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Protease Enzyme: A Safe and Effective Industrial and Commercial Alternative

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***Abstract*: Proteases are created by microbes, plants, and animals and are found in all forms of life. Bacterial proteases take the top spot among them due to their wide range of industrial uses. They are one of the most diverse classes of industrial enzymes, including uses in detergents, leather, food, pharmaceuticals, and textiles, as well as silver recovery and bioremediation. Protease is most commonly used in laundry detergents and the leather industry, where it is used to remove protein-based stains from clothing and for dehairing. Some protease-producing bacteria and protease taxonomy, as well as approaches for increasing protease productivity, are described in this article. It is described how to make bacterial proteases from inexpensive and widely available substrates. Also mentioned is the usage of bacterial proteases in a variety of industrial applications. Finally, future possibilities are discussed.**

***Keywords*: Protease, Pseudomonas aeruginosa, Bacillus subtillis, Soil, Industry, Detergent, Cathepsin B.**

1. Introduction

Proteins are the building block of human body and class of macromolecules which help to perform various function of the cell. Muscle, hairs, eyes, hormones and enzymes that is mainly composed of proteins. Proteins are made of different amino acid, which have the alpha Carbon atom which linked to the NH2 group, hydrogen atom, and with COOH group. Amino acid genrally linked together with help of peptide bonds, genrally proteins are large chain molecule which is difficult in digestion, sectretion of Protease enzyme in different zone of body causes the hydrolytic reaction on the peptide bond which result in formation of smaller fragmented amino acid. (Barrett et al, 1986). Protease enzyme are class of hydrolytic enzymes which is essential for many important biological processes in a body. Protease enzyme mainly help in digestion of protein, also play important role in blood clotting, cell devision, immune function, signal transduction, apoptosis, protein recycling, and in other vital processes. (Ward, 2019)

Previously it was estimated that the about 2% humans genes are encode for the protease enzyme also it has been seen their important biological function in vital processes it also becomes important therapeutic targets which is also used for study of antiviral agent. (C. Ganesh Kumar, 1999). Proteolytic enzymes is important because they are also used as catalytic agent in industry process, medical and in laboratory. Proteinases both microbial and non-microbial sources, are widely used in the food industry:- Baking, brewing, cheese manufacturing, meat tenderizing), tanning industry, and in the production of biological detergents. (T.Beaker, et, al., 1997). Proteases implement a large variety in pharmaceutical and function particularly in the life cycle of disease affecting organisms and led them to become a potential target for developing therapeutic agents against AIDS and cancer.The massive diversity of proteases attracted worldwide attention to exploit their physiological and biotechnological applications. (Aparna laskar, et, al., 2009).Microbes re highly versatile in production of large amount of enzymes, with special patterns of activity.They are considered to the most common source of commercial enzymes due to their physical and biochemical properties. (Barrett et al, 1986)

Characterization of protease on the basis of production conditions is described on the basis of tempreture, pH and nutrient sources. In the present study, highest alkaline and neutral producing strains were isolated and identified for various commercial applications. (Henner D J, 2016)proteases are physiologically important for various vital prosseces of life in living organisms, they are ever present and widly found in diversity of sources - plants, animals, and microorganisms and nails. Optimum pH of action of Keratinases protease enzyme is between 7-10. Some

Table 1
Protease production in distinct zone of digestive system

Distinct zone of digestive system	Enzyme produced	Region of production	Function
Stomach	Pepsin	Gastric gland	Breaksdown of protein into Amino acid
Small Intestine-Duodenam	Trypsin	Pancrease	Partial breakdown of amino acid
Small Intestine-Ileum	Peptidase	Wall of leumen	Complete breakdown into amino acid

2. Plant Protease

- *Papain:-* It is kind of protease found in carica papaya, papain produce as a crude and dried material by the latex of the fruit, which are mainly grown in

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subtropical region. Papain contains the single polypeptide with 3- disulfide and sulfhydryl group, which is necessary for action of the enzyme. Papain has optimal pH for activity is 5.0- 9.0 and it is most stable at80º C- 90ºC in the presence of substrate. Papain is a well-known ingredient for meat tenderizer. I t is also used to remove the debridement and used to remove debride or clean a wound which impede to infection. It is added to some toothpaste as tooth whitener, due to their low concentration they easily diluted in saliva. (Israel schechter et al, 1997)

- *Bromelain*: - It is cysteine proteases which is found in Ananas comosus, produce in stem and juice of pineapple. They show maximum activity at pH 5- 9 and unstable or inactivate at 70º C. Bromelain is also used to treat the osteoarthritis and it shows ability to amend the lymphocyte adhesion. ((Glaciele Maria de souza et al, 2018))
- *Keratinases*:-Some plant produce Keratinases proteases which hydrolyzed the protein the protein substrate soluble or insoluble proteins. It can be degrade soluble protein like gelatin, casein and bovine serum albumin. Insoluble protein like Wool, feather of birds, Human hair Keratinases protease also work on the pH 12 and optimum temperature is generally 40º C- 60º C. The application of Keratinases mostly use in areas of industry also it is promising tool for agriculture, food industry and waste management. (Beti vidmar et al, 2018)
- *Ficin:*- Ficin is a class of protease enzyme found in latex of a tropical Ficus septica tree. Ficin also used as medicine. It contains the single reactive cysteine at their active site. The homology of amino acid at the active site which is similar to the papain. It will cleave the proteins at the carboxyl side. Ficin show maximum activity at optimal pH=5.7 and the optimal temperature at 65 °C. Purified form of ficin is used in the preparation of animal arteries and making cheese and sausage. (Sri Wahyuni et al, 2015).

3. Microbial Protease

The failure of the plant and animal proteases to meet present world demands has increased in curiosity in microbial proteases. Microbes are brilliant source of enzymes due to their broad biochemical diversity and the insusceptibility to a genetic manipulation.

- *Bacterial protease:* They are mainly neutral and alkaline produced by microbes belong to genus Bacillus they widely known as commercial protease bacteria. Neutral bacterial protease how their optimum activity between the pH range of 5- 8 and have low thermotolarance. Neutral protease bacteria generally produce less bitterness in hydrolyzed food due to their intermediate rate of reaction then animal protease and hence, they show high value in food industry. (Abebe Bizuye et al, 2014). They are insensitive to the natural plant proteinases that's why they are mainly used in brewing industry also they show high affinity for hydrophobic pairs of amino acid, and due their low thermotolarance they beneficial ability to control reaction during production of hydro lysates of food with a low degree hydrolysis. Some neutral bacterial proteases are related to the metalloproteae which require the divalent metal ion for processing activity. Bacterial proteases which are alkaline are show their activity at high alkaline pH-10 and optimum temperature for activity is 60º C. Bacterial protease are important group of enzyme they widely applicable in industrial processes, food industry, silvery recovery, Detergent industry, in pharmaceutical also used in leather textile, and also their valuable activity in medical usage management industry and in household waste. (Balakrishnan et al, 2012).
- *Fungal protease*: - Fungal protease are widely used for hydrolyzing proteins and also used in production of other components of soy beans and production of soy sauce. Nowadays, these are attracted attention of biotechnologist due to their large application, also they secrete in large amount of enzyme in culture media and they easily grow on low cost substrate. (Paula de Souza et al, 2015)Fungal strains which widely used to produce protease are mainly related to genera of Penicillium, Rhizopus, Mucor, Humicola, Thermoscus, and Thermomyces. Fungal protease showing their activity on the pH range 4 to 11 and they have low ability to tolerate heat. Acidic fungal protease show activity on pH range of 2.5 to 6.0 and used in the production of cheese due to their narrow pH and specificities with temperature. Neutral protease fungi are metalloproteae which is active at pH of 7. Fungi having filament are widely used in industries for production of enzymes and metabolites. (Kranthi, V. S. et al, 2012)
- *Virus protease:* - Viral proteases are those enzymes which are endopeptidases and encoded by DNA and RNA of virus. The main function of these enzymes is to hydrolyze the cleavage of particular peptide bonds in viral polyprotein precursor's proteins. Viral proteases of serine, cysteine or aspartic acid residues react on the scissile peptide bond by using different catalytic mechanism. (Hrusková-Heidingsfeldová O, et, al,, 1995)Aspartyl protease of retroviral is important for the assembly of viral proteins and help in replication of homodimers, this expressed in the polyprotein precursor. Recent research is focused on the 3- dimensional structure of viral protease and their interaction with inhibitor which is synthetic and it is used to design the potent inhibitor that can fight against the non- stopping spreading and harmful epidemic AIDS. Viral protease enzymes can be easily genetically manipulated to produce new enzyme with better properties and used in several application. (Lawrence Kuo et al, 1994) Depending on the site of

action proteases are subdivided into two major groups.

Exopeptidases acts at the end side of polypeptide chain. Amino peptidase and carboxypeptidase are classified form of exopeptidases which generally act on the free N-terminus substrate of polypeptide and at the C-terminus of the chain.

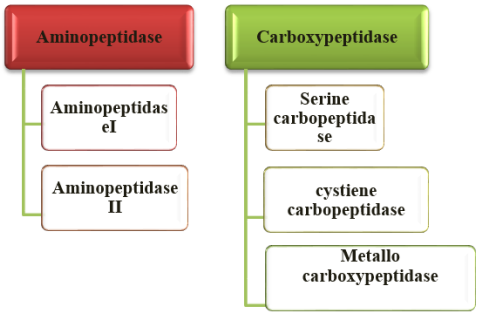

Fig. 1. Classification of exopeptidase

Endopeptidase are generally act on the inner zone of polypeptide. Endoproteases are also classified on the bases of their side chain specificity and functional group present at the site.

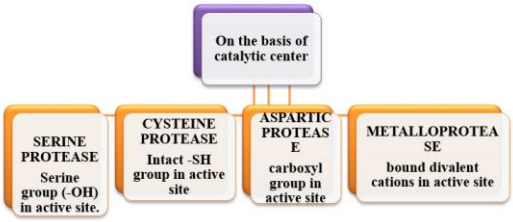

Fig. 2. Classification of endopeptidase on the basis of catalytic center

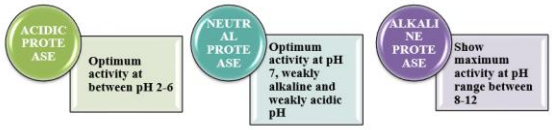

Fig. 3. Classification of endopeptidase on the basis of optimum temperature and pH range.

Protease enzyme is very specific in Proteolytic functions, found in the living organism and also they show importance for the growth and cell differentiation. Due to play roles in several physiological functions they show importance in industries, medical field, food waste management and many others. Protease gives lot of economic benefit. Sale of enzyme across the word is about to 60% in industry production also they show uses in detergent industry, silver recovery, in food industries, leather and in textile industry. (M.B. Rao et al, 1998).

4. Waste Management

Day today life of human activity caused the pollution in natural resources that require technology which reduce the pollution from environment. Well-developed recent biotechnological application in modern word used bacterial protease for treatment of waste and in bioremediation of some hydrocarbons. Protease used because of numerous potential applications in waste management of wastes from, household waste also helps to reduced hair clogged pipe lines. And have advantages to use of conventional chemical catalysts. (Shanmugavel et al, 2016). Protease also used for degradation of keratinous material in household for removal of the bad odors from the public places and house. Protease are generally solubilize the proteins in waste feather from the slaughterhouses by the multistep to recover concentrates of liquid or in dry solid of nutritional value for livestock for fishes. Several household and industrial processes cause the adverse changes in environmental and these are challenged by the society (Fekadu Alemu, 2015).

Cleaner production is widely used by many industries and in household waste to hostile their impact and to reduce the chemicals, water and raw material and decrease the risk in environment solid waste from sludge, from tannery and by the effluent from treatment of plants these are major causing agent of environment pollution. In recent days protease from bacteria used in commercial in bioremediation and also used as probiotic agent in aquaculture for importing into diet of fish and shrimp. (Santosh kumar et al, 2015)

5. Cancer Research

In normal cells protease enzyme play crucial role to carry out biological processes in a living body and when in disturbance in balance of living system it leads to cause the diseases like cancer which leads to initiation of tumor growth . (Jaideep et al, 2016). In recent reports gives the various valuable application in area of cancer research and role of protease shows the important target for designing of drug. Five class of protease, they are related with the progression of tumor and also in some cases secretion of protease by tumor cells make difficult in prognosis. Cathepsin B is kind of cysteine protease which participate in remodeling of connective tissue, dissolution and process of growth, invasion and metastasis in basement membrane. Cathepsin B in the tumor cells has been increased the expression in tumor cells which is abutting to the extracellular matrixes and protease mobilized from region of cell invasion. (Saghian, 2021).

Recent research involves the proteases in cancer therapy, protease inhibitor (PIs) that reduces the invasion and capabilities of metastatic tumor cells. PIs show effect on tumor invasion, it inhibit the extracellular matrix or by indirect inhibition of activation of Proteolytic cascade but it was reported that tumor cells is one part of the tumor, component of matrix extracellular and some stromal cells which shows important contribution of tumors Proteolytic activity. That's why study of protease and their protease inhibitors target for drug designing for clinical purpose. Protease inhibitors combination with the anticancer agents is probably proves that it has value for particular forms of cancers. (Syed Rakashanda et al, 2017).

6. Extraction of Silver from the Waste X-Ray

Silver metal show industrial importance due used in various sectors such as in x-ray films, photograph, jewelries, wares

made up of silver and in many electronic objects. Silver is light sensitive material which is widely used in photographs making and in making of x-ray. X-ray films show the radiographic pictures or images which contains the silver halide emulsion, in which silver bromide is highly used which is produces Ag+ ion and electron when it exposed to the light and then these electrons associated with the specks and they attracted the Ag+ ion. The silver can't be destroyed in X-ray and photographs and they can be reclaimed from the waste x-ray film and it will be recovered and reuse by using protease enzyme. Recovery of silver is kind of process in which pure form of silver metal can be recycled from the waste old x-ray films. Several methods are used to recover the silver from X- ray wastes burning the x-ray films directly, stripping of the gelatin and silver by using various solutions of chemicals such as, ammonium thiosulphate, sodium thiosulphate, and nitric acid. (Amira Hassan Al-Abdalall .et al, 2016). Chemical cause harm to the environment so industry using protease enzyme producing bacteria. Protease is very important in bioprocessing using in the recovery of silver from the X-ray. Using enzyme gelatin will be hydrolyzed which help to isolate silver but also help in recycling of polyester films. Recently it was reported that using protease degrade or recover gelatin and silver in a few minutes and about 99% silver which is pure are recovered. Protease gives about 0% pollution and these are easily stable in environment in silver recovering industry. (Choudhary, 2013).

7. Protease in Detergent

Normal detergent do not properly wash the stains of proteins because these stains are made of different kind of molecules, where they need wide range of enzymes to break them down the stains that's why industry involve Proteases for the breakdown of proteins stains, like blood, egg, gravy, and other stains of proteins. Protease has been added in detergents for over 50 years to facilitate removal of the proteinaceous materials stain like blood, milk also it removes proteins which secretes from body and food like- egg, fish and meat. Preparation of enzyme detergent is depends on its compatibility at a high temperature. Ideal enzyme detergent is stable and show activity in the solution of detergent and enzyme should be effective at a wide range of washing temperatures. (Renganathan Rajkumar et al, 2019)

The first enzymatic detergent is made in 1913 which consist of sodium carbonate and crude pancreatic extract. In 1956 the development of first bacterial enzyme under the trade name known as Bio-40. In early 1960, Danish companies Novo Industry first introduced Alcalase, which produced by Bacillus licheniformis and give commercial name Biotex. (Jabeen, F. et al, 2011)

- *Bacillus subtilis*: - Bacillus subtilis have capacity to secrete several proteins in the culture medium and it is lack in pathogenicity so it good host for production of foreign polypeptide by using recombinant DNA technology. Some sp. Bacillus secretes two major types of protease, one is a subtilisin or alkaline protease and other is a metalloproteae or neutral protease, these show their application in industries. They have been characterized and cloned and these proteases are synthesis 'pepro enzyme' to increase the expression of neutral protease and subtilisin. Henner replace the natural promoter 'apr' and 'npr' with promoter of amylase by B.amyloliquefaciens and promoter from neutral protease from B.subtilis. (Marie-Alice Fraiture et al, 2020)
- *Pseudomonas aeruginosa*: - It is a pathogen which Couse the severe fatal infection in host cells. It majorly secretes the various extracellular proteins but mainly two types – alkaline protease and Elastase protease. Alkaline protease encoded the gene 'apr' IF0 3455 and PAO1 and they were cloned in E.coli. The 'tac' promoter used to clone E.coli and DNA fragment of 8.8 kbp encoding for alkaline protease is well expressed in E. coli. (Guowei Yin,et,al, 2017).

8. Future Scope

Research on biochemical, regulatory and molecular aspects of proteolytic enzyme systems has been stimulated in several aspects of proteases. In terms of the commercial realisation of this enzyme class, researchers and process engineers have now begun to discover and engineer new enzymes which, using genetic and protein engineering techniques, are stronger in their pH and temperature kinetics. Protein engineering will in the future provide opportunities to produce proteases with completely new functions. Therefore, although microbial alkaline proteases play an important role in many industries already, they are significantly more likely and will likely be more widely utilised in future processes in the near future. As a result, ideal proteases with the desired application for a specific industry will be produced in the near future.

9. Conclusion

Proteases from bacteria are among the most significant hydrolytic enzymes. Enzyme and widely used since enzymological advent. Due to its wide range of applications in the detergent, bioremediation, food and leather processing industries, this industry is very important and has been widely marketed by various businesses worldwide. The use of cheaply available agro-industrial waste, highly productive strains and genetically modified bacteria has significantly improved their production.

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