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A REVIEW ON PRODUCTION OF PROTEASE

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ABSTRACT

Hydrolytic enzymes that degrade proteins into tiny peptides and amino acids are called proteases. They account for nearly hour of the general demand for industrial enzymes. Proteases are employed with the food, pharmaceutical, leather, and detergent industries. Because of their potential utility, there has been some revived interest to find proteases with uncommon characteristics, likewise as a continuing drive to extend protein potency. This analysis only touches on a small portion of the massive studies on alkaline proteases that are currently available. Isolation of alkaline protease-producing microorganisms has been reported from a variety of sources. The impact of various nutritional and environmental factors on alkaline Protease development are investigated in submerged and solid-state fermentation in various articles. The enzymatic and physicochemical properties of basic proteases from various microorganisms are discussed, which can help in recognizable proof of catalyst with high movement and strength across high pH and temperature range, so that it can be manufactured for industrial use.

Keywords: Protease production, Endoprotease, Exoprotease

INTRODUCTION

Enzymes are biocatalysts that help reactions move more quickly. In vivo, a large number of enzymes are generated, many of which are important in industry.

Protease is the most widely used enzyme in industry. Protease enzyme is used in all living things and accounts for 1-5 percent of total protein content **[1]**. Protease is the third most common group of industrial enzymes, accounting for 60% of global sales **[2]**. Proteases, also called as peptidase, proteinase, or proteolytic enzymes, can hydrolyze peptide bonds in proteins **[3]**.

Acid, alkaline and neutral proteases are the three classes of proteases based on their acid-base activity **[4]**. Fungi are the only organisms that produce acid proteases, which have a pH range of about 2.0 to 5.0. Proteases have a pH range is around 7.0-8.0 and are often derived from plants, while alkaline Protease has a pH greater than 8 **[5]**. All living things, including plants, animals, and microbes, have proteolytic enzymes **[6]**. Microbial development of proteases is favoured over other sources because microbes can be grown and cultivated in a very small space. They have a higher growth rate and are easily genetically engineered **[3]**. Protease enzymes are imperious in modern biotechnology and can also generate environmentally friendly products **[7]**. The pathogenicity of the microbial protease was resolved to be zero. As a result, they are easy to develop in culture medium and have a extensive industrial applications **[8]**. In the marine environment, Protease enzymes are produced as a bioactive compounds. Temperature, strain, salinity, and density are all factors that influence the biosynthesis of these bioactive substances. They have the opportunity to remain involved under difficult circumstances **[9]**. Pollution is regulated using marine enzymes **[10]**. Dry washing, detergents, meat processing, cheese manufacturing, silver recovery from photographic film, digestive enzyme development, and some medical treatments for inflammation and virulent wounds all use protease enzymes **[11]**. Genetic manipulations are accessed using bacterial protease **[12]**. *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus thuringiensi* are the bacteria that produces proteases **[3]**.

Proteases were previously classified based on their origin (animal, plant, or microbial), catalytic activity (endo or exopeptidases), molecular size, charge, or substrate specificity, but the Enzyme Commission (EC) suggested a more practical classification system that divides all enzymes into six major classes. The Enzyme Commission (EC) classifies proteases as hydrolases in class three and sub-group four (which hydrolyze peptide bonds) **[13]**. Serine proteases (EC 3.4.21), aspartic proteases (EC 3.4.23), serine carboxy proteases (EC 3.4.16), cysteine proteases (EC 3.4.22), metallo proteases I (EC 3.4.24), and metallo carboxy proteases

(EC 3.4.17) are the six families of proteases defined by the Enzyme Commission **[14]**. Alkaline proteases (EC.3.4.21-24, 99) are proteases that are active in the pH spectrum of neutral to alkaline. They either have a serine core or are metallo-type enzymes, and they are the most studied group of enzymes due to their widespread use in the detergent, food, pharmaceutical, and leather industries **[15]**. Considering the high importance of protease, in this review article various applications and production conditions along with the diverse types of protease are discussed.

TYPES OF PROTEASES

ENDOPEPTIDASES

The preference of endopeptidase for peptide bonds in the polypeptide chain's inner regions, away from the N and C termini, distinguishes them. Enzyme activity is reduced when free amino acids or carboxyl groups are present. Endopeptidases are classified as Serine protease, Aspartic protease, Cysteine protease and Metalloprotease based on their catalytic mechanism **[15]**.

Serine proteases

Serine proteases are active in the pH range of 7 to 11, which is neutral and alkaline. These enzymes have esterolytic and amidase activity and are highly substrate specific. The serine protease from *Blakeslea trispora*, which has a molecular mass of 126 kDa, has a molecule mass range of 18 to 35 kDa. Serine proteases isoelectric points are normally in the pH range of 4 to 6. The second largest family of serine proteases is *Bacillus*-derived subtilisins. *Subtilisins Carlsberg* and *Subtilins Novo*, also known as Bacterial Protease Nagase [BPN], are two types of alkaline proteases **[16]**.

Aspartic proteases

Endopeptidase with two aspartic acid residue [Asp32 and Aso215, pepsin numbering] are known as aspartic acid proteinases or aspartyl proteinases. And these are also known as acidic proteases. They exhibit behaviour in the pH range of 3 to 4 and have an isoelectric point of 3 to 4.5. They are categorized into pepsin-like enzymes and rennin-like enzymes **[16]**.

Cysteine proteases

These type of proteases occurs in both eukaryotes and prokaryotes. There are 20 different cysteine protease families **[17]**. At neutral pH, papain cysteine is most active, while lysosomal cysteine is most active at acidic pH.

Metalloproteases

Metalloproteases are the most complex of the protease catalytic classes. They are distinguished by the fact that they need a divalent metal ion to function. They produces collagenases from higher species. And are classified into neutral, alkaline, myxobacter-1, myxobacter-2 **[18]**.

EXOPEPTIDASES

Exopeptidase are enzymes that only work at polypeptide chain's end. They are known as amino or carboxypeptidases depending on whether they function at the N or C terminus.

Aminopeptidase

It is found in bacteria and fungi. Act at N-terminus on polypeptide chain. They are intracellular enzymes. But there is extracellular aminopeptidases by *A. oryzae*.

Carboxypeptidase

The three forms of carboxypeptidase are serine carboxypeptidase, metalloprotease carboxypeptidase, and cysteine carboxypeptidase. Serine carboxypeptidases isolated from *Penicillium* spp., *Saccharomyces* spp., and *Aspergillus* spp. have similar substrate specificity, but pH, stability, molecular weight, and inhibitor effect vary slightly.

PROTEASE PRODUCTION FROM VARIOUS RESERVOIRS

Proteases can be found in almost any biological source. Being needed for the performance of various physiological functions by living organisms. They can be present in a wide variety of places, including plants, animals, and microorganisms.

Protease production from plant

The use of plants as a source of proteases is influenced by the availability of land for cultivation and the suitability of climatic conditions for development. Furthermore, the development of proteases from plants is a lengthy process. Some well-known proteases of plant origin include papain, bromelain, keratinases, and ficin **[19]**. Papain is a long-used conventional plant protease. It's made from the latex of *Carica papaya* fruits, which are native to subtropical West and Central Africa, as well as India. Wrightin is a substance derived from the latex of the *Wrightia tinctoria* plant. 'Carnein' is derived from the latex of Ipomoeacarnea spp. fistulosa, a herb (Morning glory), and 'Milin' from the latex of *Euphorbia milii*, these three are new thermostable serine proteases named 'wrightin,' 'Carnein,' and 'Milin,' have potential applications in food and other biotechnology. A neutral protease has also been purified from *Raphanus sativus* leaves **[20]**. From potato leaves we get purified aspartic acid with a variety of physiological functions **[21]**.

Protease production from animals

The most well-known proteases of animal origin are pancreatic trypsin, chymotrypsin, pepsin, and rennins. These are made in large batches and in their purest form. Their production, however, is reliant on the availability of slaughtered livestock, which is influenced by political and agricultural policies **[18]**. Trypsin is the primary digestive enzyme in the intestine that hydrolyzes food proteins (Mr 23,300). It's a serine protease that hydrolyzes peptide bonds with carboxyl groups contributed by lysine and arginine residues. Due to the ability of protease inhibitors to inhibit the enzyme from the insect gut, this enzyme has gained popularity as a target for bio-control of insect pests. Rennet is a pepsin-like protease (rennin, chymosin) formed in the stomachs of all nursing mammals as an inactive precursor, pro-rennin. By the action of pepsin or autocatalysis, it is converted to active rennin (Mr 30,700). It's commonly used in the dairy industry to create a flavorful, healthy curd.

Protease production from bacteria

Bacillus species contain the majority of commercial proteases, which are mostly acidic and alkaline. Neutral proteases from bacteria have a narrow pH range of activity (pH 5 to 8) and low thermotolerance. Because of their intermediate rate of reaction, neutral proteases contain less bitterness in hydrolyzed food proteins than animal proteinases, making them useful in the food industry. Neutrase is a neutral protease that is resistant to plant proteinase inhibitors, making it useful in the brewing industry **[18]**.

Protease production from fungi

Fungi produce a broader range of enzymes than bacteria. Neutral acidic, and alkaline proteases, for example, are found in *Aspergillus oryzae*. Fungal proteases have a broad pH spectrum of activity (pH 4 to 11) and a broad substrate specificity. However, compared to bacterial enzymes, they have a slower reaction rate and are less heat resistant. Fungal enzymes can be easily produced in a solid-state fermentation process. Fungal acid proteases have an optimal pH of 4 to 4.5 and are stable between 2.5 and 6.0. Because of their small pH and temperature specificities, they are especially useful in the cheese-making industry. Fungal neutral proteases are metalloproteases that are active at pH 7.0 and are inhibited by chelating agents **[22]**.

Protease production from virus

Because of their role in the processing of proteins created by viruses that cause fatal diseases like AIDS and cancer, viral proteases have grown in popularity. Viruses contain peptidases for serine, aspartic, and cysteine. Retroviral aspartyl proteases are homodimers found in the polyprotein precursor and are needed for viral assembly and replication. The precursor is autolyzed, releasing the mature protease **[22]**.

EFFECT OF PRODUCTION PARAMETERS ON ACTIVITY AND STABILITY OF PROTEASE

Effect of pH

Extracellular pH is important for the cell and protein production of alkaliphilic microorganisms. For these microorganisms to thrive, a pH of around 10 is optimal. These microorganisms' cytoplasmic pH can be calculated using the ideal pH of intercellular compounds or by calculating the transport of inside and outside powerless bases that are not transported by the cells. The pH range for basic protease production is generally 9–11. **[38]** a *Bacillus firmus* (pH9), *Bacillus* sp. **[23]**. (pH11), *Bacillus* sp. (pH 10), *Aspergillus clavatus* (pH 9.5), and *Penicillium* sp. are among the bacterial and parasitic species that produce antacid protease (pH 9).

Effect of temperature

Temperature is another important factor to consider when it comes to cell growth and protease catalyst production. At temperatures ranging from 45 to 70 degrees Celsius, basic protease moves optimally. *Micrococcus* sp. (50C), *Bacillus clausii* (60C), *Pseudomonas aeruginosa* (60C), *Aspegillus oryzae* (50C), and *Actinomycete* (50C) are among the bacterial and parasitic species included. However, several microorganisms, such as *Aspergillus niger* (30°C), *Aspergillus nidulans* (35°C), and *Bacillus amovivorus* (37°C), have ideal temperatures that do not fall within the provided range **[23]**.

Effect of carbon

Glucose, sucrose, maltose, sorbitol, fructose, are the best carbon sources for *Bacillus* for producing proteolytic enzymes. According to previous research, when a carbon source is introduced to the medium, protease production peaks **[24]**.

Effect of nitrogen

Sodium nitrate is used as inorganic nitrogen source. The production of alkaline and neutral proteases increased even further as the concentration of sodium nitrate increased. *Bacillus* sp. Produced high amount of protease by utilizing $NaNO_3$ **[25]**.

GENERAL PROCESS OF PROTEASE PRODUCTION

Protease producing bacteria will form hydrolytic zone in casein agar. Now to increase the enzyme production need to do optimizing some parameters like pH, temperature, substrate concentration, etc. *Bacillus* spp. are specific producer of extracellular proteases.

Carbon sources such as casein broth, soyabean milk, and even starch or lactose are used as carbohydrate sources for protease development.

Solid state fermentation and fed batch process are the types of fermentation process. The solid or submerged influence the growth of mass and enzyme production. Growth is carried out at 30-37°C in fermenter. By using ultrafiltration and chromatographic technique purification is done **[26]**.

IMPROVEMENT IN THE YIELD OF PROTEASES

Scientists have used cloning and overexpression, strain screening, fed batch and chemostat fermentation to increase protease yield for industrial usage. For the optimization of various media and growth environments, different statistical methods, such as response surface methodology, have been used. For the improvement of raw strain UV or chemical and modern rDNA technology are used **[27]**.

INDUSTRIAL APPLICATIONS

Table 1: Industrial applications of Protease enzyme [28]

Industry	Enzyme	Application
Detergent	Alkaline proteases, subtilisin	Laundry detergent for removal os stains (food, blood, body secretions)
Leather	NnovoNorisk	For bating, dehairing and soaking
Dairy	Chymosin	Cheese production
Baking	Trypsin	Dough conditioner
Brewing	Papain	Chill proofing, removal of haze in beverages
Pharmaceutical	Curcain	Wound healing agent

CONCLUSION

Protease enzymes are a type of proteolytic enzyme that hydrolyzes peptide bonds in proteins to produce shorter polypeptides and amino acids. In the present review, general view of the wide and complex matter of the industrial applications, sources, properties of alkaline protease have been discussed. The properties of protease has been revealed that the enzyme is used in various industries for different purposes. The enzyme is widely used by microbial sources such as bacteria, fungi, and archaea. They are important for digestion and absorption of dietary proteins. They also help with blood coagulation, immunity, precursor protein activation, cell signalling, protein recycling, and apoptosis, among other things.

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