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ORIGINAL RESEARCH PAPER

Botany

A REVIEW ON FUNGAL PROTEASE AND ITS TREMENDOUS APPLICATIONS IN VARIOUS SECTORS

KEY WORDS: Fungi, protease, industrial applications

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ABSTRACT

Proteases are extracted from numerous animals, plants and microorganisms of living organisms. Microbial enzymes represent almost 90% of the total market and influence each important sector of the economy. From microbial enzymes, fungal proteases are highly specific and thus have considerable potential for many industrial applications. The uses of this enzymes in maximizing reactions accomplished in the food and paper industry, meat tenderization, detergents, leather, silk degumming, pharmaceuticals and photographic industry. In this review, the enzyme source, types, optimization method, purification and characterization techniques and various industrial applications were clearly discussed.

INTRODUCTION

The commercial proteases account for nearly 60% of the total industrial enzyme market (Jabalia *et al.,* 2014; Hamza, 2017). Proteolytic enzymes hydrolyze and degrade peptide bonds of proteins (Theron and Divol, 2014). They constitute a very large and complex group of hydrolytic enzymes that degrade proteins into small peptides and amino acids (Souza *et al.,* 2015). Several factors such as pH, ionic strength, mechanical handling, temperature, nutritional factor and inoculums concentration influence the protease activity (Sharma *et al.*, 2017).

Proteases are the most important industrial enzymes that possess a wide variety of characteristics such as substrate specificity, active site and catalytic mechanism and also possess different profiles for mechanical stress, chemical environment, pH and temperature for stability and activity (Jisha *et al.,* 2013). Because of their broad substrate specificity, proteases have a wide range of applications such as in leather processing, detergent formulations, baking, brewing, meat tenderization, peptide synthesis, cheese manufacture, soy sauce production, protein hydrolysis, pharmaceutical industry, waste treatment, silk industry, organic synthesis, recovery of silver from waste photographic film (Naveed *et al.,* 2021; Gat *et al.,* 2019; Hamza, 2017).

The common and well known proteases from plant origin are papain, bromelain, ficin, keratinase and ginger rhizome (Bekhit *et al.,* 2018; Meshram *et al.,* 2019). The drawback for producing plant proteases are the availability of land for cultivation and weather conditions for growth (Deshpande *et al.,* 1998; Malcata *et al.,* 2012). Pepsin, trypsin, chymotrypsin and rennin are some common proteases of animal origin (Mazorra-Manzano *et al.,* 2018; Parde *et al.,* 2012; Queiroga *et al.,* 2012). The production rate is critically dependent on the slaughter of livestock (Sandhya *et al.,* 2006).

The drawback of proteases from plant and animal origin creates the need for large amounts of protease production at low cost to meet the market demand. Microbes are an excellent source of protease owing to their broad biochemical diversity and their susceptibility to genetic manipulation (**Mehta** *et al.,* 2016; Mamo and Assefa, 2018). In the total worldwide enzyme sales, two-third share of commercial proteases are accounted for by microorganism. The demand for proteases has led to an interest in microbial proteases due to their rapid growth, cost effectiveness and high yield (Jisha *et al.,* 2013).

Degradation of proteins done by microorganisms and they utilize the degradation products as nutrients for their subsistence. The process of degradation is initiated by proteinases (endopeptidases) secreted by microbes followed by further hydrolysis using peptidases (exopeptidases) at the extra or intra cellular locations. A variety of proteases are produced by microorganisms depending on the species of the strains (Jisha *et al.,* 2013).

Bacteria

Several reports are available on protease production by bacteria belonging to genus *Bacillus* (Aramesh and Ajoudanifar 2017) *Pseudomonas* (Zeeshan *et al.,* 2021), *Alteromonas* (Zhang *et al.,* 2009) *Streptomyces* (Sarkar and Suthindhiran 2020) and *Thermoactinomyces* (Hanphakphoom *et al.,* 2014). Though proteases are produced by many genera of bacteria, the genus *Bacillus* contains a number of industrially important species and approximately half of the present commercial production of bulk enzymes. *Bacillus* produce a wide range of extracellular enzymes and secrete two major types of protease, a subtilisin or alkaline protease and a metalloprotease or neutral protease, which are of industrial importance. Bacterial neutral proteases are active in a pH range of 5 to 8. Whereas, alkaline proteases are active at high pH (9-10) and optimal temperature is around 60 °C.

Fungi

Fungi can effectively secrete a wider variety of proteolytic enzymes than bacteria. The filamentous fungi have a potential to grow under varying environmental conditions (Ikram-Ul-haq and Umber, 2006). Fungal proteases are advantageous over bacterial proteases because they are easy to obtain and to recover (Souza *et al.,* 2015). Fungal proteases are active over a wide range of pH ranging from 4 to11 and demonstrate broad substrate specificity. Several fungal genera including *Aspergillus* (de Castro *et al.,* 2015; Srinubabu *et al.,* 2007), *Penicillium* (Cabral *et al.,* 2013; Papagianni and Sergelidis, 2014), *Rhizopus* (Kao *et al.,* 2014; Benabda *et al.,* 2019), *Mucor* (Gomes *et al.,* 2020; Nascimento *et al.,* 2015), *Thermomyces* (Fawzi *et al.,* 2014; Da-silva *et al.,* 2007) and *Trichoderma* (Kredics *et al.,* 2005; Szekeres *et al.,* 2004) are reported to produce proteases.

Viruses

Proteases from viral origin have been involved in the functional processing of proteins that causes certain fatal diseases including AIDS and cancer. The well known proteases such as serine, aspartic and cysteine peptidases are found in various viruses (Mamo and Assefa, 2018; Pandey *et al.,* 2016). All the virus encoded peptidases are endopeptidases, there are no metallopeptidases (Rao *et al.,*

1998). Extensive research has focused on the expression, purification and enzymatic analysis of retroviral aspartic protease.

Classification of proteases

According to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology proteases are classified in subgroups of 4 of group 3 (hydrolases). Microbial proteases differ widely in their properties, based on various criteria including the type of reaction catalyzed, the active site functional group, molecular structure and evolutionary relationships they are classified into various groups (Landi *et al.,* 2011; Pratush *et al.,* 2013). This includes

1. Based on their active site of action proteases are subdivided into two major groups.

I. Exopeptidases
a. aminopeptidases
b. carboxypeptidases
ii. Endopeptidases
a. serine endopeptidases
b. aspartic endopeptidases
c. cysteine endopeptidases
d. metallo endopeptidases

2. Based on the optimum pH they are grouped as
i. Acid proteases
ii. Neutral proteases
iii. Alkaline proteases

Exopeptidases

Exopeptidases are enzymes that break peptide bonds proximal to the amino or carboxyl terminus of the substrate and they act only near the ends of the polypeptide chains. The exopeptidases are classified mainly on the basis of their actions at the N or C terminus. Accordingly they are classified as amino and carboxy peptidase (Mahajan and Badgujar, 2010).

Aminopeptidases

Aminopeptidases are active at the free N terminus of the polypeptide chain and liberate a single amino acid residue, a dipeptide or a tripeptide. Aminopeptidases are widely distributed in bacteria and fungi (Nampoothiri *et al.,* 2008). Aminopeptidases are generally intracellular enzymes, however fungal species of *A. oryzae* have reported early to secrete extracellular aminopeptidase (Kumar *et al.,* 2021).

Carboxypeptidases

Carboxypeptidases are active at C terminals of the polypeptide chain and liberate a single amino acid or a dipeptide (Barrett, 2000). Based on the nature of the amino acid residues at the active site of the enzymes, carboxypeptidases are divided into three major groups such as serine carboxypeptidases, metallo carboxypeptidases and cysteine carboxypeptidases.

Endopeptidases

Endopeptidases catalyze the hydrolysis of the internal bond of the peptides (Landi *et al.,* 2011). Based on the catalytic mechanism of endopeptidases, they are divided into four subgroups such as serine proteases, aspartic proteases, cysteine proteases and metallo proteases (Mienda *et al.,* 2014; Pandey *et al.,* 2016).

Serine endopeptidases

Serine proteases are widely reported from bacteria, fungi and viruses and are characterized by the presence of a serine group in their active site. They are found in exopeptidase, endopeptidase, oligopeptidase and omega peptidase groups. Serine proteases are inactivated by organic phosphate esters which catalyze the active serine residue. Serine proteases are generally active at neutral and alkaline pH, with an optimum between 7 and 11 (Mienda *et al.,* 2014). They have broad substrate specificities, which include esterolytic and amidase activity and its molecular masses range between 18 and 35 K Da. They hydrolyze a peptide bond which has tyrosine, phenylalanine, or leucine at the carboxyl side of the splitting bond. Serine protease produced by some of the fungal species (Casalongue *et al.,* 2002; Zhang *et al.,* 2005; Rodriguez *et al.,* 2011).

Aspartic endopeptidases

Aspartic endopeptidases are commonly known as acidic proteases and depend on two highly conserved aspartic acid residues for their catalytic activity. Most of the aspartic proteases show their maximum activity at low pH of 3 to 5 (Kao *et al.,* 2014; Garcia-carreno *et al.,* 2006). They have an isoelectric point in the range of pH 3 to 4.5 and molecular masses of aspartic proteases are in the range of 35 to 50 kDa usually consisting of 320 to 340 amino acid residues (Theron and Divol, 2014). Microorganisms produced aspartic proteases can be divided into 2 groups which includes Pepsin like enzymes which produced by *Aspergillus* (Singh *et al.,* 2009) and *Rhizopus* (Kumar *et al.,* 2005) and Rennin-like enzymes produced by *Endothia* and *Mucor* and *Rhizomucor* sp. (Kao *et al.,* 2014). *Saccharomyces, Mucor, Aspergillus, Candida* and *Shewanella* sp. are produced aspartic endopeptidases (Rana and Gupta, 2023; Singh *et al.,* 2009).

Cysteine/Thiol endopeptidases

Cysteine proteases are produced by both prokaryotes and eukaryotes. So far, nearly twenty families of cysteine proteases have been recognized (Grzonka *et al.,* 2007). Cysteine protease activity mainly depends on cysteine and histidine containing catalytic dyad. The order of Cys and His (Cys- His or His- Cys) residues differ among the families. The cysteine proteases process through the formation of a covalent intermediate and involve a cysteine and a histidine residue. Based on their specificity of the side chain, cysteine proteases are divided into four groups such as (i) papain- like (ii) trypsin- like with preference for cleavage at the arginine residue (iii) specific to glutamic acid, and (iv) others. Papain is the best known cysteine protease. Cysteine proteases are active at a neutral pH however Lysosomal proteases showed maximally active at acidic pH (Mienda *et al.,* 2014).

Metallo endopeptidases

Metalloproteases are the most miscellaneous of the catalytic types of proteases and are characterized by the requirement for a divalent metal ion for their activity (Nagase, 2001). So far, about 30 families of metalloproteases have been recognized; of which 17 contain only endopeptidases. Based on the specificity of their action, metalloproteases can be divided into four groups - neutral, alkaline, Myxobacter I, and Myxobacter II (Kim *et al.,* 2005; Rani *et al.,* 2011). The neutral proteases show specificity for hydrophobic amino acids, whereas the alkaline proteases have a very wide specificity. *Myxobacter* protease I is specific for small amino acid residues on either side of the cleavage bond, whereas protease II is specific for lysine residue on the amino side of the peptide bond. All of them are inhibited by chelating agents such as EDTA but not by sulfhydryl agents or DFP. Propeptides metallo endopeptidases removal is dependent on other proteases or autocleavage (Formanek *et al.,* 2013).

Classification of protease based on their optimal pH

Based on their optimal pH, proteases are classified into three groups. They are acid protease, neutral protease and alkaline protease. Table 1 shows the acid, neutral and alkaline protease producing some of the fungal sources

Acid proteases

Acid proteases are active in the pH range of 2 to 6 and are mainly of fungal origin. General examples in this subclass include aspartic proteases of the pepsin family. Several metallo proteases and cysteine proteases are also categorized as acidic proteases (Aguilar and Gutierrez, 2008;

Martinez-Medina *et al.,* 2019).

Neutral proteases

Neutral proteases are active at neutral pH and possess specific function in hydrolyzing hydrophobic amino acid bonds. Majority of the cysteine, metallo and some of the serine proteases are classified under neutral proteases. They are mainly of plant origin, except few fungal and bacterial neutral proteases (Aguilar and Gutierrez, 2008). Guerard *et* al. (2002) reported that a commercial neutral protease Umamizyme is prepared by *A. oryzae* and it has high peptidase activity in contrast to other proteinase preparations.

Alkaline proteases

Alkaline proteases are a commercially important group of enzymes used primarily as detergent additives. Alkaline proteases are active in the alkaline range of pH 8 to 13. Though they maintain some activity in the neutral pH range as well. The molecular mass of the alkaline protease ranges from 20 to 130 kDa (Gimenez *et al.,* 2000; de Castro *et al.,* 2001). They are obtained mainly from *Trichoderma reesei* (Nilegaonkar, 2010), *Myceliophthora* sp. (Da-silva *et al.,* 2011) and *Aspergillus strain* (Balagurunathan *et al.,* 2013).

Production of proteases

Proteases have been generally produced in submerged (SmF) and solid state fermentation (SSF). The production of proteases has been improved by screening for hyper-producing strains and or by optimization of the production medium. The important factor has to be screened while optimizing a production medium is the cost-effectiveness of the fermentation medium (Pandey *et al*., 2005). Several methods have been implemented for the optimization of both media components as well as physical factors for protease production. The well known common conventional method used for optimization of protease production is one factor at a time method. The conventional method of optimization involves varying one parameter and keeping the others constant. The major disadvantages of this method are it does not include integrative effects among the factors and does not describe the complete effects of the parameters on the process. Moreover, conventional methods of optimization are time consuming. In recent times, industrial production of enzymes is moving away from conventional methods of optimization (Oskouie *et al,* 2008; El-Hersh *et al*., 2017). Statistical methods offer a perfect approach for process optimization studies in biotechnologically important enzyme production (Tripathi *et al.,* 2017; Ryu *et al*., 2008). Statistical method is a useful tool for studying the interaction of several factors on the response simultaneously and large number of data can be obtained for a less number of experiments

Submerged fermentation (SmF)

Submerged fermentation is defined as the cultivation of microorganisms in liquid nutrient broth (Barragan *et al.,* 2016). The growth of the microorganism at a large scale involves the use of closed large vessels containing a rich nutrient broth and enough oxygen (Al-Maqtari *et al.,* 2019). The major factors influencing the production such as carbon and nitrogen sources, vitamins and metal ions, environmental factors such as pH, temperature, agitation/ aeration (Dey *et al.,* 2012; Garg *et al.,* 2011). Most of the commercial microbial proteases are generally produced by submerged fermentation (Aguilar and Gutierrez, 2008). Furthermore, there is no universal medium for the production of protease and composition of media varies from species to species. SmF is intrinsically less problematic and heat transfer is better and homogeneity is maintained. In addition, SmF has benefits in process control and easy recovery of enzymes, mycelia and spores. However, the products are dilute and enzyme extracts might be less stable than those from SSF.

Solid State Fermentation (SSF)

Solid State Fermentation is defined as the growth of microorganisms on moist solid substrate in the absence of free flowing water. The microorganism obtains water, carbon, nitrogen, minerals and other nutrients from the solid substrate. In solid state fermentation the necessary moisture content for the microbial growth exists in an absorbed state or complex within the solid matrix (Krishna, 2005). SSF has been a developed method for fungal protease production and its advantages include simplicity, inexpensive yield of higher amount enzymes, less energy consumption and low wastewater output (Elumalai *et al.,* 2017; Krishania *et al.,* 2018). Agricultural residues such as wheat bran, rice bran, oil cakes, husks or combination of two or more substrates are used as substrates which are economical (Tripathi *et al*., 2013; Sumantha *et al.,* 2006; Negi *et al.,* 2020). The drawbacks of SSF are the incomplete utilization of the nutrients, due to poor oxygen and heat transfer in the substrate.

Purification and characterization of proteases

Crude enzyme preparations are used for commercial purposes, whereas purified enzymes are employed for pharmaceutical and other medical applications further required for property studies and better understanding of structure function relationship.

Recovery of enzymes

The first step in downstream processes is the recovery of crude cell free preparation at the end of fermentation process. There are several methods for recovery of pure enzymes which includes filtration and centrifugation (Westphal and van Berkel *et al.,* 2021). pH of the fermentation broth also plays a major role in better separation. It is important to perform some chemical pretreatment of the fermentation broth to prevent the denaturation of enzyme activity and to avoid the clogging of filters (Mukhopadhyay *et al*., 1990; Venkata Naga Raju and Divakar, 2014).

Concentration and precipitation of enzymes

The concentration of the culture filtrate can be obtained by various methods such as ultrafiltration, ammonium sulphate precipitation, dialysis, lyophilization (Naidu, 2011) and Centrifugation (Ullah *et al.,* 2022). The most common method used for recovery of enzymes from fermentation broth is precipitation. Precipitation is generally influenced by the addition of salt or an organic solvent to the fermentation broth, which lowers the solubility of the desired enzymes in an aqueous broth (Dey *et al.,* 2012).

Chromatographic techniques

Protease can also be purified by a combination of different chromatographic procedures such as ion exchange and hydrophobic interaction chromatography (Khare *et al.,* 2005), gel filtration and ion exchange chromatography (Bayoudh *et al.,* 2000) affinity chromatography (Sareen and Mishra, 2008), ion-exchange chromatography (Carmona *et al.,* 2011), Sephadex G 100 column chromatography (Savitha *et al.,* 2011). Depending on the pH of the protease, the choice of chromatography techniques varies.

Industrial application of proteases

Proteases discover wide range of applications in industries such as food, detergent, leather and pharmaceutical industries. Stringent pollution control norms have resulted in enzyme based development of environmental friendly technologies.

Food Industry

Dairy Industry

In the food industry, proteases are used extensively in the manufacture of cheese. The proteases produced by GRAS (Genetically Regarded as Safe) cleared fungi are gradually replacing chymosin in cheese making (Mamo and Assefa, 2018; Mercado-Flores *et al.,* 2016). In 1988, chymosin produced through recombinant DNA technology was first

introduced to cheese makers for evaluation. Genencor International increased the production of chymosin *Aspergillus nigar* var. awamori as host. At a commercial level the most important native enzyme for cheese making is isolated from the mold of *Rhizomucor miehei* and *Rhizomucor pusillus* (Alahmad Aljammas *et al.,* 2022; Sambo *et al.,* 2021).

Baking Industry

In baking processes wheat flour is a major component and it contains insoluble protein (gluten), which determines the properties of the bakery dough. In the baking process, wheat gluten modified by Endo- and exo proteases from *Aspergillus oryzae.* The dough mixing time is reduced by protease addition and the result is increased in loaf volume (Souza *et al.,* 2015). The enzyme is used in the baking industry as a processing aid to strengthen gluten in the dough system. Protease is used in protein degradation for the manufacturing of baked wafers, cookies and biscuits from the flour (Fazilat, 2016; Naveed *et al.,* 2021).

Synthesis of aspartame

The use of aspartame as a non caloric synthetic sweetener has been approved by the Food and Drug Administration. Aspartame is a dipeptide composed of L-aspartic acid and methyl ester of L-phenylalanine. The L configuration of the two amino acids is responsible for the sweet taste of aspartame. The chemical method of aspartame production is expensive and stereo specificity maintenance is crucial. Enzymatic amalgamation of aspartame is therefore preferred (Rao *et al.,* 1998).

Brewing Industry

The brewing industry is a major user of proteases. In this industry proteases are used to remove the haziness in the brewing industry. Presence of certain proteins in the beer, it looks a little hazy at the freezing temperature and also affects the beer shelf life. Removal of these proteins from the beer helps it to look clear (Thakur *et al.,* 2018). In the making of fruit juices and certain alcoholic beverages are produced from *A. saitoi* used to degrade the proteins that cause turbidity (Pandey *et al.,* 2006). Recently Byarugaba-Bazirake *et al.* (2013) proved commercially available proteases used in the production of banana wine. The enzymes of proteases are used in chill proofing, a treatment designed to prevent the precipitate formation during cold storage. Lopez and Edens (2005) found addition of proline-specific proteases from *A. niger* effectively prevented chill-haze formation in beer. It suggests the hydrolysis of proline-rich proteins resulted in a peptide fraction that is unable to interact with the polyphenols.

Soy sauce production

Soybeans serve as a rich food source, due to their high content of good quality protein. Proteases have been used from ancient times to prepare soy sauce and other soy products. The alkaline and neutral proteases of fungal origin play an important role in the processing of soy sauce. Proteolytic modification of soy proteins helps to improve their functional properties. Treatment of soy proteins with alcalase at pH 8 results in soluble hydrolysates with high solubility, good protein yield and low bitterness. The hydrolysate is used in protein-fortified soft drinks and in the formulation of dietetic feeds (Mahajan and Badgujar, 2010).

Meat tenderization

Worldwide, India is endowed with the largest buffalo population. It accounts for 59.08% of the world buffalo population. Concerning 10.66 million buffaloes are slaughtered annually producing 1.47 million MT buffalo meat. They are slaughtered chiefly for meat. The byproducts from slaughtered animals are also good value. Tripe of buffalo is one of the important edible offal and weighs about 4.36 to 5.45 kg/ animal. Commercial exploitation of tripe for development of processed product manufacture is limited. The reason is its poor functional properties and inherent toughness due to high content of collagen. It is vital to develop technologies for utilization of tripe into processed product manufacture by reducing its toughness. The treatments with proteolytic enzymes are one of the popular methods to increase meat tenderization (Mahajan and Badgujar, 2010). During the heating process, different meat proteins are denatured and its cause the structural changes in the meat like cell membrane destruction, meat fiber shrinkage, Shrinkage of aggregation and gel formation of myofibrillar and sarcoplasmic proteins and solubilize of the connective tissue (Tornberg, 2005; Xu *et al.,* 2013). The degree of tenderness can be related to those of connective tissue, myofibrils and sarcoplasmic protein (Lawrie, 1991).

Detergent Industry

Major microbial protease finds application in the detergent industry, which accounts for around 25% of the total worldwide enzyme sale. In all kinds of detergents, proteases are one of the standard ingredients. Those used for household laundering to reagents used for cleaning contact lenses or dentures. Beg and Gupta (2003) reported proteases were used for the first time in 1914 as detergent additives. These proteases also help in removing the stains such as of blood, proteins secreted from our own body and food such as milk, egg, meat, fish etc. (Gupta et al., 2002). In detergent industry proteases are considered as the best due to its ionic strength. Protease ionic strength coincides with the pH of detergent solution and therefore increases the effectiveness of the detergent (Thakur *et al.,* 2018). Proteases used as detergent additives should be effective in very small amounts over a broad range of pH and temperature; it has a longer shelf life (Cherif *et al.,* 2011).

Leather Industry

In leather processing the chemical methods lead to environmental pollution (Solid wastes and liquid effluents) and also affect living organisms. This chemical processing of leather is overcome by the usage of protease enzymes (Aruna *et al.,* 2014). Leather processing involves a number of stepladders such as soaking, dehairing, bating, degreasing and tanning etc. More than a century proteases have been used for bathing, their use for soaking and dehairing is more recent only. The major problem of using chemicals in leather processing causes suffocation with the smell and also releases some unpleasant and harmful gases which can even cause death also (Jha *et al.,* 2004). Increasing the usage of enzymes in the dehairing and bating process not only prevents the problem of pollution, but is also effective in time saving with better quality of leather (Nilegaonkar *et al.,* 2011). The protease enzymes are used for selective hydrolysis of non collagenous constituents of the skin, removal of non fibrillar proteins like albumins and globulins in the pre tanning operations. The soaking process purpose is to swell the hide (Puvanakrishnan *et al.,* 1999). The needed amount of enzyme depends on the type of the leather whether it is soft or hard (Mahajan and Badgujar, 2010).

Photographic Industry

Silver is one of the expensive and noble metals used in large quantities in the photographic industry. Approximately 25% of the world's silver requirement is met through recycled silver of which 75% is obtained from photographic waste. The X-ray waste films contain 1.5 to 2% of silver by weight in their gelatin layer, which can be used as a good source of silver for a variety of purposes (Laxman *et al.,* 2010). The silver is recovered from photographic/X-ray film wastes by following methods like burning the films directly, oxidation of the metallic silver following electrolysis, stripping the gelatin-silver layer using various chemicals and enzymatic hydrolysis of gelatin (Nakiboglu *et al.,* 2003). The conventional and most primitive method of burning the films directly for silver recovery generates the undesirable foul smell, causing environmental pollution and the silver and gelatin coated

emulsion cannot be recovered from the polyester film (Laxman *et al.,* 2010). Usage of chemical methods causes environmental hazards, time consuming and very expensive. For these disadvantages of the above methods, enzyme based methods are the best alternative option, which minimize the environmental hazards. Ingale *et al.,* (2002), Nakiboglu *et al.,* (2003), Masui *et al.,* (2004) using microbial proteases for the silver recovery from photographic/X-ray films.

Pharmaceutical Industry

Proteases in pharmaceutical industries used to eliminate the keratin in acne or psoriasis, elimination of human callus and degradation of keratinized skin, depilation and preparation of vaccines (Brandelli *et al.,* 2010; Millet *et al.,* 2001). Oral administration of proteases has been used as a digestive aid to correct lytic enzyme deficiency syndromes, these proteases derived from *Aspergillus oryzae* (Luizym and Nortase). Subtilisin or clostridial collagenase is used in combination with broad-spectrum antibiotics in the burns and wounds treatment. Alkaline protease to be able to replace trypsin in animal cell cultures, which is produced from *Conidiobolus coronatus* (Chiplonkar *et al.,* 1985; Jabalia *et al.,* 2014). Microbial proteases are used for the treatment of dermal ulcers, cancer, cystic fibrosis, cardiac problems, digestive disorders and inflammation (Chanalia *et al,* 2011).

Therapeutics

In the medicinal field protease for diagnostic and therapeutic use is widely accepted. The proteases are mainly related with the development of anticancer, anti-inflammatory, antimicrobial and clot dissolving agents. Both strains of bacteria and fungi have contributed equally in defining therapeutic potential of protease (Banik and Prakash, 2004). The most obvious use of protease enzymes is to assist digestion. Injection of some foreign proteases into humans reduces tissue inflammation and pain. Protease usage helped reduce the discomfort of breast engorgement in lactating women. The use of immobilized alkaline protease from *Bacillus subtilis* possessing therapeutic properties has been studied for development of soft gel based medicinal formulas, ointment compositions, gauze, non-woven tissues and new bandage materials (Furhan and Sharma, 2014). Alkaline fibrinolytic protease has been reported to preferentially degrade fibrin suggesting its future application in thrombolytic therapy and anticancer drugs (Yoo *et al.*, 2010; Mukherjee and Rai, 2011). Alkaline proteases with collagenases activity are increasingly used in therapeutic applications in the form of the slow-release dosage preparation. A new semi-alkaline protease by *Aspergillus niger* LCF9, which has high collagenolytic activity (Furhan and Sharma, 2014).

Silk degumming

Raw silk of the threads must be degummed to remove sericin, which is a proteinaceous substance that covers the silk fiber. Traditional method of degumming performed in an alkaline solution containing soap. Usage of this method the fiber is attacked due to the harshness. The usage of proteolytic enzymes is the best method because the enzyme removes the sericin without attacking the fiber. In recent studies, tests with high enzyme concentrations show no fiber damage. The enzyme treated silk threads are so strong compared with the traditional one (Romsomsa *et al.,* 2010; Furhan and Sharma, 2014).

Table 1 Major fungus producing various types of proteases

Type of protease	Sources	References
	Aspergillus oryzae MTCC 5341	Singh et al., 2010
	Monilinia fructigena	Hislop et al., 1982
	Aspergillus niger I1	Siala et al., 2009
	Aspergillus niger	Mukhtar, 2009
Acid protease	*Aspergillus awamori*	Sinha and Sinha, 2009
	Aspergillus oryzae	Amrane et al., 2013
	Fusarium sp.	Pekkarinen et al., 2000
	Penicillium sp.	Papagianni, 2014
Neutral protease	*Pleurotus sajor-caju*	Uma et al., 2012
	Arthrobotrys oligospora	Minglian et al., 2004
	Aspergillus flavus	Malathi and Chakraborty, 1991
	Trichoderma koningii	Ciloci et al., 2014
Alkaline protease	*Rhizopus oryzae*	Irfan et al., 2014
	Penicillium sp.	Revathi et al., 2022
	Aspergillus flavus AS2	Roja Rani and Prasad, 2013

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