

PRTOEASE – THE ENZYME WITH A BOON TO MANY INDUSTRIES – A REVIEW

Manisha Sellappan¹, Sellakiruthika Subramani²

^{1,2}V.S.B Engineering College, Karur, India.

DOI : <https://www.doi.org/10.56726/IRJMETS30626>

ABSTRACT

Microbial protease is an enzyme used in various industries such as food, textile, waste, detergent, animal feed, leather, dairy, brewing and others. It also used in baking industry for the production of baked foods, bread, crackers and waffles. Microbial proteases are widely used because of its nature friendly indicator and they can handle extreme conditions and control autoproteolytic activity. It was considered an alternative to chemicals. The enzymes were used in food preparations by many food industries. Proteases plays important role in food industry. It was produced from many sources such as soil, plant(vegetable) and microbial sources. From soil, bacillus subtilis (bacteria) are collected and used for the production of proteases and optimized in food industry. Due to high demand for proteases, production line is increased. How the microbial proteases are produced from bacillus subtilis (from soil sample) and its application in the food industry were discussed in this review. Microbial proteases have become more important in the food industry due to its specific properties, such as high production yield, substrate specificity, high activity, pH and temperature optima. Proteases have wide range of temperature (20°C-80°C) which increases the fields application. Proteases can be obtained from animal, plants, and microorganisms such as Bacteria, fungi, viruses. In industry, various enzymes are produced using bacillus subtilis. Protease enzymes are the main enzymes produced from bacillus subtilis. The main sources for the production of protease is found mainly in soil. The proteases are widely used in food, detergents, pharmaceuticals, leather, agriculture industries.

Keywords: bacillus subtilis, protease sources, classification, types, application food, textile, leather, pharmaceutical industries.

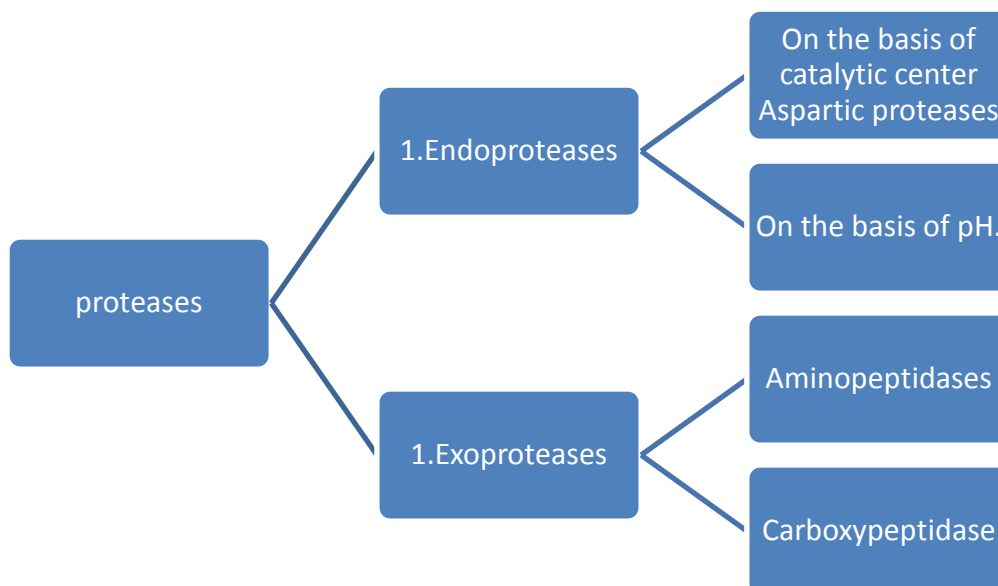
1. INTRODUCTION

Protease

Proteases are the enzymes which breakdown the proteins into smaller polypeptides or amino acids(proteolysis). Proteases, as also known as peptidases or proteinases. Proteolytic enzymes break down proteins in the body or on the skin. This helps with digestion. The term 'protease' was introduced by british botanist Sydney Howard Vines (1849-1934) in "Proteolytic Enzymes in Plants," Annals of Botany, vol. 17, no. 65 (January, 1903), p.237.

Classification

- Endoproteases
- Exoproteases



Types of proteases

There are different types of proteases. They are alkaline, endo serine, alkaline and neutral and acidic, heat stable, two acid, one alkaline, acid, cysteine and less extent aspartic proteinase. Protease are classified into six classes aspartic, glutamic, serine, cysteine, metalloproteases, and threonine.

2. METHODOLOGY

Sources Of Protease

Sources

Proteases are produced from many natural sources such as bacteria, fungi, yeasts, protozoa.

In **bacteria**, protease was found in *Aeromonas salmonicida* A-7301[1], *Arthrobacter aureus* [2], *Bacillus alclophilus* PB 92 [3], *Bacillus brevis* HPD 31[4], *Bacillus amyloliquefaciens* [5]. *Bacillus pumilus* strain MK6-5 [6], *Bacillus subtilis* Alkaline [7], *Pseudomonas fluorescens* P 26[8], *Pyrococcus furiosus* [9].

In **fungi**, protease was found in *Aspergillus flavus* var. *columnaris* [10], *Mucor pusillus* strain S7 407 [11], *Streptomyces rectus* var. *proteolyticus* [12], *Xanthomonas campestris* pv [13].

In **yeast**, protease was found in *Aureobasidium pullulans* [14], *Candida digboensis* SRD yeast 1 [15], *Candida humicola* [16], *Saccharomycopsis lipolytica* [17],

In **protozoa**, protease found in *Rumen protozoa* [18].

In **plants**, protease was found in Nepenthes-Nepenthesin I & II Neprosin[19], Papaya-Papain[20], Pineapple-Bromelain, Fig (*Ficus carica*)-Ficin[21], Kiwifruit, Banana-Actinidin, Zinger-Zingipain, Cardoon-Cardosin A, Rice-Oryzasin, Barley-Phytapsin[22].

3. APPLICATIONS OF PROTEASES

Microbes are important sources including protease in the production of industrial product such as food, pharmaceutical, detergents, leather, dairy, brewing, management of industrial and household waste, photographic, silk degumming, proteases in peptide mapping and sequencing, baking process, meat tenderization, animal feeds and other products in industries.

Baker industry

Microbial proteases are useful in baking industry. The proteases used on large scale in the production of breads, baked foods, crackers and waffles due to it reduce mixing time, decrease dough consistency, regulate gluten strength in bread, assure dough uniformity, control bread texture, and improve flavour. proteolytic enzyme treatment are used in baking process. The bread production is usually obtained from *Aspergillus oryzae*.

Dairy industry

Mostly the proteases used in dairy industry for the manufacture of cheese. The important function of protease is to hydrolyze the specific peptide bond to generate macro peptides and para k casein. There are some disadvantages (i. e) the development of bitterness in cheese after storage and poor yield. In cheese ripening stage, the protease is important and alkaline proteases produced off-flavors was tested [23].

Brewing industry

Bacterial protease called neutrase is used in alcohol production for high Yeast growth. Protease plays important role in alcohol production due to break down of large proteins into smaller ones. It also used in the finishing stages of beer to remove the chill haze. *Streptomyces fradiae* was used for clarification.

Meat industry

Protease is used for meat tenderization. After the animal is dissected, protease degrade the muscle fiber protein by series of complex reactions, then it increases the tenderness of the meat.

Medical usage

Alkaline proteases are used for the preparation of elastoterase. Elastoterase was applied for the treatment of burns, furuncles and deep abscesses. *Bacillus* spp was approved to be safe to human [24].

Leather industry

In leather industry, many hazardous chemicals are used such as sodium sulphide and the chemicals are responsible for pollutions. Therefore, the use of protease is beneficial to the production and environment because protease is eco-friendly and less harmful than chemicals [25]. *Bacillus* spp like *bacillus altitudinad* GVC11, *Bacillus cereus* MCM B-326, *Bacillus subtilis* and *Bacillus subtilis* KT004404 are used for dehairing in the leather industry [26].

Detergents industry

In this industry, the enzymes are widely used. Protease also One of the important enzymes in the detergents production. Protease have advantages such as removal of proteins stains. Several *bacillus* spp such as *B. subtilis*, *B. brevis*, *B. cohnii* are used in the detergent additive protease production [27]. There are some disadvantages also such as allergic reactions, expensive production. It can also overcome by using prominent compatible enzymes [28].

Waste management

In leather and poultry industry, wastes are densely packed and stabilized by chemicals are not eco-friendly [29]. By enzyme degradation, the wastes are easily solved. It also eco-friendly and *Bacillus* species is widely used in degradation process [[30]].

4. CONCLUSION

Since the need of enzymes especially protease have been broadly used in industries such as food, textile, detergent, pharmaceuticals, leather. The production of proteases must be increasing with the use of cheap raw material and by incorporating genetic manipulation. Now with technology that provides clean production without the addition of chemicals and we can achieve high yield of protease with low production cost. We can control the risk of eco-pollution and provide better protease for various production of products like cheese, baking products, alcohol production etc.

5. REFERENCES

- [1] Sakai, D.K. 1985. Significance of extracellular protease for growth of a heterotrophic bacterium, *Aeromonas salmonicida*. Applied and Environmental Microbiology. 50(4): 1031-1037.
- [2] Blanco, C. and Michotey, V. 1994. Characterization of an endoserine protease secreted by *Arthrobacter aureus*. Applied and Environmental Microbiology. 60: 341 – 343.
- [3] Van Der Laan J.C., Gerritse G., Mulleners, L.J., Van Der Hoek. R.A. and Quax, W.J. 1991. Cloning, characterization and multiple chromosomal integration of a *Bacillus* alkaline protease gene. Applied and Environmental Microbiology. 57(4):901-909.
- [4] Vasantha N., Thompson, L.D., Rhodes, C., Banner, C., Nagle, J. and Filpula, D. 1984. Genes for alkaline protease and neutral protease from *Bacillus amyloliquefaciens* contain a large open reading frame between the regions coding for signal sequence and mature protein. Journal of Biotechnology. 159(3):811-819.
- [5] Shiga, Y., Hasegawa, K., Tsuboi, A., Yamagata, H. and Udaka, S. 1992. Characterization of an extracellular protease inhibitor of *Bacillus brevis* HPD31 and nucleotide sequences of the corresponding gene. Applied and Environmental Microbiology. 58(2):525-531.
- [6] Kumar, C.G. 2002. Purification and characterization of a thermostable alkaline protease from alkalophilic *Bacillus pumilus*. Letters in Applied Microbiology. 34(1):13-17.
- [7] Irvine, D.M. and Puhon Z. 1969. Proteolysis by proteases of *Bacillus subtilis* used to make Canadian cheddar cheese. Journal of Dairy Science, 56:317-322.
- [8] Mayerhofer, H.J., Marshall, R.T., White, C.H. and Margaret, L.U. 1973. Characterization of a heat-stable protease of *Pseudomonas fluorescens* P26. Applied Microbiology. 25(1):44-48.
- [9] Halio, S.B., Bauer, M.W., Mukund, S., Adams, M. and Kelly, R.M. 1997. Purification and characterization of two functional forms of intracellular protease PfpI from the hyper thermophilic archaeon *Pyrococcus furiosus*. Applied and Environmental Microbiology. 63(1): 289–295.
- [10] Impoolsup, A., Bhumiratana, A. and Flegel, T.W. 1981. Isolation of alkaline and neutral protease from *Aspergillus flavus* var. *columnaris*, a soy sauce koji mold. Applied and Environmental Microbiology, 42(4):619-628.
- [11] M.B., Tanksale, A.M., Ghatge, M.S. and Deshpande, V.V. 1998. Molecular Rao, and biotechnological aspects of microbial proteases. Microbiology and Molecular Biology Reviews. 62(3):597-635.
- [12] Mizusawa, K., Ichishima, E. and Yoshida, F. 1969. Production of thermostable alkaline proteases by thermophilic Streptomyces. Applied Microbiology, 17(3):366-371.
- [13] Dow, J.M., Daniels, M.J., Carke, B.R., Milligan, D.E. and Tang, J.L. 1990. Extracellular proteases from *Xanthomonas campestris* pv. *campestris*, the black rot pathogen. Applied and Environmental Microbiology. 56(10):2994-2998.
- [14] Chi, Z., Ma C., Wang P. and Li. H.F. 2007. Optimization of medium and cultivation conditions for alkaline protease production by the marine yeast *Aureobasidium pullulans*. Bio resource Technology, 98(3):534-538.

- [15] Patel, M.J., Tipre, D.R. and Dave, S.R. 2009. Isolation and identification of *Candida digboiensis* strain from acid mine drainage of lignite mine, Gujarat. *Journal of Basic Microbiology*. 49(6):564-571.
- [16] Ray, M.K., Devi, K.U., Kumar, G.S. and Shivaji, S. 1992. Extracellular protease from the Antarctic yeast *Candida Humicola*. *Applied and Environmental Microbiology*.58(6):1981-1923.
- [17] Simms, P.C. and Ogrydziak, D.M. 1981. Structural gene for the alkaline extracellular protease of *Saccharomycopsis lipolytica*. *Journal of Bacteriology*. 145(1):404-409.
- [18] Forsberg, C.W., Lovelock, L.K., Krumholz and Buchanan-smith, J. G. 1984. Protease activities of rumen protozoa. *Applied and Environmental Microbiology*.47(1):101-110.
- [19] Kadek et al (2014a), Kadek et al. (2014b) and Yang et al. (2015) Schrader et al. (2017) Rey et al. (2016).
- [20] Amri & Mamboya (2012),Canay, Erguven & Yulug (1991),Choudhury et al. (2009).
- [21] Chanalía et al. (2011), Mazorra-Manzano, Ramírez-Suarez d,Yada (in press),Malone et al. (2005), Karnchanatat et al. (2011),Feijoo-Siota & Villa (2011),Frazao et al. (1999), Simões & Faro (2004),Runeberg-Roos & Saarma (1998).
- [22] Richards, M., Kock, De.R., Duodu, G. and Buys, E. (2014). LWT - Food Science and Technology The effect of legume protease inhibitors on native milk and bacterial proteases. *LWT - Food Science and Technology*, 57(2): 628–633.
- [23] Kumar, C.G. and Takagi, H. 2002. Microbial alkaline proteases. *Biotechnology Advances*. 17(7): 561–594.
- [24] Singh, S. and Bajaj, B.K. 2017a. Agro industrial/forestry residues as substrates for production of thermo active alkaline protease from *Bacillus licheniformis* K-3 having multifaceted hydrolytic potential. *Waste Biomass Valorization*. 8(2): 453–462.
- [25] Singh, S. and Bajaj, B.K. 2017b. Potential application spectrum of microbial proteases for clean and green industrial production. *Energy, Ecology and Environment*. 2(6): 370–386.
- [26] Guleria, S., Walia, A., Chauhan, A., Shirkot, C.K. 2016. Purification and characterization of detergent stable alkaline protease from *Bacillus amyloliquefaciens* SP1 isolated from apple rhizosphere. *Journal of Basic Microbiology*. 56(2):138–152.
- [27] Singh, R., Kumar, M., Mittal, A. and Mehta, P.K. 2016. Microbial enzymes: industrial progress in 21st century. 3 *Biotech*. 6(2), 1–15.
- [28] Kudrya VA, Simonenko IA. Alkaline serine proteinase and lectin isolation from the culture fluid of *Bacillus subtilis*. *Applied microbiology and biotechnology*. 1994;41(5):505–509.
- [29] O. Okparaorcid, Department of Biochemistry, Federal University of Technology, Akure, Nigeria. DOI: 10.4236/aer.2022. 101002 Microbial Enzymes and Their Applications in Food Industry.
- [30] Singh R, Mittal A, Kumar M, Mehta PK (2016). Microbial proteases in commercial applications. *J Pharm Chem Biol Sci* 4:365–374.
- [31] Sindhu Raveendran, Binod Parameswaran, and Ashok Pandey. Applications of Microbial Enzymes in Food Industry.
- [32] Riddhi Sawant and Saraswathy Nagendran (2014), protease: an enzyme with multiple industrial application.