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Short Review on the Production of Protease: New Trends and Methodologies

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ABSTRACT

Proteases are classified as highly demanded enzymes with numerous plant, animal, and microbial sources. They play important roles in industrial and biotechnological applications, which create money making opportunities. Finding novel and emerging methods by which the cost could diminish is in demand. Thus, the target of this review is a sufficient presentation on physiological and biological activates of this enzymes, introduction and different types, and finally, recent studies on the pronunciation of agroindustry and other inexpensive sources and methods to produce proteases.

Keywords: Protease, submerged fermentation, solid state fermentation, biotechnological applications.

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INTRODUCTION

Primarily, protease is classified among the most important enzymes, which is produced by biotechnology industries, and aligned 60% of entire market throughout the world [1,2]. This enzyme participates in different industries, food industries such as beverage, as well as cosmetics to perform proteolytic reactions in proteins and polypeptides [3]. While application of protease could be useful to catalyze high specificity reactions, but still, due to biochemical issues and being cost-needed, protease exploitation is in lower amounts [4].

Thus, finding inexpensive sources of production by means of submerged fermentation, and solid state fermentation methods using agroindustry wastes are in demand [5]. In solid state fermentation method, due to the special shape of their filaments, higher rate of fungal growth is observed, and thus, more enzyme yield [6].

Among fungal species, Aspergillus spp. are capable to produce a group of bioactive substances [7]. Aspergillus is a member of Ascomycota phylum and Deuteromycetes fungi. Aspergillus oryzae is used to degrade starch into its structural units, and also to produce rice vinegar [8-12].

This genus is also GRAS approved by FDA [13, 14]. Other wastes used in solid state fermentation are soybean meal, wheat and rice bran, apple, and banana [15, 16]. Therefore, SSF may reduce the costs of protease production and at the same time could be considered as environmentally friendly method [17].

In general, proteases are the extracellular enzymes possessing catalytic properties, and belong to hydrolases (i.e. through addition of water to peptide bonds and degrade proteins). Primarily, proteases are highly dependent on active sites, the type of substrates, and mechanisms of action. They play important roles

in physiochemical and commercial targets (including 65% of enzyme market). Other than that, proteases are highly demanded in different industries such as detergent, food industries in cheese-making, beer clarification, beverage, etc., due to their specific properties like extreme pH profiles. Since daily need to proteases in different industries, the obligation of creating new properties in enzymes to serve requested targets are needed [18].

Proteinases are employing 3 different Amino Acids including serine, histidine and aspartate, to perform their catalytic roles. The critical parameters of microbial production of protease are culture conditions in order to achieve commercial scale of production.

Food industries

Protease extracted from Aspergillus oryzae, considered as "safe", are being widely used in processing of foods. Aspergillus orzye enjoys having glycosyl hydrolases to decompose insoluble cellulose, and hereupon, makes this fungus as a major producer of mentioned enzyme [19].

Application of agro solid by-products (as a novel method) in order to produce inexpensive protease enzyme is a trend of rapid recycling agriculture waste products [20]

From the standpoint of economic, selecting microorganisms with ability to produce large scale of enzymes is important. For instance, Bacillus spp. are being recognized as economical species of extra cellular alkaline protease producers.

Bacillus strains derived proteases are used a lot due to their high pH range and temperature resistance. Among different protease species, serine types are more industrially used.

Protease enzymes are abundant in almost all of living organisms, and even in higher organisms 2% of gene codes are formed by these enzymes. These enzymes degrade large protein molecules to release smaller pieces of proteins (peptides) and amino acids, which are needed by human body. Some proteinases specialized in this regards are trypsin, chymotrypsin, and pepsin and the lysosome enzymes cathepsin B and cathepsin D (as depicted in table).

Some proteolytic activities or proteases are to remove clotting status of fibrin, transfer secreted proteins from cell content and surrounded media, activate zymogenic forms of enzymes [20].

proteases are among the three largest important enzyme groups and contains almost 60% of overall global market [21].

Sources

Plant sources

Mainly, growth factors govern the process of protease production in plant sources like climate and cultivation conditions. Papain, bromelain, keratinases and ficin are examples of plant originated proteases [21]. Papain, secreted from Carica papaya fruits, has more specific enzyme preparation due to the presence of several proteinase and peptidase isozymes.

Table 1. some of the plant based protease

Enzyme name	Sources	Ref
Bromelain	pineapples	[18]
Papain	Carica papaya fruits	[18]
wrightin	Wrightia tinctoria,	[18]
Carnein	Ipomoeacarnea spp. Fistulosa (latex)	[23]
Milin	Euphorbia Milii (latex)	[23]
A neutral protease	Raphanus sativus leaves	[23]
An aspartic protease	Potato leaves	[24]

Proteases from Animals

Animal originate proteases are pancreatic trypsin, chymotrypsin, pepsin, and rennins, produced in bulk amount and pure form. But their production amounts depend on the population of slaughtering livestock [26].

Proteases from Microbes

Microbial originating proteases are considered as good sources due to their broad biochemical pathways. In contrast to other sources, microbial proteases have all biotechnological related properties. Neutral and alkaline proteases produced by Genus Bacillus are active in pH 5 to 8. Bacterial neutral proteases are low thermostable and have high potential of hydrolyzing hydrophobic amino acids. Former property causes proper control of protease in food processes. Two groups of enzymes are defined, metal needed and serine types. Alkaline types are characterized as being active in high pH (10) and optimum temperature of 60°C. Members of the genus Pseudomonas like Pseudomonas aeruginosa are producers of Fungal type alkaline proteases. Proteases, mostly metallo types, are being produced by fungi, like Aspergillus oryzae, have broader range of pH activity, and lower temperature range of resistance. Fungi type proteases may be produced by current methods such as solid-state method. This type of protease mostly exploits in dairy industry and making cheese, owing to their properties of narrower pH and Temperature range. viral types (Serine, aspartic and cycsteine) involve in the vital protein production of fatal disease like AIDS [18].

Protease types

Proteases could be divided in 2 groups, exo and endo peptidase, based on action site; or divide in 4 groups namely serine proteases, aspartic proteases, cysteine proteases and metallo-proteases based on their function. However, still some proteases remained which would not exactly fit to any of aforementioned classifications [27].

Exopeptidases

This type classified in 2 groups, Amino and carboxyl peptidase based on terminals of action sites. Aminopeptidases removes N-terminal Met and mostly act on N-terminal to release amino acids [18].

Table 2. some of the amino and carboxyl peptidase properties

Aminopeptidase	Source	Required ions
Aminopeptidase I	Aspergillus oryzae	requiresMg +2 or Mn+2
leucine aminopeptidase	Hordeum vulgare	
aminopeptidase	acillus licheniformis	Co+2 ions
Aminopeptidase	stearothermophilus	Zn+2, Mn+2, or Co+2 ions.
serinecarboxypeptidases	Penicillium sp., ccharomyces sp., and Aspergillus sp	
Metallocarboxypeptidases	Saccharomyces sp. and Pseudomonas sp.	Zn+2 or Co+2

Endopeptidases

With respect to the inner peptide bonds, and the fact that free amino acids are reducing enzyme activity of this type, four subgroups are classified in this group as follow: serine protease, Aspartic protease, cysteine/ thiol protease, metalloproteases [26].

Production

Extracellular Microbial originating proteases are influenced by physiochemical conditions and component in media, like the amount of sugar, metals, pH, concentration of some ions or cations. Production by solid-state fermentation or submerged fermentation are two methods using raw materials (like agroindustry wastes). Later is more general in protease production because of its advantages, however, it is expensive. To date, considering more inexpensive sources, a bulk of studies have been performed by scholars to find novel protease sources [28, 29]. Table 3 is a summary of recent studies on different microbial genera, and sources to feed them in order to produce proteases.

Table 3. recent studies on protease production.

Aspergillus oryzae NRRL 1808	Wheat bran	31 U/g	[30]
Aspergillus oryzae NRRL 1808	Wheat bran	3 U/g	[30]
Aspergillus flavus	Wheat bran, soy protein	1894 U/g	[31]
Aspergillus oryzae MTCC 5341	Wheat bran	43 U/g	[32]
Beauveria felina	Wheat bran	20000 U/g	[31]
Beauveria felina	Wheat bran, soy protein	8211 U/g	[31]
Engyodontium album	Wheat bran	3186 U/mL	[33]
Engyodontium album BTMFS10	Wheat bran	4351 U/g	[33]
Bacillus cereus strain AT	Cow dung substrate	4,813 U/g	[34]
Bacillus licheniformis ZB-05	Rice husk	469,000 U/g	[35]
Bacillus sp.	Green gram husk	9,550 U/g	[36]
Bacillus sp.	Red gram husk	350 U/g	[37]

Recent literatures on protease production

Cell immobilization is the process of limiting cell movements, which is performed in a continuous fermenting bioreactor. Maghsoodi et al. [38], showed that application of cell immobilization on carriers like corn cob enzyme was 119.67U/ml. In another study, Murthy (2015) studied the application of producing acid protease by solid state fermentation (potato wastes) method using A. oryzae RIB 40 treated by UV mutagenesis with a lethality 1.8 × 10−4. Wherein mutant strain had produced protease 5.6 fold higher than regular strains [39]. Application of novel strains made scholars to find better ways of production along with testifying new raw material sources. For instance, Mechria et al. [40] employed submerged fermentation using Lysinibacillus fusiformis strain C250R to produce protease on wheat bran, and found that in optimized conditions (70 ◦C and pH 10) the yield was 4.5 fold higher than initial conditions. Along with application of aforementioned parameter, statistical methods are also important. Abdel Wahab [41] selected Aspergillus niger WA as producer of protease. Additionally, statistical methodology by Plackett-Burman design (PB) help to identify significant production factors. The optimum conditions to produce the enzyme was 10 min incubation at pH 10.0 and 60°C (4.7 fold) [41]. Furthermore, Sudha et al. showed that peptone and glucose are effective on production of protease. They also found the optimum conditions from Exiguobacterium profundam MMI MG951843.1 was 35°C and pH=9 [42]. In another study, Benmard et al, discovered Penicillium chrysogenum as a high producer of serine alkaline protease. The combined treatment they offered was heating 10 min at 80°C, an ammonium sulfate dialysis, and a final UNO q-12 anion exchange chromatography. They found optimum conditions as pH 10 and 80°C [43]. Shakilanishi et al. tried to derive collagen from Chrome shavings to produce dehairing protease. The yield of the protease production in their study was 88.1% using 22% (w/v) of 2-30 propanol and 14% (w/v) of K2HPO4 aqueous two phase system. Additionally, they showed that their production method was more efficient than ultrafiltration system [44].

Some other studies focused on the optimization of growth condition of producer microorganism, which directly affects the amount of produced protease. For instance, Liu et al., studied on sludge fermentation and found that protease may reduce bacterial richness while promote the growth of those ones with ability of catalyzing polysaccharides as well as prevent the growth of a group of probiotic bacteria [45]. Sethi et al. employed a new fungus strain Aspergillus terreus NCFT4269.10 to produce protease by means of both solid state and liquid state surface culture methods. Results showed the highest rate of growth under conditions of riboflavin (10 mg/100 ml), i.e., 256.45 mg l−1h−1. In addition, the highest amount of protease produced when using Fe2+ [46]. Decastro et al. studied the synergistic conditions of using different agro-wastes together on protease production by Aspergillus niger LBA 02. They used wheat bran, soybean meal, cottonseed meal, and orange peel. They found the highest protease amount in media containing wheat bran and soybean meal which was 262.78 Ug^{-1} after 1 hr. [47]. Salihi et al. studied the production of protease from A. oryzae CH93, and it yields a 47.5 KDa extracellular protease. Purification method was by means of ammonium sulfate and Q-Sepharose chromatography. The optimum conditions of production were pH 8, and temperature of 50◦C [48].

CONCLUSION

Regarding the growing interests to produce more environmentally friendly products, using biocatalysts such as protease enzyme is gaining considerable attention. Therefore, finding new microbiota strains with higher rate of growth, and consequently more protease production is a point of interests. Some challenges would face to scholars are activity period of produced enzymes, which needs more costs, as well as precise control of bioreactor conditions, that need to be studied in detail.

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