IJMPR 2021, 5(6), 01-04

International Journal of Modern Pharmaceutical Research

www.ijmpronline.com

ISSN: 2319-5878

IJMPR

Review Article

SJIF Impact Factor: 5.273

A REVIEW ON PROTEASES: THEIR SOURCES, TYPES AND APPLICATIONS

Farheen Aslam*, Maryam Rasheed[a] and Saima Iftikhar Bajwa[B]

*Assistant Professor, Department of Biotechnology, Lahore College for Women University, Lahore.
[a]Department of Biotechnology, Lahore College for Women University, Lahore.
[b]School of Biological Sciences, University of the Punjab, Lahore.

Received on: 28/09/2021
Revised on: 19/10/2021
Accepted on: 08/11/2021

***Corresponding Author**
Farheen Aslam
Assistant Professor,
Department of Biotechnology,
Lahore College for Women
University, Lahore.

ABSTRACT

The protease producing bacteria are widely explored for the high quality production of various industrial products of high commercial values. These enzymes catalyze hydrolysis of peptide bond present in proteins. They are widely used commercially in pharmaceuticals, leather, food and detergent industries. Most of microbes produce more than one kind of enzymes. The nature of protease producing bacteria depends on the nature of media and optimum conditions used for their growth. Proteases are produced by a variety of organisms like plants, animals, fungi and bacteria. Most commonly, bacteria are used for efficient production of proteases due to their high multiplication rates. The cost of protease production is a major hurdle in their way of use in industries. This obstacle can be overcome by improving the yield of proteases. The extensive screening techniques are performed to screen the overproducing strains of microbes and they are further purified through other selection methods. The protease production is enhanced with the advancement in genetic engineering techniques like expression of recombinant proteins in a variety of host organisms, advancement in manipulation procedures and enhancement in the processes such as transcription, translation and secretion. Applications of proteases have been enhanced by improvement in the fields of producing recombinant proteins for modifying protease characteristics in terms of their specificity, stability, mode of action and kinetics. The techniques like random mutagenesis and site directed mutagenesis are being performed for constructing and designing the new structures of protease with increased stability.

INTRODUCTION

Proteases are one of the group of enzymes which are used for the hydrolysis of proteins into peptides or amino acids. They can be either highly specific in their catalysis when they hydrolyze the peptides with specific amino acid sequences or they can be non-specific in nature where they can hydrolyze a wide range of protein substrates.[1]

These proteins account for the worldwide sale of 60% of total enzymes.[2]

The proteinases, peptidases and proteolytic enzymes are grouped as peptidases. These enzymes use water molecules for breaking the peptide bonds of proteins so they are also known as hydrolases.

Functions of Proteases

Proteases are involved in performing various body functions including cell division, cell differentiation, cell motility and cell death. They also play role in intracellular degradation of proteins in the form of organelles like lysosomes which are membrane bounded organelles that contain proteases. They also perform physiological functions such as in proteasome system. The proteases serve as causative agent of some diseases, they can also act as therapeutic agents.

Proteases perform various roles from cellular to organ and to organism level in hemostasis and inflammation. They have also become potential targets for the production of therapeutic drugs against lethal diseases such as AIDS and cancer.[2]

Sources for Protease production

The proteases are produced by a variety of organisms like plants, animals, micro-organisms and fungi. However, the proteases obtained from bacteria are considered most significant as compared to plant, animal or fungal proteases. And in bacteria, the *Bacillus* sp. are considered significant due to their ability to produce extracellular proteases.

Microbes

Most often, micro-organisms are used as a source of enzymes because they require low spaces for growth and have high multiplication rates. They can also easily genetically manipulated to produce modified versions of various enzymes with increased commercial value.[3]

Bacillus can produce a wide range of extracellular enzymes. Various strains of Bacillus are used for this purpose. These are used for the production of alkaline proteases of industrial importance.[4]

Bacillus are most widely used bacteria for the production of various chemicals and industrial enzymes including amylase and protease. Proteases are also used in brewing and baking industries, in tenderization of meet, synthesis of peptides, cheese making industries, medical diagnosis. It is also involved in various biochemical processes.[5,6]

They are widely distributed in water and soil and can survive extreme environmental conditions. Bacillus species screened from different environments can have different proteases with different physiochemical properties.

Animals

The enzymes have also been extracted from the stomach of various animals. For example, pepsin and rennet enzymes have been obtained from the stomach of calves since ancient times, but they have low pepsin content as compared to rennet enzyme. The tissues that contain enzymes are being preserved at the slaughterhouses using techniques like drying, salting or freezing. These tissues before enzyme extraction are washed and dried properly and then they are sliced or grounded. The enzymes are extracted by washing with water or 5-10% NaCl solution.Trypsin is present in the intestines of animals and humans and is involved in the digestion of food proteins. It is a serine protease. Chymotrypsin is found mostly in the pancreatic extracts of animals. The pure form of chymotrypsin is very expensive. Pepsin is present in the stomach of vertebrates. It is acidic in nature. It works optimally at the pH range of about 1-2 and become inactivated above 6 pH.

Plants

The protease enzymes can also be produced from plant sources. For example, Papain enzyme is being produced from papaya plant and Bromelain is produced from pineapple plant.

Papain is mostly produced from the green fruit. The latex is obtained by forming incisions on the surface of fruit. This latex become congealed on the surface of fruit from where the enzyme can be extracted using the techniques like water extraction and solvent precipitation after drying the latex at temperatures of about 55˚C. The enzyme can then be purified using purification techniques. It is a cysteine protease and work actively in the pH range of about 5 to 9.

Similarly, Bromelain is obtained from the stem of pineapple. After harvesting the fruit, the stem is peeled and blended or crushed and is pressed to obtain the bromelain enzyme-containing juice extract.

Types of Proteases

Enzymes can be classified into following types on the basis of their activity, presence of functional group and acid-base nature:

Depending on the site of action

The principal types of enzymes include:
1. Exopeptidases
2. Endopeptidases

1. Exoproteases

Exoproteases are those proteases that attack at the terminal end or near the ends of peptide chains.

2. Endopeptidases

Endoproteases are involved in the cleavage of internal peptide bonds from within the peptide chain instead of C or N termini.

Depending on the nature of functional group

They can also be divided into following types depending on the basis of the nature of the functional groups present at their active site:
- Aspartic proteases
- Serine proteases
- Cysteine proteases
- Metalloproteases
- Glutamic acid and Threonine proteases

- **Aspartic proteases**

Aspartic endoproteases work at low optimum pH that is about 3-4. They have a pair of aspartic acid residues present at their active site that is involved in catalytic reaction. They are classified into the following two groups:
- Pepsin and pepsin-like enzymes
- Rennet and rennet-like enzymes

- **Serine proteases**

Serine endoproteases are those type of endoproteases that contain a serine residue at their active site for performing catalytic actions. The well recognized members of this sub-group are chymotrypsin. They have optimum pH range of 7-12.They are further delinearized into the following types:
- Serine alkaline proteases which have optimum pH range of 9-10.
- Serine high alkaline proteases which have optimum pH range of 10-11.

The subtilisins which come under commercial *Bacillus* proteases are among the important family of Serine proteases produced by *Bacillus* species of bacteria.

- **Cysteine proteases**

Cysteine or thiol endoproteases contain cysteine-histidine dyed at their active centers for performing catalysis. They require reducing agents for catalysis and are inhibited by the sulfhydryl agents. The important subgroups of this family include papain or papain-like proteases like streptopain and clostripain.

- **Metalloproteases**

Metalloendoproteases require bound divalent cations. They are usually inhibited by reagents like diaminetetraacetic acid. Thermolysin which is a highly thermostable enzyme is a neutral zinc protease and a well-recognized member of this sub-group. Some other members include metalloprotease collagenase and metalloprotease elastase.

- **Glutamic acid and Threonine proteases**

Glutamic acid and Threonine endopeptidase have glutamic acid and threonine residue respectively at their active centers for performing catalytic activity.[2]

Depending on acid-base nature

Depending on their acid-base nature, they are classified into three categories including acid, alkaline or neutral peptidases. Acid proteases show best activity at a pH of about 2-5, and they are mostly produced by fungal sources. The proteases showing maximum efficiency at pH 7 are referred to as neutral, and they are mostly produced by plants sources. While the proteases showing maximum efficiency at pH 8 or above are called alkaline proteases, and they are mostly originated from micro-organisms. The proteases which are produced through micro-organisms are commercially important in food, pharmaceutical, tanning, and detergent industries.[6]

Applications of Proteases

There is rising interest in the study of proteolytic enzymes because of their importance in not only in the metabolic processes of cells but also due to their importance for industrial processes. These extracellular proteases are of high commercial value in various sectors of industries.[7]

In detergent industry

High alkaline proteases find their applications as commercial detergent proteases, for example, M-proteases and Novozymes. Novozymes are highly active at low temperatures, therefore, they are highly effective in cold water washings. However, thermostable proteases contribute a major portion worldwide in industries as compared to other proteases.[6]

In brewing industry

Proteases are used in the mashing of cereals for brewing purposes. They are also used for removing chill haze in brewing processes which results from the decreased solubility of proteoglycans. Papain is highly effective in removing haze through the protein polymer hydrolysis.

In baking industry

Proteases are used in baking industry for the modification of gluten protein, for flavor development and as a nutritional source. For the manufacturing of biscuits a floor with low gluten (a protein in wheat) content is required which is achieved through gluten hydrolysis with proteases. Neutral proteases from bacterial sources are used for the production of biscuits, crackers and cookies.

In leather industry

Proteases are used in various processes in leather industries. For example, they are used for dehairing of hides, where they have served as a good substituent for toxic chemicals and increased the importance of biotechnology. They have improved the quality of leather and have reduced the environmental pollution. They have resulted in the significant reduced production of waste water quantities. In addition, they have also been used in industries like leather tanning industries from the species like *Aspergillus* and *Bacillus*.

In silver recovery

X-ray films contain about 1.5-2% silver in their gelatinous layers by weight. This silver can be recovered through the burning of x-ray films, however, it results in the production of large quantities of environmental pollution. That's why, protein hydrolysis is used for the recovery of silver from the gelatinous layers of x-ray films without much production of environmental pollution.[7]

For waste treatment

It was reported that alkaline proteases from *Bacillus subtilis* have been used for processing of feathers waste from the slaughter houses. Approximately 5% body weight of poultry comprises of feathers and they are the high source of protein in feeds and foods after their structures containing rigid keratin are completely destroyed. Treating feathers with NaOH, following mechanical disruption and enzymatic hydrolysis causes the solubilization of feathers. The resultant heavy and greyish powder is used as an additive in feed. Similarly, a variety of keratinolytic alkaline proteases are used for the production of amino acids.[7]

In pharmaceutical industry

Proteases are also used for pharmaceutical purposes. The proteases from *Aspergillus oryzae* are used to cure the syndromes due to lytic enzyme deficiency. They are orally administered. Clostridial collagenases are used to cure burns and wounds along with some broad spectrum antibiotics.

Yield improvement

The cost of protease production is a major hurdle in their way of use in industries. This obstacle can be overcome by improving the yield of proteases. In order to improve the yield of proteases, the hyper-producing strains of microbes are screened and fermentation media is required to be optimized. Strain improvement can be done either by conventional mutagenesis or by recombinant DNA technology for getting improved production of proteases. Enhancement in the yields of viral proteases are considered important due to their vital roles for developing therapeutic agents against some hazardous diseases like malaria, AIDS and cancer.

REFERENCES

1. Gupta R, Beg Q, Lorenz P. Bacterial alkaline proteases: molecular approaches and industrial applications. Applied microbiology and biotechnology, 2002; 59(1): 15-32.
2. Rao MB, Tanksale AM, Ghatge MS, Deshpande VV. Molecular and biotechnological aspects of microbial proteases. Microbiology and molecular biology reviews, 1998; 62(3): 597-635.
3. Kocher GS, Mishra S. Immobilization of Bacillus circulans MTCC 7906 for enhanced production of alkaline protease under batch and packed bed fermentation conditions. Internet J. Microbiol, 2009; 7(2): 359-78.
4. Ferrari E, Jarnagin AS, Schmidt BF. Commercial production of extracellular enzymes. Bacillus subtilis and other gram-positive bacteria: Biochemistry, physiology, and molecular genetics, Apr 27, 1993: 917-37.
5. Anwar A, Saleemuddin M. Alkaline protease from Spilosoma obliqua: potential applications in bio-formulations. Biotechnology and applied biochemistry, 2000; 31(2): 85-9.
6. Gupta R, Beg Q, Lorenz P. Bacterial alkaline proteases: molecular approaches and industrial applications. Applied microbiology and biotechnology, 2002; 59(1): 15-32.
7. Jellouli K, Bougatef A, Manni L, Agrebi R, Siala R, Younes I, Nasri M. Molecular and biochemical characterization of an extracellular serine-protease from Vibrio metschnikovii J1. Journal of Industrial Microbiology and Biotechnology, 2009; 36(7): 939-48.
8. Jisha VN, Smitha RB, Pradeep S, Sreedevi S, Unni KN, Sajith S, Priji P, Josh MS, Benjamin S. Versatility of microbial proteases. Adv Enzyme Res., 1: 39–51.