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Review Article

Commercial Potential of Fungal Protease: Past, Present and Future Prospects

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ABSTRACT

Proteases are one of the most important classes of proteolytic enzymes widely distributed in the animal kingdom, plant and as well as microbes. These enzymes possess the enormous commercial potential and have been used in several industrial processes, including food industry, leather processing, silk processing, detergent industry and therapeutic applications. The major source of commercial enzymes including protease in microbial world not only yields high quality of enzyme but also enzyme with diverse substrate specificity. Bacterial and fungal enzymes including proteases are widely accepted for different industrial process in last few decades. However, fungal protease are more promising for commercial application as these microorganism are more resistant to harsh climatic conditions and produces proteins/enzymes in their habitats. Fungal species are competent in expression of enzymes in psychrophilic, mesophilic and thermophilic conditions. In last few decades, psychrophilic and thermophilic enzymes were identified for their commercial potential worldwide. Several fungal strains have been isolated and characterized for ideal source of protease production. In this author has given emphasis on commercial potential of protease from fungal source.

Keyword: Protease; thermophilic and psychrophilic protease; fungal protease; food processing; silk degumming; leather processing

INTRODUCTION

Proteins are the most versatile molecules in biological world meant for several function including structural organization and catalytic capabilities [1]. Enzymes are the biocatalyst with higher precession and accuracy while performing biochemical reactions. The entire biological world has been evolved with diverse enzymes and microbial population shown tremendous potential in

production of these amazing molecules. Both bacterial and fungal species are competent in production of these versatile molecules which have served mankind since several decades [2-3]. In recent years there has been a phenomenal increase in the use of proteases as industrial catalysts. There are several advantages while using enzyme as biocatalyst over conventional chemicals. Most significant achievement made using these biocatalysts in last one decade is chemical free industrial process which led to environmental damage and pollution. These amazing molecules offer a high degree of substrate specificity which leads to efficient biochemical process with negligible error in product formation [4]. Further, enzymes obtained from diverse biological sources perform several catalytic reaction ideal for modern revolutionary industrial demand. Thermal and chemical stability further boosted significance of these molecules as these enzymes can catalyse reaction in different temperature and in the presence of various chemicals [5].

Another aspect which efficiently drives modern industrial operation is reuse of catalyst to cut down production cost. Here enzyme emerged as key player which can be used for several times in continuous reaction after immobilization on appropriate material [6]. These enzymes had made remarkable land mark in several industries including food processing, detergent industry, leather and textile industry [7-8]. To meet commercial demand numerous biological sources were explored and tonnes of enzyme had been produced in last few decades. Among these potential sources used for enzyme production microbial (bacterial and fungal) species lies on the top with enormous capability of enzyme production [9]. Further, advancement in biological science especially bioengineering, molecular biology and protein engineering enabled to produce enzyme in large scale and specific to reaction [10]. Significance in using enzyme from microbial sources over conventional catalyst is ease in downstream process where purification of product became easier. Food processing, leather making and silk degumming are driven by microbial protease and fungal protease constitutes more than 30% of enzyme in all these process. It is widely accepted that protease are contributing their potential in modern therapeutics including development of anti-inflammatory drugs, clot dissolving agents, antimicrobial and cancer treatment [11]. Finding a novel and suitable source for such amazing molecule has been daunting challenges for decades which led to exploration of microbial diversity and use of modern recombinant DNA technology to fulfil commercial demand.

Protease

Proteases are abundantly and widely distributed in biological world including plant, animal and microbes [12]. A protease also called as peptidase or proteinase is group of enzyme that performs proteolysis known as hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein [13]. Proteases constitute more than 70% of industrial enzyme alone and microbial sources (bacterial and fungal) are leading supplier of these enzyme. These enzymes possess catalytic activity in broad range of temperature and pH [14-15]. The entire protease group has been evolved during organic evolution where catalytic site for each enzyme became key factor in driving biochemical reaction. Such catalytic promiscuity has emerged as key tool for modern commercial industry where several biochemical reactions can be catalysed by single group of enzyme [16].

However, discovery of psychrophilic and thermophilic enzyme including proteases enhanced their catalytic spectrum. In current prospect, several proteases have been isolated and produced in a recombinant way to catalyse reactions in temperature ranges 5^0C-100^0C and in pH range 2-10 [17-18]. The physiological role of protease has been

established and these molecules participate in various physiological reactions including digestion, defence mechanism. All the protease exhibits common mechanism of action as acting on carbonyl- carbon bond of peptide group. However, different protease utilize different strategies to generate nucleophile to attack of carbonyl-carbon (O=C-C) bond. The most acceptable classification of enzyme is based on the molecular mechanism of enzyme. The core amino acids in process of catalysis as active site of enzyme define specificity and catalytic efficiency of any biocatalyst [19].

Classification of Protease

The protease group is vast and constitute more than 70% of commercial enzymes with diverse substrate and catalytic capabilities. The proteases have been classified based on several criteria such as targeted amino acid for hydrolysis, chemical environment and type of substrate [20]. However, most convincing mode of classification is based on amino acid involve in hydrolysis and on that basis proteases have been classified into six major groups:

1. Serine Proteases (EC 3.24.21)
2. Threonine proteases (EC 3.4.25)
3. Cysteine proteases (EC 3.4.22)
4. Aspartate proteases (EC 3.4.23)
5. Glutamic acid proteases (EC 3.4.19)
6. Metalloproteases (MMPs) (EC 3.4.24)

Serine proteases (EC 3.24.21)

The serine proteases contribute major industrial and therapeutic protease where serine serves as the nucleophilic amino acid. In this class of enzyme chymotrypsin/trypsin and subtilisin are commercially available serine protease [21]. With their tremendous scope in industry and medicine several recombinant serine protease have been produced and are in commercial use. Till date, 16 proteases superfamilies' have been reported and MEROPS database which is protease database was developed as repository of all serine proteases from various sources [22]. Each protease superfamily uses the catalytic triad or dyad in a different protein fold and so represents convergent evolution of the catalytic mechanism. Among all these protease super-families, four distinct and most significant from commercial point of view are trypsin-like, chymotrypsin-like, elastase-like and subtilisin-like proteases [23].

Threonine proteases (EC 3.4.25)

However, threonine protease is another industrially significant protease where threonine (Thr) residue lies on catalytic site. These proteases have much significance in physiology and proteasome and acyltransferases are classical example [24]. Till date, five families along with two separate superfamilies identified from various sources. The two major threonine protease superfamilies classified as the Ntn fold proteosomes (superfamily PB) and the DOM fold ornithine acyltransferases (superfamily PE). This classification is based on organic evolution of threonine protease denotes an independent, convergent evolutions of the same active site [25].

Cysteine proteases (EC 3.4.22)

Further, cysteine proteases also known as thiol protease possess great importance in industrial applications. These proteases perform catalysis associated with nucleophilic thiol in a catalytic triad or dyad. These proteases are primarily present in all the fruits including papaya, pineapple, fig and kiwi fruit. Cysteine proteases had shown their potential in poultry industry and are key competent of meat tenderizers. Additionally, cysteine proteases have been employed as therapeutics enzyme in controlling viral infection [26]. The 14 different superfamilies have been reported under cysteine protease and as per MEROPS protease database the

evolution of all these families denotes convergent evolution of catalytic site [27].

Aspartate proteases (EC 3.4.23)

Aspartic acid proteases are quite different from other proteases as their contribution in maintaining physiological functions. The classical examples are pepsins, cathepsins, and renins key enzyme which maintain physiology [28]. This group of enzyme shows their activity in lower pH and showing resemblance with acid proteases. Till date four different families under aspartase proteases have been identified from various sources and are denoted as Family A01 (Pepsin family), Family A02, Family A22 and Family Ax1. The organic evolution of aspartate protease families as per MEROPS database is due to ancestral gene duplication [29].

Glutamic acid proteases (EC 3.4.19)

Most important glutamic acid proteases which are widely present in fungal species are key tenzyme for food processing and modern therapeutics such as antitumor and anticancer [30]. Recently, the glutamic protease family re-classified as a sixth catalytic type of peptidase (family G1) in the MEROPs database. [31]. The presence of glutamic acid protease in fungi is unclear as there is complete lack of such enzyme in fungal physiology. But with large number of fungal isolates and the divergence of glutamic acid protease suggested concept of gene conservation and the evolution of protein families.

Metallo-proteases (MMPs) (EC 3.4.24)

A novel protease group called metalloprotease which enormously used in drug development involve metal ion for catalysis. Due to wide range of substrate affinity and diverse sources of proteases the applications of proteases are unique and widely applicable in different industries [32-33]. Further, thermostable protease discovery solve many problems encountered while using conventional proteases such as storage and industrial operation running in higher temperatures. Further, immobilization techniques of modern enzyme engineering technology enhanced commercial significance of these enzymes [34]. Apart from these major groups proteases also classified based on reaction environment. The catalytic capability of enzyme including protease affected by pH in larger extent and hence proteases have been classified based on maximum catalytic activity shown in particular pH. The three major sub classifications have been made as acid proteases, alkaline proteases and neutral proteases. This classification of protease lies on principle of reaction condition of enzymes, acid proteases perform enzymatic catalysis in lower pH range 2-6, while alkaline protease in higher pH 8-10. However, neutral protease shows their maximum activity near a pH of 7.0. This classification does not fall in any scientific criteria precisely used for enzyme classification however it offer ease in selection of enzyme for desired industrial process. Further, such classification is also useful in designing reaction conditions in order to avoid production loss [35].

Historical background and present demand

Since the ancient time period, the enzyme technology was associated in fermented products as part of food and medicine. Over the last few centuries, bakery products, alcohol and vinegar have served mankind as part of conventional food and liquor across the globe [36]. Fungi played a crucial role to produce these products available for human consumption, even without knowing exact mechanism. However, several mushrooms species were known as food stuff with high nutritional values [37-38]. Apart from these applications, numerous fugal species were used as part of folk medicine to combat several life threatening disorders. As for concern of industrial applications, the use of fungal extract of hunting animals (toxin), cleaning natural fabric such as silk and recycling of waste material was known since ancient time

period. In the last century with advancing technology in molecular biology these potential enzymes for the commercial application from various fungal sources were defined [39-40]. Several novel species of fungi were isolated and characterized for large scale production of proteases. Among these species Aspergilous, Yeast, Candida, Penicillium and Cephalosporium are major one and were employed for large scale production of proteases and other enzymes [41-42]. Over the last few decades, several fungal strains were isolated and characterized for the source of the proteases which can catalyze biochemical reactions in different pH, temperatures [43-44]. Further, recombinant DNA technology and enzyme engineering technology has played crucial role in large scale production and precise reuse of the proteases produced [45]. Proteases have a large variety of applications in various industries and many investigations are focused on the discovery and characterization of novel naturally occurring proteases from sources that have been overlooked. The proteases produced from different fungal species are in the use of several industrial operations, including, food processing, making of leather, fabric industry, meat tenderazation and as therapeutics [46].

Sources of Commercially Significant Proteases

To meet commercial demands, large scale production of these enzymes became necessary and hence several sources were explored including fungi. The fungi possess diverse habitat, grow ubiquitously and produce various proteins/enzymes for their survival. Among these enzyme proteases constitutes a major class along with other enzymes such as lipase, cellulase, xylinase and pectinase. Based on habitat fungi are classified as psychrophilic, mesophilic and thermophilic emerged as potential sources of commercial enzymes including proteases.

Psychrophilic Fungi

The microbial world has been key source of various enzymes for industrial and therapeutic application since many decades. Bacterial species are on the top concern for large scale production of enzyme worldwide [47]. However, fungi also have contributed tremendous scope as potential source of proteases and other enzymes. Fungi are widely distributed organism are capable in producing several class of proteases including cold tolerant protease, protease acting on normal temperature and thermostable protease acting in higher temperature [48]. Several deep sea fungi are capable in producing psychrophilic protease including *Aspergillus terreus, Beauveria brigniartii* and *Acremonium butyri.* Another class of fungi called as thermophiles such as *Canariomyces thermophila, Thermomyces ibadanensis, Talaromyces thermophiles, Myriococcum thermophilum and Dactylomyces thermophiles* are capable in producing commercial enzyme including protease that act in higher temperature (table 3) [49]. Several strain of yeast including *Candida lipolytica, Yarrowia lipolytica* and *Aureobasidium pullulans* have been reported in large scale production of protease [50].

Mesophililic fungi

The mesophilic fungal strains are also competent in production of large scale of commercial protease enzymes. The major class of mesophilic fungi is *Aspergillus* which contribute more than 25% of protease produced from fungal source which includes *Aspergillus candidus, A. flavus, A. fumigatus, A. melleus, A. niger, A. oryzae, A. sojae, A. sulphurous and A. sydowi.* Such diverse fungal biodiversity results in various kinds of protease catalyse numerous biochemical reactions [51-52]. Industries such as food processing basically utilized cold tolerant enzymes including protease and marine environment is enormous sources of

protease producing enzymes. Similarly, hot springs and marshy area are the key source for thermophilic fungi ideally produces protease and other enzyme for commercial application (table 3) [53]. It is widely accepted that mesophilic fungi are distributed ubiquitously and need to explore for ideal candidate source [54].

Thermophilic fungi

Among the eukaryotic organisms, only a few species of fungi have the ability to thrive at higher temperature. Such fungi comprise thermophilic and thermotolerant forms, which are arbitrarily distinguished on the basis of their minimal and maximum temperature growth. In such habitat, these strains produces heat shock proteins (HSPs) allow organism to sustain in hostile environment. Thermophilic proteases are produced by thermophilic microbes including bacteria and fungi possess great scope for commercial application [55-56]. These proteases withstand with their proteolytic activity at higher temperature ideal for several industrial process. Deep Ocean and hot spring are key geographical area for hunting these proteases which meet commercial demands [57-58]. Several fungal strains naturally build their habitat in such harsh condition and produce thermostable proteases. A list of fungal strains isolated from such harsh conditions and protease produced for commercial application is shown in table 2. Till date more than 150 different fungal strains have been isolated and characterised that are capable in producing such enzymes [59].

These proteases not only offer catalytic activity in higher temperature but also possess longer shelf life and provide ease in storage [60]. There is tremendous potential in fungal biodiversity to isolate such proteases with higher catalytic potential. Enzymes of thermophilic fungi have been studied primarily to explore their suitability in bioprocesses and, to a lesser extent, to probe similarities and differences in physicochemical properties between enzymes from mesophilic and thermophilic fungi [61]. Thermophilic fungi also synthesize heat shock proteins (HSPs) and acquire thermo tolerance. They observed that conidia of *Thermomyces lanuginosus*, germinated at 50°C and heat shocked at 55°C for 60 min prior to exposure to 58°C (Table 3) [62]. Later, *Thermomyces lanuginosus* strains were characterized as excellent source of xylanase production another commercial significant enzyme.

Present study

With the increasing potential of fungal derived enzymes, a study was carried out to isolated novel fungal strains and enzyme production by solid state fermentation. To isolate novel fungal strain various samples were explored including cow dung, snuff, birds nesting material, municipal refuse and compost. The fungal isolated were examined by chemical characterization and microscopically. Further, genomic characterization was achieved by 16 S RNA analysis. Four different strains of fungi were isolated and characterized as *Aspergillus flavus, Humicola grisea (Thermomyces lanuginosus), Rhizomucor pusilis* and *Aspergillus fumigatus* by biochemical and 18 S RNA analysis [63]. To produce desire proteases from the novel isolated fungal strains solids state fermentation was carried out. Different growth media and parameters were selected for large scale enzyme production including macro and micro nutrients. The enzyme (protease) produced from solid state fermentation was purified, quantified and characterized. The proteolytic activity of purified protease was evaluated by proteolytic assay using casein as substrate. Further, the enzyme was immobilized to evaluate activity, proteases produced to enable the reuse of these potential proteases [64] (table 4 & figure 1). The kinetics parameters were determined for purified protease by different kinetics studies including, Michaelis–Menten kinetics (MM plot) and Lineweaver–Burk kinetics (LB plot).

Table 1: Cold tolerant fungal strains and enzyme produced for commercial important along with protease

Sl No	Fungal Stain	Habitat	Enzyme Produced	Commercial Application
1.	*Debaryomyces hansenii*	5-10^{0}C	β-Glycosidase and cellulase	Food and milk processing
2.	*Saccharomyces cerevisiae*	10-15^{0}C	Protease and lipase	Food processing
3.	*Mrakia frigida*	5-10^{0}C	Cellulase and Trehalase	Paper and milk processing
4.	*Candida parapsilosis*	5-10^{0}C	Protease and lipase	Food and Fruit processing
5.	*Candida maltosa*	10-15^{0}C	α-amylase, cellulase, glucoamylase,	Milk and bakery Industry
6.	*Debaryomyces castellii*	10-15^{0}C	lipase, protease, and xylanase	Food and milk processing
7.	*Candida mogii*	5-10^{0}C	Xylanase, Protease and lipase	Fruits, Food and milk processing
8.	*Kluyveromyces marzianus*	5-10^{0}C	Xylanase, Protease and lipase	Fruits, Food and milk processing
9.	*Saccharomyces pombe*	5-10^{0}C	Lipase and alkaline protease	Food Processing
10.	*Saccharomyces sp.*	5-10^{0}C	All major protease and lipase	Fruits and Food Processing

Table 2: Mesophilic fungal strains, enzyme produced including protease for commercial application

Sl No	Fungal Stain	Habitat	Enzyme produced	Commercial Application
1.	*Aspergillus candidus*	Mesophilic	Alkaline Protease	Animal feed, Textile, Leather
2.	*Aspergillus. flavus*	Mesophilic	Cellulase and Protease	Pulp, paper and Textile
3.	*Aspergillus. fumigatus*	Mesophilic	Amylase and Protease	Agriculture, Animal feed, Leather
4.	*Cephalosporium sp. KSM*	Mesophilic marine	Cellulose and Trehalase	Pulp and paper production
5.	*Chrysosporiumkeratinophilum*	Mesophilic marine	Protease and β-Glycosidase	Paper industry and detergent
6.	*Conidioboluscoronatus*	Mesophilic	Invertase and Cellulase	Food processing, Pulp and paper
7.	*Entomophthoracoronata*	Mesophilic marine	Lipase and alkaline Protease	Food processing and Leather
8.	*Fusariumeumartii*	Mesophilic marine	Alkaline Protease	Textile, Leather and Detergent
9.	*Aspergillus. oryzae*	Mesophilic	Alkaline Protease	Textile, Leather and Detergent
10.	*Tritirachium album Limber*	Mesophilic marine	Alkaline Protease	Textile, Leather and Detergent

Table 3: Thermophilic fungal strains, enzyme produced including protease and commercial applications

Sl No	Fungal Strain	Habitat	Enzyme Produced	Commercial application
1.	*Canariomyces thermophila*	45-60^{0}C	Serine and alkaline protease	Leather and detergent
2.	*Chaetomium mesopotamicum*	50-60^{0C}	Alkaline Protease	Leather and detergent
3.	*Coonemeria verrucosa*	45-50^{0}C	cellulase, lipase, phytase, protease	Animal feed and agriculture
4.	*Malbranchea cinnamomea*	45-55^{0}C	Serine and alkaline protease	Detergent and leather
5.	*Myriococcum thermophilum*	40-60^{0}C	α-amylase, cellulase, lipase protease	Detergent, animal feed
6.	*Talaromyces thermophilus*	60-75^{0}C	cellulase, glucoamylase, protease, xylanase	Biofuel, leather and detergent
7.	*Thermomyces ibadanensis*	40-55^{0}C	Serine Protease and Lipase	Waste treatment
8.	*Thielavia australiensis*	40-45^{0}C	cellulase, glucoamylase, protease	Biotechnology application
9.	*Myceliophthora thermophila*	48-56^{0}C	Serine and alkaline Protease	Detergent and leather
10.	*Melanocarpus albomyces*	50-55^{0}C	α-amylase, lipase, protease, xylanase	Textile and fabric

Table 4: Large scale production by solid state fermentation and analysis of protease activity in free and after immobilization of novel isolated fungal strains. The wheat bran was source of carbon for fungal growth and proteolytic activity was determined by protease assay using casein as substrate from enzyme.

Substrate wheat bran (%)	*Protease activity (U/ml) Aspergillus fumigates*		*Protease activity (U/ml) Humicola gricea*		*Protease activity (U/ml) Rhizomucor pusilis*		*Protease activity (U/ml) Aspergillus flavus*	
	Free Enzyme	**Immobilized**	**Free Enzyme**	**Immobilized**	**Free Enzyme**	**Immobilized**	**Free Enzyme**	Immobilized
2.5	315.25	325	718.00	615.00	350.00	318.20	18.00	25
5.0	321.75	330	761.00	518.55	325.00	290.70	21.00	28
7.5	335.25	320	833.00	760.25	365.00	310.55	33.00	30
10	354.00	374	946.00	862.33	315.00	270.00	46.00	44
12.5	346.25	329	921.00	840.30	344.00	310.25	41.00	40

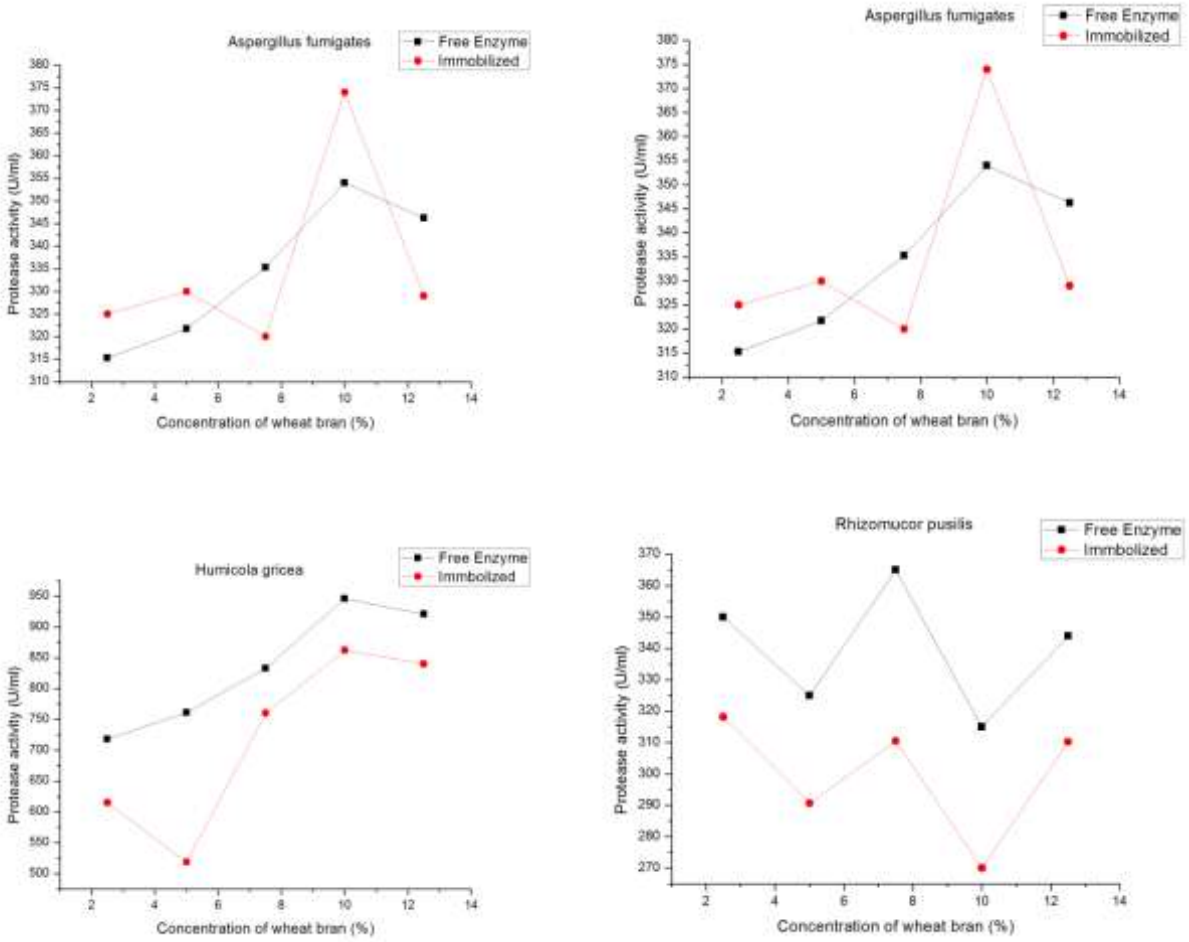

Fig. 1. Proteolytic activity in free and immobilized protease produced from four novel isolated fungal strains. The Proteases were produced using wheat bran as source of carbon to growing culture and proteolytic activity was determined by protease assay using casein as substrate.

Commercial application of fungal proteases

Proteases are most significant enzyme group with several commercial applications employed in food processing, leather making, as detergent, mean tenderization and for therapeutic applications [65-66]. In case of food processing, milk industry, beverages and processing of grains, bakery utilizes enormous amount of protease and other enzyme from several sources including fungi. Ethanol fermentation, production of detergent for biological application has increased exponentially in last decades [67-68]. Large scale processing of meat and silk fabric also need protease which fetch with climatic issues and quality of meat and silk fabric [69-70]. Some of significant applications of protease are listed in table 1, 2 and 3 derived from various fungal sources.

i. Protease in food processing

Food processing utilizes several enzymes for production of quality and nutritional food products. Entire bakery industry relies on single fungi known

as yeast: Saccharomyces that delivers several kinds of food products [71]. Further, processing of fruits and storage of their juices for long shelf life need enzyme treatment and primarily protease are employed. Preparation of milk products and candies need complete processing catalysed by series of enzyme including protease [72]. Beverage industry both soft and hard drink need a complete processing of substrate for elegant flavour and shelf life of product. Processing of tea, coffee and coca needed a precise oxidation of raw material (ripen seeds, leaves and berry) to produce complete products and enzyme such as protease play crucial role. Ethanol principal element of hard drink is product of sugar fermentation carried out by series of biochemical reactions catalysed by group of enzyme including different type of proteases [73]. Hence, proteases have emerged as key enzyme for food industry and offer catalysis in different environment form low temperature to harsh high temperature [74].

ii. Protease in Leather making

Leather is prime commodity for Indian in earning foreign currency and India is the second largest exporter of processes and unprocessed leather on world map after china. Leather making a complex process involves several unit operation and dehairing and other protein from dermis region of raw hide [75]. Old and conventional methods used in leather making are less efficient and utilized massive amount of chemical that leads lead to environmental pollution. Several studies have suggested that the enzymatic processing of leather not only solves environmental issues but also yield better quality leather. Leather making which primarily carried out at higher temperature involves several thermostable proteases [76-77]. Thermophilic fungi have emerged as potential sources for leather processing along with bacterial proteases. Further, cleaning of hair and their biological digestion had yielded in several vital amino acids used as dietary supplement [78]. The use of thermostable protease became integral part of modern leather making and thermophilic fungi are vital source of such commercially significant enzymes [79]. Several studies have been carried out to reuse of these potential enzyme after immobilization for leather making [80].

iii. Protease in Textile Industry

Another most significant area of commercial application of protease is textile industry where a final elegant texture is provided by enzyme treatment [81]. Silk is most precious fabric in world used for several applications processed by thermostable protease to remove gum and other impurities lies of core protein fibre on silk. Silk degumming a growing industry, enormously utilize protease and results in high quality silk [82-83]. Synthetic fabric also subjected to protease treatment for elegant and smooth finish. Fungal proteases are key enzyme in fabric industry since decade and growing tremendously. Indian sericulture has grown double in last one decade and consumption of protease also enhances in several folds [84]. Use of such method not only offer good quality fabric but also impart mechanical strength to fibre [85]. Further use of enzyme including protease minimizes chemical detergent associated with large scale of environment pollution [86].

iv. Protease as detergent

Biological determent had great attention in current scenario where massive amount of enzymes have been implemented in detergent making primarily protease [87]. Both domestic and industrial detergents are having major constituents from protease. The biological detergent are primarily used in cleaning of large boiler in industries, hospital are another key consumer of biological detergent along with poultry industry [88]. Discovery of thermostable protease further enhanced efficiency of biological detergent. In modern days both domestic and industrial detergent are utilizing protease in higher extent [89]. In order to meet

commercial demand fungal enzymes have become key source for these amazing molecules.

v. Therapeutic application of proteases

The medicinal application of protease for diagnostic and therapeutic is widely accepted and several enzymes are in use since many years. Proteases are primarily associated with development of anti-cancer, anti-inflammatory, antimicrobial and clot dissolving agents. Both bacterial and fungal stains have contributed equally in defining therapeutic potential of protease [90]. Some of major therapeutic applications are –

a) Anti-Inflammatory

Inflammation is physiological response against microbial infection and mechanical injures which is associated with large scale of plasma accumulation along with immune cells. The available treatment to combat inflammation lies on use of Non-Steroidal (NSAID) drug in acute cases while steroidal drugs in chronic conditions. However, such management of inflammation is associated with several complications including side effects [91]. To combat these complications COXII specific drugs have been formulated and launched in market. These COX II specific drugs are much expensive and are not choice for inflammation. Enzymes, especially protease emerged as better option to combat both acute and chronic inflammation in reasonable price. Serratiopepetidase is most effective protease available for use in management of inflammation [92]. This enzyme is generally produced from Serratia species of bacterium but several fungal species were also explored for anti-inflammatory proteases. Additionally, a group of serine protease from Indian Earthworm has been studied for its anti-inflammatory potential and is underway for further molecular findings [93].

b) Anti-Cancer

Protease impart significant role in maintaining normal physiology and enzyme like caspase primarily involved in killing of abnormal cells. Caspase is a protease that is produced naturally and serves as key element of immune system. Several proteases are in trials for their anti-cancer therapeutics application [94]. These protease cytotoxic and imparts selective cancer management. Proteases from different biological sources became key enzyme in development of new generation anti-cancer drug molecules [95]. Advantage of using enzyme in cancer management over chemotherapeutic agents is to reduce toxicity impart by chemical based drugs. In the future these enzymatic drugs for cancer management will serve several cancer patients efficiently. In the year 2014, a serine protease from Indian earthworm was evaluated for its antitumor activity against breast cancer cell lines and result shown tremendous scope for protease in development of anti- cancer therapeutics. [96].

c) Clot dissolving agent

Blood vascular disorders both cardiac and cerebral are the leading cause for death worldwide. A blood clot in vascular pipeline is the result of abnormal functioning of plasma proteins meant for clot formation and dissolution. Blood clot and thrombus leads to blockage of blood supply and which further lead to several other severe physiological consequences including ischemia, embolism and tissue necrosis [97-98]. To combat these vascular hurdles an external clot dissolving agent need to perfuse in vascular pipeline. The available external clot dissolving agents called as thrombolytic are basically proteases [99]. In last few decade several protease from animal and microbial origin have been developed as refined external thrombolytic and are in clinical use. Tissue plasminogen activators (t-PA), Urokinase (u-PA), Streptokinase (SK), Staphylokinase (SAK), Earthworm fibrinolyitc Enzyme (EFE) and their recombinant variant are developed for clinical application [100]. These agents are efficient and act differently to dissolve blood clot but still there are several complications with these agents including their specificity, stability

and compatibility. Hence, there is an immense need of other protease for the development of external thrombolytic agents. Fungal proteases are having much scope in refining them into therapeutic molecules. Advantage of using fungal protease as clot dissolving agents is their stability and substrate selectivity. In the future these sources will emerge as key supplier for life saving drug [101].

d) Antimicrobial

The antimicrobial potential of proteolytic enzyme is well studied in last decade and protease has emerged as key enzyme. The cytotoxic nature of several protease work as efficient antimicrobial agents and many of enzyme based antimicrobial agents are in clinical use [102]. Several protease form animal and microbial origin have been characterized as antimicrobial and earthworm protease is among of these [103]. However, several antimicrobial peptides both from bacteria and fungi have been reported for antimicrobial nature. There is an immense need to explore fungal biodiversity to isolate these potent molecules.

Future prospects

Current prospect of commercial enzyme and future demand are increasing enormously and conventional method of enzyme production might fail to fulfil the demand. Hence, novel recombinant methods including large scale production of these proteases on high level expression system will be an answer [104]. Further, protein engineering is key technology that led to design of desired enzyme for commercial application in both industrial and therapeutic applications. Development of high catalytic values and kinetic parameter is only possible by refinement at gene level or through protein engineering [105]. Enzyme immobilization another tool, enables to design biocatalyst for reuse after immobilization of inert material. However, these practices are already in use and benefits enzyme industry in several aspects. The reuse of enzyme to cut down production cost and other provide stability to conventional enzyme for industrial process running in harsh conditions [106]. In future modern biological techniques will be severing enzyme industry for large scale production of protease and other enzymes.

CONCLUSION

Proteases are the leading enzymes with immense commercial potential are widely used in industrial and therapeutic applications. The thermophilic fungi comprise a group of twenty-three species belonging to nine genera. This is vast number in context with commercial demand and need to reanalyse to meet industrial demand. Fungal protease are not limited to industrial application, there therapeutic potential have been classified in last few decade constitute one of the largest groups of industrially important enzymes and modern biological techniques are essential to explore these amazing molecules for mankind. Thermophilic fungi are the chief components of the micro flora that develops in heaped masses of plant material, piles of agricultural and forestry products, and other accumulations of organic matter wherein the warm, humid, and aerobic environment provides the basic conditions for their development. There is an immense need to explore marine fungal biodiversity to find out fungal species competent in producing vast number of proteases.

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CONFLICT OF INTEREST

There is no conflict of interest.

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