisin Maxacal Maxatase	*B. alcalophilus* *Bacillus* sp. *Bacillus* sp.	Detergent Detergent Detergent
Solvay Enzymes, Germany	Opticlean Optimase Maxapem HT-proteolytic Protease	*B. alcalophilus* *B. licheniformis* Protein engineered variant of *Bacillus* sp. *B. subtilis* *B. licheniformis*	Detergent Detergent Detergent Alcohol, baking, brewing, feed food, leather, photographic waste Food, waste
Amano Pharmaceuticals, Japan	Proleather Collagenase Amano protease S	*Bacillus* sp. *Clostridium* sp. *Bacillus* sp.	Food Technical Food
Godo Shusei, Japan	Godo-Bap	*B. licheniformis*	Detergent, food
Advance Biochemicals, India	Protosol	*Bacillus* sp.	Detergent

Industrial Application of Microbial Proteases

The microbial proteases are considered the leaders of the industrial enzyme market worldwide and constitute almost 60% of the whole enzyme sale worldwide (Savitha *et al.*, 2011). The enzyme market of the world of industrial level was estimated to be $3.3 billion in 2010, and is expected to be approximately $4.4 billion by 2015 (Abidi *et al.*, 2011). The industrial enzymes sold in the world market contain almost 75% of the hydrolytic enzymes, while the proteolytic enzymes are the two third of the total sale (Savitha *et al.*, 2011). Fungal enzymes are largely utilized in industries because of many technical issues which include the capability of the enzyme to be used in the fermentation medium at a very high concentration and give a distinct benefit over the bacterial enzymes due to easing the downstream processing (Hajji *et al.*, 2010). Due to the wide specificity of the hydrolytic action of the proteases, they have vast applications in different industries like food, leather, laundry detergent, silk, pharmaceutical, for waste management and for the recovery of silver from the used X-ray films and in the structural elucidation of proteins, where their synthetic capabilities are utilized for the synthesis of the proteins (Rao *et al.*, 1998; Johnvesly & Naik, 2001; Gupta *et al.*, 2002; Abidi *et al.*, 2011; Savitha *et al.*, 2011; Qing *et al.*,2013). An overview of protease applications is presented by Qing *et al.* (2013) in Figure.

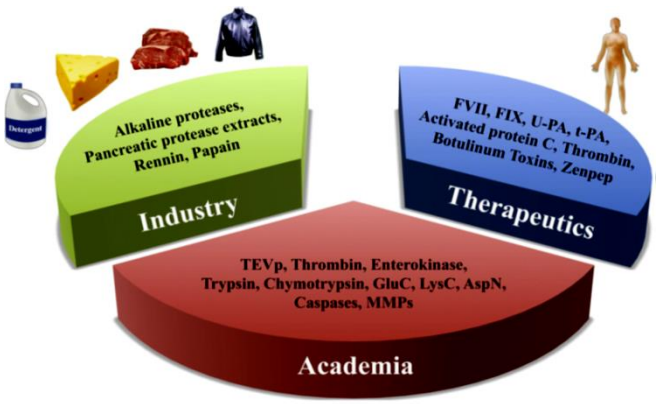

Figure 2. An overview of the protease applications.

Food Industry

Protease enzymes have a large number of applications in the industry of food. These applications include improving the sensory quality and digestibility of the food. It also provides health benefits via reducing the allergenic components (Tavano, 2013). The potential applications of the proteolytic enzymes in the food processing are in beer haze clarification, cereal mashing, brewing, in coagulation step in the cheese production, in the manufacture of the hydrolysates of the protein and in baking altering the viscoelastic features of the dough (Ward, 2011). The hydrolytic quality of the proteolytic enzymes are also used extensively for the breakdown of the turbidity complex due to its hydrolytic quality which results from the protein in alcoholic liquors, the improvement of quality of the protein rich food products, fruit juices and soy protein breakdown, gelatin breakdown, whey and casein protein breakdown, meat tenderization and the recovery of meat protein (Tomar *et al.*, 2008; Kumari *et al.*, 2012). The most important use of the protease enzymes in the dairy industry is in the cheese production. Here the basic function of the enzymes is to breakdown the particular peptide bond to manufacture macro peptides and casein (Rao *et al.*, 1998). The protease enzymes manufactured via *Endothiaparasitica* and *Mucormichei* are quickly taking the place of rennin in the cheese production (Demain & Adrio, 2008). Nowadays latest patents are arising for the sake of lactic acid bacteria related to the applications of the endoproteases and exoproteases in the dairy industry. These proteolytic enzymes are utilized as the catalysts for the ripening of the cheese and in the production of EMC (enzyme modified cheese). It also accelerates the production of dairy constituents which contain bioactive peptides. In addition, the proteolytic enzymes also play an important role in the tenderization of meat (particularly of beef) because they possess capability to breakdown the proteins of the muscle fiber and connective tissue (Kumar & Takagi, 1999). In the baking procedures for the modification of the wheat gluten, some exo and endoproteinases are obtained from the *Aspergillus oryzae*. The addition of proteolytic enzymes in the baking make the dough's time of mixing time of less which increases the loaf volumes (Rao *et al.*, 1998).

The processing of soy sauce and other products is carried out with the help of some neutral and alkaline proteolytic enzymes from the fungal source. The treatment via enzymes helps to cultivate hydrolysates which have high soluble rate, low bitterness and good yield of protein. Hydrolysates of the protein are usually produced from soyprotein, whey protein and casein and they have important applications because they are the dietetic constituents of the products of health, in the formulae of infant, beverages focused for lactating and pregnant women and the supplements of clinical nutrition, use in flavoring agents and also people that are allergic to the proteins of milk (Ramamurthy *et al.*, 1991; Rao *et al.*, 1998; Kumar & Takagi, 1999). Alkaline proteolytic enzymes are also utilized in the cultivation of hydrolysates of protein which have high value of nutrition. Hydrolysates of the protein also have an essential role in the regulation of blood pressure and areutilized in the formulation of infant food and the dietary products of particular therapeutic range, fortification of the soft drinks and fruit juices (Ward, 1985). Rebeca *et al.* (1991) has reported the manufacture of hydrolysates of the fish that have a very high value of nutrition via *B. subtilis* protease enzyme. Some proteases that can be exploited for their potential applications on industrial level are given in the Table. 1.

Table 2. Some industrially important proteases along with their applications.

Industry	Protease	Application	Bibliography
Baking	Neutral Protease	Dough conditioner	Gupta *et al.*, 2002
Beverage	Papain	Removal of haze in beverages, chill proofing.	Gupta *et al.*, 2002
Dairy	Fungal proteases, Chymosine, Other proteases	Production of enzyme modified cheese (EMC)	Tavano, 2013
Sweetner	Thermolysin	Reverse hydrolysis in aspartame synthesis	Gupta *et al.*, 2002
Detergent	Alkaline protease, Subtilism,	Laundry detergents for protein	Rathindra & Monika, 2004

	Several proteases	stain removal	
Leather	Trypsin, Other Proteases	Bating of leather, dehairing of skins	Varela *et al.,* 1997
Food processing	Several proteases	Modification of protein rich material i.e., soy protein or wheat gluten	Tavano, 2013
Medicine	Trypsin	Dead tissue removal, blood clot dissolution	Chao *et al.,* 2007

Leather Industry

The traditional methods that are used in the processing of leather utilize hydrogen sulfide and some other chemicals which create safety hazards and pollution in the environment. Therefore, due to these environmental issues, the leather biotreatment by using an enzyme based approach is better because it gives many benefits, such as reduction of waste, speed and easy control that is why it is ecofriendly. Some alkaline proteolytic enzymes which also have keratinolytic and elastolytic activity may be utilized in the industries of leather processing. Different stages of making the skins and hides use proteolytic enzymes during the procedures of bating, soaking and unhairing. These treatments via enzymes degrade the unwanted pigments and it also increases the area of skin, thus the clean hides are manufactured. During the enzymatic procedures of the bating, pancreatic proteolytic enzymes are usually used. Nowadays the usage of microbial proteases has gained popularity (Varela *et al.*, 1997).

Some alkaline proteases of the microbial origin catalyze the procedure of unhairing, due to the presence of the alkaline conditions which favor the swelling of the roots of hair, and the rapid attack of proteolytic enzymes on follicle protein of the hair that facilitate hair removal. Varela *et al.* (1997) has reported the usage of *B. subtilis* alkaline proteolytic enzymes for dehairing the skin of the sheep. George *et al.* (1995) has reported the usage of *B. amyloliquefaciens* alkaline proteolytic enzymes for dehairing skins and hides. Hameed *et al.* (1999) has described the usage of *B. subtilis* (K2) alkaline proteolytic enzymes in the processing of leather and bating of the leather. Further studies have shown the successful usage of the alkaline protease enzymes in the tanning of the leather from *Conidiobolus coronatus* and *Aspergillus flavus* (Laxman *et al.,* 2005).

Detergent Industry

The enzymes utilized as the additives in detergents show the vast application of the enzymes used at the industrial level. The total worldwide sales of enzymes constitute about 25% proteases used in laundry detergents (Demain & Adrio, 2008). The usage of the enzymes in the formulations of the detergents elaborate the ability of the detergents to eradicate the tough stains, it also makes the detergent safe environmentally. Nowadays, various detergents used in the laundry products constitute the mixtures of the enzymes that include cellulases, lipases, amylases, and proteases (Hmidet *et al.,* 2009). The alkaline proteolytic enzymes that are added to the detergents used in the laundry enable proteinaceous substances to release from the stains. The capacity of the alkaline proteolytic enzymes in the detergents is affected by many features like composition of the detergent, washing solution temperature and its pH. Under ideal conditions proteolytic enzymes utilized in the formulation of detergents also have good stability and activity within a wide spectrum of temperatures and pH. It should also have compatibility with the many components of the detergent also with sequestering and oxidizing (Kumar & Takagi, 1999; Jaouadi *et al.,* 2008; Savitha *et al.,* 2011). Although, rather than their use in the detergents of laundry, the proteases have too found popularity in the making of detergents used in the household dishwashing and both in the cleaning detergents of institutions and industries (Godfrey & West 1996; Showell, 1999).

Pharmaceutical and Cosmetic Industry

In the pharmaceutical and cosmetic industries, protease enzymes can be used in the removal of the keratin in psoriasis and acne, eradication of callus of human or the destruction of keratinized skin, in increasing of ungual drug delivery, degradation and formation of the vaccine for the therapy of dermatophytosis (Vignardet *et al.,* 2001; Brandelli *et al.,* 2010). Further, these keratinases can regenerate the epithelia and speed up the healing processes, eradicate the scar and can act also in the

trauma medicine (Chao *et al.,* 2007). In the cosmetic products, proteases can hydrolyze the peptide bonds of keratin, collagen and elastin of skin. Enzymes like papain, bromelain and other proteases are also used on the skin for performing smoothing and peeling. The quick action of these proteases is related to cell renewal, exercising keratinolytic activity, promoting removal of dead cells in the epidermis and also restoring the same (Sim *et al.,* 2000). In addition, the formation of elastoterases was performed to treat the purulent wounds, burns, furuncles, carbuncles, and deep abscesses (Gupta *et al.,* 2002). The proteases of the collagenolytic origin are being straightly utilized in the clinical therapy, including healing of wound, treatment of retained placenta, treatment of herniated intervertebral discs in sciatica, and also for the enhancement of the adenovirus influenced cancer gene therapy (Watanabe, 2004).

Medical use

Proteases are also utilized for the development of the important medical products. Kudrya and Simonenko (1994) have utilized extensively the elastolytic activity of the *B. subtilis* (316M) for the elastoterase formation that was utilized for the therapy of purulent wounds, furuncles, deep abscesses, carbuncles, and burns. Kim *et al.* (1996) has reported the usage of the alkaline proteolytic enzymes of the *Bacillus sp*. strain like a thrombolytic agent which also has activity of fibrinolytic origin.

II. CONCLUSION

The use of proteases in different industries has become popular from many years and for the supreme production of the protease enzymes, a lot of microbial sources exist in nature. Their wide diversity, specific range of action and the property of being active to a very wide spectrum of pH and temperature have attained attention of biotechnologists all over the world. However they have vast distribution in nature, microorganisms are used preferably for these enzymes in fermentation bioprocesses due to their fast growth rate and also due to their specific ability of being genetically engineered to cultivate new enzymes with desirable qualities or simply for the enzyme over production. The scientists are continuously searching for the new microorganisms for protease production. Proteases have many uses in the basic areas of the processing of food, pulp and paper, leather and nutrition of animals, production of beverages, detergents and textiles. With the emergence of new inventions in the biotechnology, the range of applications of the protease enzymes has been divided into various new fields like analytical, medicinal, and clinical chemistry.

III. REFERENCES

[1]. Abidi, F., J.M. Chobert and T. Haertle. 2011. Purification and biochemical characterization of stable alkaline protease Prot-2 from Botrytis cinerea. Process Biochem., 46: 2301-2310.

[2]. Andrade, V.S., L.A. Sarubbo and K. Fukushima. 2002. Production of extracellular proteases by Mucor circinelloides using D-glucose as carbon source / substrate. Braz. J. Microbiol., 33: 106-110.

[3]. Anitha, T.S. and P. Palanivelu. 2013. Purification and characterization of an extracellular keratinolytic protease from a new isolate of Aspergillus parasiticus. Protein Expr. Purif., 88: 214-220.

[4]. Banerjee, U.C., R.K. Sani, W. Azmi and R. Sani. 1999. Thermostable alkaline protease from Bacillus brevis and its characterisation as a laundry detergent additive. Proc. Biochem., 35: 213-219.

[5]. Biesebeke, R.T, G. Ruijter and Y.S.P. Rahardjo. 2002. Aspergillus oryzae in solid-state and submerged fermentations progress report on a multi-disciplinary project. FEMS Yeast Res., 2: 245-248.

[6]. Brandelli, A., D.J. Daroit and A. Riffel. 2010. Biochemical features of microbial keratinases and their production and applications. Appl. Microbiol. Biotechnol., 85: 1735-1750.

[7]. Chao, Y.P., F.H. Xie, and J. Yang. 2007. Screening for a new Streptomyces strain capable of efficient keratin degradation. J. Environ. Sci., 19: 1125-1128.

[8]. De Souza, P.M. and M.L.A. Bittencourt. 2015. A biotechnology perspective of fungal proteases. Brazil. J. Microbiol., 46(2): 337-346.

[9]. Demain, A.L. and J.L. Adrio. 2008. Contributions of microorganisms to industrial biology. Mol. Biotechnol. 38: 41-55.

[10]. Dhandapani, R. and R. Vijayaragvan. 1994. Production of thermophilic, extracellular alkaline protease by B. stearothermophilus AP-4. World J. Microbiol. Biotechnol., 10: 33-35.

[11]. Geisseler, D. and W.R. Horwath. 2008. Regulation of extracellular protease activity in soil in response

to different sources and concentrations of nitrogen and carbon. Soil Biol. Biochem., 40: 3040-3048.

[12]. George, S., V. Raju, M.R.V. Krishnan, T.V. Subramanian and K. Jayaraman. 1995. Production of protease by Bacillus amyloliquefaciens in solid-state fermentation and its application in the unhairing of hides and skins. Process Biochem., 30: 457-46.

[13]. Germano, S., A. Pandey and C.A. Osaku. 2003. Characterization and stability of proteases from Penicillium sp. produced by solid-state fermentation. Enz. Microb. Technol., 32: 246-251.

[14]. Godfrey, T. and S. West. 1996. Introduction to Industrial Enzymology. In: Industrial Enzymology, (2nd ed) Macmillan Press, London, UK. pp 1-8.

[15]. Gupta, R., Q.K. Beg and P. Lorenz. 2002. Bacterial alkaline proteases: molecular approaches and industrial applications. Appl. Microbiol. Biotechnol., 59: 15-32.

[16]. Gupta, R., Q.K. Beg and P. Lorenz. 2002. Bacterial alkaline proteases: molecular approaches and industrial applications. Appl. Microbiol. Biotechnol., 59:15-32.

[17]. Hajji, M., N. Hmidet and K. Jellouli. 2010. Gene cloning and expression of a detergent stable alkaline protease from Aspergillus clavatus ES1. Process Biochem., 45: 1746-1752.

[18]. Haki, G.D. and S.K. Rakshit. 2003. Developments in industrially important thermostable enzymes: a review. Bioresour. Technol., 89: 17-34.

[19]. Hameed, A., M.A. Natt and C.S. Evans. 1996. Production of alkaline protease by a new Bacillus subtilis isolate for use as a bating enzyme in leather treatment. World J. Microbiol. Biotechnol., 12: 289-291.

[20]. Hameed, A., T. Keshavarz and C.S. Evans. 1999. Effect of dissolved oxygen tension and pH on the production of extracellular protease from a new isolate of Bacillus subtilis K2, for use in leather processing. J. Chem. Technol. Biotechnol., 74: 5-8.

[21]. Hmidet, N., A.N. El-Hadj and A. Haddar. 2009. Alkaline proteases and thermostable amylase co-produced by Bacillus licheniformis NH1: Characterization and potential application as detergent additive. Biochem. Eng. J., 47: 71-79.

[22]. Jaouadi, B., S. Ellouz-Chaabouni and M. Rhimi. 2008. Biochemical and molecular characterization of a detergent-stable serine alkaline protease from Bacillus pumilus CBS with high catalytic efficiency. Biochim., 90 : 1291-1305.

[23]. Johnvesly, B. and G.R. Naik. 2001. Studies on production of thermostable alkaline protease from thermophilic and alkaliphilic Bacillus sp. JB-99 in a chemically defined medium. Process Biochem., 37: 139-144.

[24]. Kalisz, H.M. 1988. Microbial proteinases. Adv. Biochem. Eng. Biotechnol. 36:1-65.

[25]. Kranthi, V.S., D.M. Rao and P. Jaganmohan. 2012. Production of protease by Aspergillus flavus through solid state fermentation using different oil seed cakes. Int. J. Microbiol. Res., 3: 12-15.

[26]. Kucera, M. 1981. The production of toxic protease by the entomopathogenous fungus Metarhizium anisopliae in submerged culture. J. Invertebr.Pathol. 38: 33-38.

[27]. Kudrya, V.A. and I.A. Simonenko. 1994. Alkaline serine proteinase and lectin isolation from the culture fluid of Bacillus subtilis. Appl. Microbiol. Biotechnol., 41: 505-509.

[28]. Kumar, C.G. and H. Takagi. 1999. Microbial alkaline proteases: From a bioindustrial viewpoint. Biotechnol. Adv., 17: 561-594.

[29]. Kumari, M., A. Sharma and M.V. Jagannadham. 2012. Religiosin B, a milk-clotting serine protease from Ficus religiosa. Food Chem., 131: 1295-1303.

[30]. Laxman, R.S., A.P. Sonawane and S.V. More. 2005. Optimization and scale up of production of alkaline protease from Conidiobolus coronatus. Process Biochem., 40: 3152-3158.

[31]. Li, Q., L. Yi, P. Marek and B.L. Iverson. 2013. Commercial proteases: present and future. FEBS lett., 587: 1155-1163.

[32]. Lopez-Otin, C. and J.S. Bond. 2008. Proteases: multifunctional enzymes in life and disease. J. Biol. Chem., 283: 30433-30437.

[33]. Macchione, M.M., C.W. Merheb and E. Gomes. 2008. Protease production by different thermophilic fungi. Appl Biochem. Biotechnol., 146: 223-230.

[34]. Maheshwari, R., G. Bharadwaj and M.K. Bhat. 2000. Thermophilic fungi: their physiology and enzymes. Microbiol. Mol. Biol. Rev., 64: 461-488.

[35]. Merheb, C.W., H. Cabral and E. Gomes. 2007. Partial characterization of protease from a thermophilic fungus, Thermoascus aurantiacus and its hydrolytic activity on bovine casein. Food. Chem. 104: 127-131.

[36]. Neklyudov, A.D., A.N. Ivankin and A.V. Berdutina. 2000. Properties and uses of protein hydrolysates. Appl. Biochem. Microbiol., 36: 452-459.

[37]. Ogawa, A., A. Yasuhara and T. Tanaka. 1995. Production of Neutral Protease by Membrane-Surface of Aspergillus oryzae IAM2704. J. Ferment. Bioeng. 80: 35-40.

[38]. Ramamurthy, V., C.M. Upadhyay and R.M. Kothari. 1991. An optimized protocol for the preparation and application of acid protease. J. Biotechnol., 21: 187-196.

[39]. Rao, M.B., A.M. Tanksale and M.S. Ghatge. 1998. Molecular and biotechnological aspects of microbial proteases. Microbiol. Mol. Biol., 62: 597-635.

[40]. Rebeca, B.D., M.T. Pena-Vera and M. Diaz-Castaneda. 1991. Production of fish protein hydrolysates with bacterial proteases; yield and nutritional value. J. Food Sci., 56: 309-314.

[41]. Sabotic, J. and J. Kos. 2012. Microbial and fungal protease inhibitors-current and potential applications. Appl. Microbiol. Biotechnol., 93: 1351-1375.

[42]. Savitha, S., S. Sadhasivam and K. Swaminathan. 2011. Fungal protease: Production, purification and compatibility with laundry detergents and their wash performance. J. Taiwan Inst. Chem. Eng., 42: 298-304.

[43]. Showell, M.S. 1999. Enzymes, detergent. In: Flickinger MC, Drew SW (ed) Encyclopedia of bioprocess technology: fermentation, biocatalysis and bioseparation, vol 2. Wiley, New York, pp 958-971.

[44]. Singh, J., N. Batra and R.C. Sobti. 2001. Serine alkaline protease from a newly isolated Bacillus sp. SSR1. Proc. Biochem., 36: 781-785.

[45]. Siota , L.F., L. Blasco1, J.L.R. Rama1, J.B. Velazquez, T. Miguel, A.S. Perez and T.G. Villa. 2014. Recent patents on microbial proteases for the dairy industry. Recent Adv. DNA Gene Seq., 8: 44-55.

[46]. Sun, S.Y., and Y. Xu. 2009. Membrane-bound 'synthetic lipase' specifically cultured under solid-state fermentation and submerged fermentation by Rhizopuschinensis: a comparative investigation. Bioresour. Technol., 100: 1336-1342.

[47]. Tavano, O.L. 2013. Protein hydrolysis using proteases: An important tool for food biotechnology. J. Mol. Catal. B. Enz., 90: 1-11.

[48]. Tomar, R., R. Kumar and M.V. Jagannadham. 2008. A stable serine protease, wrightin, from the latex of the plant Wrightia tinctoria (Roxb.) R. Br.: purification and biochemical properties. J. Agric. Food Chem., 56: 1479-1487.

[49]. Varela, H., M.D. Ferrari, L. Belobradjic, A. Vazquez and M.L. Loperena. 1997. Skin unhairing proteases of Bacillus subtilis: production and partial characterization. Biotechnol. Lett. 19: 755-758.

[50]. Vignardet, C., Y.C. Guillaume and L. Michel. 2001. Comparison of two hard keratinous substrates submitted to the action of a keratinase using an experimental design. Int. J. Pharm. 224: 115-122.

[51]. Vishwanatha, K.S., A.G. Rao and S.A. Singh. 2010. Acid protease production by solid-state fermentation using Aspergillus oryzae MTCC 5341: optimization of process parameters. J. Ind. Microbiol. Biotechnol., 37: 129-138.

[52]. Vishwanatha, K.S., A.G.A Rao and S.A. Singh. 2010. Production and characterization of a milk-clotting enzyme from Aspergillus oryzae MTCC 5341. Appl. Microbiol. Biotechnol., 85: 1849-1859.

[53]. Vishwanatha, K.S., A.G.A. Rao and S.A. Singh. 2009. Characterisation of acid protease expressed from Aspergillus oryzae MTCC 5341. Food Chem., 114: 402-407.

[54]. Walsh, K.A. and P.E. Wilcox. 1970. In: Perlmann, G.E., Lorand, L. (ed). Methods Enzymol.,pp 31–226.

[55]. Ward, O.P. 1985. Proteolytic enzymes. In: Moo-Young M (ed) Comprehensive biotechnology, the practice of biotechnology: current commodity products, vol 3. Pergamon Press, Oxford, pp 789-818.

[56]. Ward, O.P. 2011. Proteases. In: Moo-Young, M. (ed.), Comprehensive Biotechnology, Waterloo, Canada, pp 571-582.

[57]. Watanabe, K. 2004. Collagenolytic proteases from bacteria. Appl. Microbiol. Biotechnol. 63: 520-526.

[58]. Wu, T.Y., A.W. Mohammada and J.M. Jahim. 2006. Investigations on protease production by a wild-type Aspergillus terreus strain using diluted retentate of pre-filtered palm oil mill effluent (POME) as substrate. Enz. Microb. Technol., 39: 1223-1229.

[59]. Yang, F. and I. Lin. 1998. Production of acid protease using thin stillage from a rice-spirit distillery by Aspergillus niger. Enz. Microb. Technol., 23: 397-402.

[60]. Zambare, V., S. Nilegaonkar and P. Kanekar. 2011. A novel extracellularprotease from Pseudomona aeruginosa MCM B-327: enzyme production and its partial characterization. N. Biotechnol., 28:173-181.