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RECENT BREAKTHROUGHS IN THE MANUFACTURE OF MICROBIAL PROTEASES AND THEIR APPLICATIONS: A REVIEW

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ABSTRACT: Enzymes are biocatalysts that accelerate biotic reactions positively or negatively. Proteases are small types of enzymes that cleave peptide bonds. These proteases can be produced by microbes (fungi, bacteria, and viruses), plants, and animals. The catalytic site is only visible from the outside and is broadly dispersed on the surfaces of different cell types. Based on the position of the cleavage site in the putative substrate, these peptidases can be classified as endopeptidases or exopeptidases. These peptidases are used in a variety of industries, including laundry detergents, leather and fabric processing, food processing, baking, flesh tenderization, food additives, artificial sweetener synthesis, feed, cheese making and the dairy industry, soy sauce production, brewing and cereal processing, photography, environmental bioremediation, medical and pharmaceutical industries, plant pathology, nitrogen production, and so on. Proteases are classified based on physiological roles. Extracellular proteases breakdown down large proteins into smaller parts on the other hand, intracellular proteases helps in the regulation of metabolism.

INTRODUCTION: Peptidases, often known as proteases, are enzymes that can break peptide bonds. They release small peptides and amino acids that the body requires and engage in cellular protein turnover. Because of their industrial applicability in various goods, these enzymes are being studied. Proteases are one of the three most common types of industrial enzymes, accounting for over 60% of all enzyme sales globally [1]. These are found everywhere in nature and are important in both physiological and economic domains.

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To address industrial application needs, novel protease sources are urged to be developed. These enzymes may be found in various plants, animals, and microbes. Trypsin, chymotrypsin, pepsin, and the lysosome enzymes cathepsin B and cathepsin D are the greatest proteinases found in mammals' digestive systems. These are beneficial for a variety of biological activities. They play an active role in the regulation of specific physiological processes.

Proteases are required for a large range of catalytic actions in the normal metabolism of a live organism. Proteases are a class of big, complex enzyme molecules specializing in performing highly targeted proteolysis. Because of their high selectivity for biomolecules, proteases are widely used in the medical and pharmaceutical industries. Gurumallesh *et al.* (2019) [2] say that substrate-specific protease are used to treat a wide range of

diseases in people. Proteolytic enzymes can rapidly alter proteins by restricted cleavage, such as zymogenic enzyme activation, blood clotting and fibrin clot lysis, and secretory protein processing and transport across membranes [3]. These proteolytic enzymes are utilised as reagents in laboratory, clinical, and industrial operations, as well as in many medicinal treatments. Proteases come in various shapes and sizes, which has led to attempts to harness their physiological and biotechnological uses [4]. This article gives an overview of the latest changes in protease production and how they are used in many different fields [5].

Production of Protease: Peptidases or protease enzymes are being investigated due to their industrial applications in various products. Proteases represent one of the three largest groups due to their substrate selectivity, stability, and manufacturing costs; limitations exist in manufacturing innovative and desired microbial proteases. New-generation technologies are increasingly used to identify or engineer novel proteases that overcome these barriers. In contrast, dedicated evolution is used to improve existing enzymes, and rational redesigning techniques are used to create new ones [6]. Efforts to replace harmful industrial methods with enzyme or protease-based bacterial methods will help both the environment and people. Its usage and manufacturing are growing due to low-cost raw materials and genetic modification. Instead of using harmful chemicals, the proteases promise cleaver manufacturing [7]. Industrial enzymes account for about 60% of the worldwide sale of enzymes [1]. These are ubiquitous and play a major role in physiological and commercial fields. The vast diversity of proteases has attempted to exploit their physiological and biotechnological applications [4].

In nature and under typical culture conditions, proteases generated by microorganisms are constitutive or partly inducible. During the post-exponential and stationary phases, Bacillus species secrete extracellular proteases. Variations in the carbon/nitrogen ratio, readily metabolizable carbohydrates (glucose), and metal ions impact extracellular protease synthesis in bacteria. Rapidly metabolizable nitrogen sources in the media, such as amino acids, impact protease production. The quantity of protease is also affected by aeration, inoculum density, pH, temperature, and incubation. Biochemical processes and engineers employ numerous ways to achieve high yields of protease in a fermenter in large-scale protease synthesis from microorganisms at the industrial level. Controlled batch and fed-batch fermentations with different microorganisms and simultaneous control of glucose, ammonium ion concentration, oxygen tension, pH, salt availability, and chemostat cultures helped increase protease production for long-term incubations.

Separating the biomass and protease production phases and applying occasional depression and induction throughout the organism's development, the alkaline protease output from *B. mojavensis* was enhanced up to fourfold in semi-batch and fed-batch operations. Cell immobilisation of *B. firmus* aqueous two-phase (ATPase) systems composed of polyethylene glycol (PEG)-(4000, 6000, 9000) and potassium phosphate using *B. thuringiensis*, and the ATPase system composed of PEG-6000 and dextran T-500 using *B. licheniformis* solid state fermentation methods and biphasic fermentation.

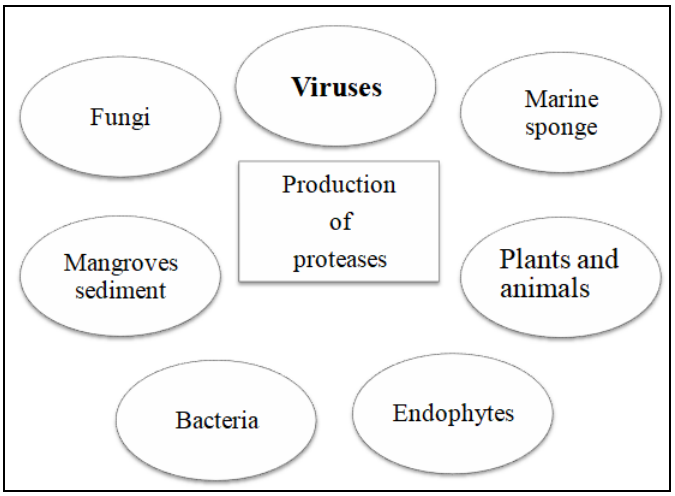

FIG. 1: PRODUCTION OF PROTEASES ENZYME BY VARIOUS BIOLOGICAL RESOURCES

Proteases from Microbes: Most protease enzymes are produced by the microbial fermentation method. Plant and animal proteases cannot fulfil current global needs, which have sparked an interest in microbial proteases. Because of their wide metabolic variety and vulnerability to genetic modification, microorganisms are a good source of enzymes. Microbial proteases account for roughly 40% of all enzyme sales globally. Proteases derived

from microorganisms are favoured over enzymes derived from plants and animals because they have almost all the properties needed for biotechnological applications. Animal, plant and, in particular, microbial proteases comprise the biggest and most significant section of the industrial enzyme market, with applications in detergents, food processing, the leather industry, organic synthesis biocatalysts, and pharmaceuticals. Protease inhibitors have been created as a class of treatments since proteases are also causal factors in certain disorders. Since the 1950s, commercial fermentation techniques for manufacturing microbial proteases have been established. Their proteases helped break down proteins and develop flavours in solid mash substrates made of soy beans, cereal grains, and other plant-based foods [3-10].

Fungi: Fungi produce a broader range of enzymes than bacteria. Acid, neutral, and alkaline proteases are produced by *Aspergillu soryzae* [11] Alternaria [12]. Fungal proteases are active over a wide pH range (pH 4 to 11) and have wide substrate specificity. They have a slower response rate and are less heat resistant than bacterial enzymes [13]. In a solid-state fermentation approach, fungal enzymes may be easily generated. The ideal pH for fungal acid proteases is between 4 and 4.5 and stable between 2.5 and 6.0. Because of their restricted pH and temperature specificities, they are very valuable in the cheese-making sector. Fungal-neutral proteases are made of metalloproteases that work at a pH of 7.0 and are stopped by chelating substances [14].

Because of the high oxygen tension, *Aspergillus species* usually develop as moulds on the surface of a substrate. Fungi often thrive on carbon-rich substrates like glucose and polysaccharide. *Aspergillus species* are typical pollutants of starchy foods, and they may be found in or on a wide variety of plants and trees. *Aspergillus niger* and *Aspergillus flavus* [15] may be found in soil and indoor settings and is sometimes mistaken for black *Stachybotrys* colonies (black mold). Some strains of *A. niger* have been found to generate ochratoxins, which are employed in the synthesis of a variety of valuable enzymes in industrial fermentation [16]. The glucoamylase generated by *A. niger* is utilised to make high fructose corn syrup, while the pectinases are used to clarify cider and wine. A galactosidanse, an enzyme that breaks down certain complex sugars, is included in beans and other medications that promise to reduce flatulence. In the biotech industry, *A. niger* is used to make magnetic isotope-containing versions of biological macromolecules for NMR investigation. It is used as a vegetarian glucosamine source. The United States Food and Drug Administration have classified *A. niger*'s fermentation products as "Generally Regarded As Safe" (GRAS). *A. niger* secreted RNase, possesses antiangiogenic and anti-carcinogenic properties. The enzymes glucose oxidase (GO) and alpha-galactosidase are extracted from *A. niger* cultures (AGS). Because of its strong affinity for-D-glucose, glucose oxidase is employed in developing glucose biosensors.

Fermentation of *A. niger* makes alpha-galactosidase, which breaks alpha 1-6 bonds in melibiose, raffinose and stachyose. At the height of the opium trade, Chandoo opium was manufactured by the long-term fermentation of *A. niger* and other moulds on raw opium. The major agent in the fermentation of Puerh tea is *Aspergillus niger*. All living things depend on fungus, which generates fungal proteases, which have been linked to a variety of disorders and might be used as therapeutic targets. Building selective inhibitors for fungal proteases is difficult owing to a lack of understanding of substrate specificity and endogenous and natural inhibitors. Genetic manipulation *via* transcriptional regulation of fungal extracellular protease expression might be viable.

As CRISPR/Cas9 technology advances, it is expected to play a larger role in future genetic modification research for filamentous fungus. Tools for functional genomics in filamentous fungal species will make it simpler to put CRISPR gene-editing technology into practise quickly [17].

Bacteria: *Bacillus bacteria* generate commercial proteases, particularly neutral and alkaline proteases. Neutral proteases have a restricted pH range of activity (pH 5 to 8) and have poor thermo-tolerance. Neutrase is a neutral protease resistant to plant proteinase inhibitors, making it valuable in the brewing business. The strong affinity of bacterial neutral proteases for hydrophobic amino acid pairs distinguishes them. Their poor

thermotolerance makes it easier to manage their reactivity during the synthesis of low-hydrolysis food hydrolysates. Metalloproteases, such as neutral proteases, need divalent metal ions to function, while serine proteinases are unaffected by chelating chemicals. Bacterial alkaline proteases are known for their strong activity at alkaline pH and wide substrate specificity, with an ideal temperature of 60°C. Pseudomonas generates a variety of proteolytic enzymes, most of which are alkaline proteases. *Pseudomonas aeruginosa* makes many different proteases, found in different strains and studied [18-24].

Currently, a variety of Bacillus strains are in use, and most of them possess the necessary activity before being boosted by mutagenesis or genetic engineering to create strains that successfully produce the needed alkaline protease. Bacillus-based expression systems are needed because these strains need to use directed and statistical mutagenesis to improve the produced enzyme's properties and level of expression [25]. Cereus RS3 isolated from desert soil samples, from *Bacillus cereus* strain RS3 was identified as an extracellular alkaline protease-generating bacterial isolate. The greatest synthesis of alkaline protease was reported at pH 8 and a temperature of 45°C. As per Shine and Kanimogh *et al*. (2016) [26] that *B. cereus* RS3 could be used to make a lot of alkaline proteases.

Bacillus nealsonii PN-11 generates thermo-alkaliphilic mannanase and protease that are active at a broad range of temperatures and pH levels. The coproduction of protease and mannanase from this strain was optimised, and a cocktail of these enzymes was used as a detergent additive [27-28]. Purification and characterization of mannanase were completed before, and protease was completed during this investigation at a pH of 10 and a temperature of 65°C.

It was completely stable at 60 °C for three hours and had >80% activity for one hour at pH 11. Individually and in combination, both enzymes were compatible with detergents. When protease or mannanase was applied separately, the detergent's wash effectiveness on various types of stains increased. David *et al.* (2018) [29] found that destining worked better when using mannanase and protease.

Viruses: Viral proteases become relevant because of their role in the digestion of proteins produced by viruses that cause deadly diseases such as AIDS and cancer. Viruses include peptidases for serine, aspartic, and cysteine. Retroviral aspartyl proteases are homodimers expressed as part of the polyprotein precursor and are essential for viral assembly and replication. Autolysis of the precursor releases the mature protease [4, 30-31].

Marine Sponges: Fibrinolytic enzymes are receiving increased attention due to their therapeutic potential for thrombolytic diseases. Purified protease-producing *Streptomyces radiopugnans* VITSD8 from maritime brown tube sponges *Agelas conifera* has a fibrinolytic nature *in-vitro*. Casein plasminogen plate and fibrin plate techniques were used to screen and test the fibrinolytic activity of Streptomyces sp. Ammonium sulphate fractionation, affinity, and ion-exchange chromatography were used to purify the crude caseinolytic protease. For making fibrinolytic protease [32], maltose and peptone were the best places to get carbon and nitrogen.

This fibrinolytic enzyme was extremely active and stable at a moderate pH and temperature. Clinical studies might further explore it as a possible source of thrombolytic treatment. A clot-busting enzyme gives cancer, stroke, and heart sufferer's fresh hope. Dhamodharan *et al.* (2019) [32] reported that enzymes made from natural marine isolates are important pharmaceutical compounds that can be used to make new and effective fibrinolytic agents, especially for treating heart problems.

Mangroves Sediment: The development of novel compounds relies heavily on the functional screening of metagenomic libraries. Microorganisms' metabolic variety allows them to survive in severe settings and is linked to enzyme production. A protease-producing clone was obtained from mangrove silt in a metagenomic library. The protease was purified with ammonium sulphate precipitation and gel filtration chromatography, yielding 77.27 percent. Nine peptides related to a *Bacillus safetynsis* peptidase were discovered using MS/MS in ESI-Q-TOF. The aligned partial sequence matched the conserved domains of a glutamyl aminopeptidase from the human gut met genome with 47.48 percent identity

and 82.74 percent similarity. The protease worked best at a pH of 8.5 and a temperature of 60°C. It had more than 80% activity at pH 9-12. Between 40 and 50°C, it demonstrated excellent thermotolerance and thermostability. For azocasein, the K_M and V_{max} values were calculated to be 0.92 mg ml^{-1} and 13.15 mmol min^{-1}, respectively. According to the substrate specificity study, PR4A3 was shown to be active on gelatine, blood, egg yolk, and milk. These results show that PR4A3 could be used in biotechnological applications [33-34].

Endophytes: Endophytes have been identified as potential sources of new secondary metabolites, such as enzymes and medicines, with medical, agricultural, and industrial uses. The three fungal endophytes (*Alternaria alternata*, *Phoma herbarum*, and an unidentified fungus) were isolated from *Eremophilia longifolia* (an Australian native plant), and tested for protease production. Using enzyme activity tests, the lyophilized growth medium produced following fungal fermentation was examined for protease production. The effects of temperature, pH, carbon supply, and nitrogen source on activity were studied in order to optimize protease synthesis. *A. alternata* has the highest protease activity over a broad pH range (3–9). The activity ranged from 9 to 50°C on pH 7, indicating a neutral protease. Zaferanloo *et al.* (2014) [35] looked at the properties of this fungal endophyte and found that it might be a source of an enzyme that could be useful in the dairy industry [36]. Proteasome proteolytic processes have a threonine active site and three separate peptidyl glutamyl peptide-hydrolyzing substrate specificities as chymotrypsin-like, trypsin-like, and peptidyl–glutamyl peptide-hydrolyzing substrate specificities. Proteolysis is used in the biochemical cascade that leads to blood coagulation and in insulin generation for covalent activation, control, and inhibition of enzymes and other protein-based or related effectors. Proteases take out signal sequences after membranes move or secrete peptides and take out N-terminal methionine residues after translation.

Plants and animals: Plant proteases have long been recognized as important components of plant immunity. DAMPs and MAMPs, effect recognition regulation of PRRs and NB-LRRs (R-Proteases action), signal transduction (including MAPK cascade activation), involvement in the synthesis of signal molecules, cell priming, regulation of RR protease expression, SAR and ISR establishment, and finally RNA silencing, all seem to involve proteases.

Plant proteases have unique properties that might be employed in biotechnology and biomedicine; for example, papain from *Carica papaya* and bromelain from *Ananas comosus* are currently in use in various industries. Bromelain is made from the pineapple stem [37], which is peeled, crushed, and pressed after the fruit is harvested to provide a juice extract containing the soluble bromelain enzyme. There is an incision in papaya's green fruit to extract papain's latex yields. The gel-like latex was collected and dried on trays with forced air at temperatures as high as 55°C, or it could be melted, filtered, and spray-dried to make the crude enzyme, which was then cleaned up using water extraction and solvent precipitation. To eliminate cloudy and viscous plant polysaccharides from crude aqueous extracts, carbohydrate/plant gum-depolymerizing enzymes may be added. When the final product is a liquid or when it is being made, sodium bisulfite is added to protect the unstable sulfhydryl groups at the active site. Balakireva and Zamyatniu (2018) [38] reported that the development of science and biotechnology would be helped by discovering new proteases and a better understanding of how plant proteases work physiologically [39-40].

Since ancient times, when milk contained in calf stomachs was found to coagulate, an enzyme has been isolated from animal sources (due to the activity of calf rennet or chymosin). The fourth stomach of unweave calves is the greatest source of the enzyme, which it shares with pepsin, although the ratio of pepsin to rennet is low. Christian Hansen began the commercial manufacture of chymosin in Denmark by removing calf stomachs with saline, just as bovine pepsin is extracted. For this reason, the enzyme-containing tissues are maintained by salting, freezing, or drying at the slaughterhouse. Single-strength rennet may be made by slicing, grinding, or grinding dry calf stomach and extracting it with water containing 5%–10% NaCl.

Proteases and their Physiological Roles: Protein catabolism, blood coagulation, cell growth and

migration, tissue arrangement, morphogenesis in development, inflammation, tumour growth and metastasis; activation of zymogens; release of hormones and pharmacologically active peptides from precursor proteins, and transport of secretory proteins across membranes are just a few of the many complex physiological and pathological processes that proteases carry out. Extracellular proteases accelerate the breakdown of big proteins into smaller molecules for cellular absorption, while intracellular proteases regulate metabolism. Proteinases are also involved in protein turnover, germination, gene regulation, sporulation, and the release of conidia [4].

Types of Proteases: Proteases, both intracellular and extracellular, are produced by microorganisms in vast quantities. Intracellular proteases have a role in sporulation and differentiation, protein turnover, enzyme and hormone maturation, and the preservation of the cellular protein pool, among other cellular and metabolic activities. Extracellular proteases are necessary for protein hydrolysis in cell-free settings, allowing the cell to receive and use hydrolytic products. Similarly, extracellular proteases have been commercially used to aid protein breakdown in a variety of industrial applications [41]. Based on where they work, exopeptidases and endopeptidases are the two primary types of proteases. Exopeptidases cleave peptide bonds close to the substrate's amino or carboxy termini, whereas endopeptidases cleave peptide bonds farther away. Serine proteases, aspartic proteases, cysteine proteases, and metallo-proteases are the 4 major families of proteases based on their functional groups. However, certain proteases, such as ATP-dependent proteases, do not fit well into this group [3].

Exopeptidases: the exopeptidases are enzymes that break down exopeptidases. These are classed as aminopeptidases (act at the free N terminus of the polypeptide chain) and carboxypeptidases (act at the C terminus of the polypeptide chain), respectively, depending on their site of action at the N or C terminus.

Aminopeptidases: The aminopeptidases are found in a broad range of bacterial and fungal species. *Aspergillus oryzae, Bacillus licheniformis, Botulinum stearothermophilus,* and *E. coli* are examples of industrial organisms that manufacture aminopeptidases. There is just one report on an extracellular aminopeptidase generated by *Aspergillus oryzae*, so it's mostly intracellular enzymes. The big protease aminopeptidase-I is from *E. coli*. It needs Mg+2 or Mn+2 for optimal action and pH range of 7.5 to 10.5 for activation. Aminopeptidase is made by *B. stearothermophilus*. The ion Co+2 boost aminopeptidase activity in *Bacillus licheniformis*. The ions Zn+2, Mn+2, or Co+2 ions activate this enzyme, which is composed of two subunits. A leucine aminopeptidase enzyme was isolated from barley-germinated grains [42].

Carboxypeptidases: Serine carboxypeptidases, metallocarboxypeptidases, and cysteine carboxy-peptidases are the three primary families of carboxypeptidases based on the type of amino acid residues at the active site of the enzymes. The substrate specificities of the serine carboxypeptidases isolated from speces of *Penicillium*, *Saccharomyces*, and *Aspergillus* are comparable, while other parameters, including pH optimum, stability, molecular weight, and inhibitor action, vary somewhat. It was necessary for the action of metallocarboxypeptidases from species of *Saccharomyces* and *Pseudomonas*.

Endopeptidases: The endopeptidases are distinguished by their preference for peptide bonds in the polypeptide chain's inner regions, away from the N and C termini. The presence of a free amino or carboxyl group reduces the activity of the enzyme [1]. Based on their catalytic mechanism, endopeptidases are split into four subgroups:

Serine Proteases: The enzyme serine proteases have a serine group in their active site, distinguishing them from other proteases. The typical catalytic triad of serine proteases is composed of three residues: serine, histidine, and aspartate.

They are abundant and pervasive in viruses, bacteria, and eukaryotes. Exopeptidases, endopeptidases, oligopeptides, and omega peptidase are several types of serine proteases. Serine proteases have wide substrate specificities, including esterolytic and amidase activity, and are normally active at neutral and alkaline pH, with an optimum between pH 7 and 11. Bacteria, moulds,

yeasts, and fungi all generate serine alkaline proteases [43]. They hydrolyze a peptide bond with tyrosine, phenylalanine, or leucine on the carboxyl side. The chymotrypsins and subtilisins are examples of serine endproteases. Bacillus species generate subtilisins, which are a class of serine proteases [44-46].

Subtilisins: The subtilisins are the second biggest family of serine proteases. Two different types of alkaline proteases isolated by *Bacillus licheniformis* makes Sulfonisin Carlsberg; and *Bacillus amyloliquefaciens* makes subtilisin Novo (also called bacterial protease Nagase), BPN9.

Aspartic Proteases: The acidic proteases rely on two highly conserved aspartic acid residues and have isoelectric values ranging from pH 3 to 4.5. Microbial aspartic proteases come in two types: enzymes that act like pepsin and are made by *Aspergillus, Penicillium, Rhizopus,* and *Neurospora*; and enzymes that act like rennin and are made by *Mucor pusillus, M. miehei* and *Endothia* species. Aspartic endoproteases have a low pH optimal range (3-4) and have two aspartic acid residues in their active sites.

Cysteine/Thiol Proteases: Cysteine proteases are found in both prokaryotes and eukaryotes, and there are roughly 20 different families of them. All cysteine proteases rely on a catalytic dyad of cysteine and histidine for their action. Although certain cysteine proteases (lysosomal proteases) are optimally active at acidic pH, most cysteine proteases have neutral pH optima.

They are classified into 4 classes based on their side chain specificity like papain-like, trypsin, cleavage at the arginine residue, specific to glutamic acid, and others. Papain, which is made up of clostripain and streptomycin from *Clostridium histolyticum* and Streptomyces species, is the most well-known cysteine protease.

Metalloproteases: The most diversified of the catalytic forms of proteases, these enzymes need a divalent metal ion to function. Collagenases from higher species, hemorrhagic toxins from snake venoms, and thermolysin from bacteria are among the enzymes found there [47]. Metalloproteases are classified into 4 classes based on the specificity of their actions: neutral, alkaline, Myxobacter I, and Myxobacter II. The neutral proteases have a narrow selectivity for hydrophobic amino acids, but the alkaline proteases have a much wider range of targets [1]. Four calcium atoms improve protein stability. The other group members are the neutral metalloprotease elastase (*P. aeruginosa*) and Myxobactersp alkaline's cell wall lytic protease I. Ethyl-enediaminetetra acetic acid inhibits these enzymes but not sulfhydryl reagents or di-isopropyl-fluorophosphate. *B. stearothermophilus* makes thermolysin, a neutral zinc protease that is very stable at high temperatures and is a well-known member of this subgroup.

Glutamic acid and Threonine Endproteases: these are newly discovered enzyme families with reaction mechanisms involving active-site glutamic acid and threonine.

Applications of Proteases: Temperature, pH, ion need, specificity, activity, and stability are all characteristics shared by all proteolytic enzymes. These biochemical properties govern the use of protease in the industry aside from other criteria, which include the cost of manufacturing and research, markets, and the economics of application. Proteases play an important role in a broad range of businesses [4].

Laundry Detergents Industry: Proteases are an important component of current laundry detergents. In the laundry detergent business, subtilisin proteases have been created via directed evolution and rational design to match their features to industrial needs during the last 30 years. Among the advances in protease engineering for laundry detergents, there is a good way to change pH profiles and a general idea of encouraging promiscuous activity toward forming peroxycarboxylic acids as mild bleaching agents. Detergent proteases with broad substrate specificity are suitable for removing various stains (food, blood, grass, and body secretions). Protease activity, stability at high pH and temperatures, and compatibility with additional chelating and oxidizing agents added to the detergent are all important considerations. A protease's pI (ionic strength) is the most important factor in determining how well it performs in a detergent. Most detergent proteases on the market today are serine proteases generated by Bacillus strains.

However, fungal alkaline proteases are useful since they are simple to treat downstream to produce a microbe-free enzyme. Combining lipase, amylase, and cellulose should improve the way proteases work in laundry detergents [48].

Leather and Fabric Processing:

There are Four Primary Phases in Leather Processing: soaking, dehairing, bating, and tanning. Alkali solutions are added during the soaking process to help the hide swell. The use of high alkali concentrations coupled with a hydrogen sulphide treatment phase for protein hair root solubilization aids in removing hair and/or wool from hides using traditional chemical methods.

Because of the hazards to employees handling these materials, there is a significant movement away from using harsh chemicals in processing. These approaches raise the danger of spills and contamination while adding considerably high costs to effluent treatment and waste disposal. The proteases selectively target protein parts that don't contain collagen. This improves the quality of leather more than chemical treatment can.

Microbial alkaline proteases are now being employed to assure quicker water absorption and minimize soaking time by 10-20 hours. The use of non-ionic and, to a degree, anionic surfactants to speed up the process is compatible with enzyme usage. Dehairing is now done using alkaline proteases including hydrated lime and sodium chloride, which results in a considerable decrease in the quantity of wastewater produced. For baiting, trypsin is combined with other Bacillus and Aspergillus proteases. The enzyme is chosen based on its selectivity for matrix proteins like elastin and keratin, and the quantity of enzyme required is determined by the kind of leather to be produced (soft or hard). Increased enzyme use for dehairing and bating reduces pollutants and saves energy. Novo Nordisk makes three different proteases called aquaderm (soaking), NUE (dehairing), and pyrase (bating). Proteases may be used in silk production techniques. Raw silk fibres have a rough texture because of a protein in silk called sericin. To get rid of sericin from the core silk fibre proteins, expensive methods have been used, such as applying starch during shrink-proofing and twist-setting [49-54].

Food Processing Industry: Since, antiquity, protein has been used in the food business. They've been used for a variety of things, including cheese manufacturing, baking, producing soy hydrolysates, and meat tenderization.

Baking: Wheat flour is an important ingredient in baking. Gluten is an insoluble protein found in flour that controls the qualities of baking dough. *Aspergillus oryzae* endo-and exoproteinases were utilized to alter wheat gluten by restricted proteolysis. The dough is treated with enzymes to make it easier to handle and machine, allowing for the creation of a larger variety of items. Including proteases shortens the mixing time and increases the volume of the bread. Using proteases to partly hydrolyze the gluten helps speed up the dough-making process. Bacterial proteases are employed to increase the dough's flexibility and strength. Protease-catalyzed gluten breakdown in more easily available high-gluten-containing wheat might remedy this issue. Heat-labile fungal proteases are utilized in baking for gluten hydrolysis, and the enzyme denatures as the temperature increases early in the baking process. The alterations associated with taste and nutritional developments are a second use of proteases in baking. Biscuits, cookies, and crackers may all benefit from bacterial neutral proteases.

Tenderization of Flesh: The majority of buffaloes are slaughtered for their meat. The by-products of butchered animals have a high value as well. Because of its low functional qualities and intrinsic hardness owing to its high collagen content, commercial exploitation of buffalo tripe for the development of processed product production is relatively restricted [47]. Chemical or proteolytic enzymes, such as papain, may be used to tenderise meat slices. Papain has a great deal of potency when it comes to hydrolyzing fibrous protein and connective tissue. During tenderization, uniform penetration of the tenderizer enzyme was always a difficulty. Plant proteases like papain and bromelain, as well as the neutral protease made by *B. subtilis*, are good for making meat tenderer.

Industry of Food Additives: In the formation of xanthan gum by *Xanthomonas campestris*, proteases may be utilised to remove the cell mass. Food concentrates, protein hydrolysates, and soy

sauces are all made using proteolytic enzymes. Many lactic acid bacteria exhibit probiotic-synthetic activity, which benefits consumer health. Probiotics are living microorganisms that exist in a particular proportion in food and, when taken, improve the intestinal microflora of the host. Adding these microorganisms to food could be a great way to control the proteolysis activity of food.

Artificial Sweetener Synthesis: The Food and Drug Administration has authorised the use of aspartame as a non-calorie artificial sweetener. Aspartic acid and the methyl ester of L-phenylalanine make up aspartame, a dipeptide. The sweet taste of aspartame is due to the L-configuration of the two amino acids. The preservation of stereospecificity is critical, yet it raises the cost of chemical manufacturing. Aspartame production by enzymes is hence favoured. Proteases or hydrolytic enzymes may catalyse the opposite process under particular kinetically regulated circumstances. The production of aspartame, an immobilised thermolysin preparation derived from *Bacillus thermoprotyolyticus* is employed. The two largest industrial manufacturers of aspartame are Toya Soda in Japan and DSM in the Netherlands [55-59].

In Feed Industry: Keratinolytic peptidase might be utilised to degrade fibrous animal protein (keratin) found in natural waste such as feathers, horns, hair, and nails. These goods might be used to augment animal diets. Eggs may be processed into very nutritious animal feed using proteolytic enzymes. It is reported that one of the most important ways to get safe food is to use pro-ecological technologies that take advantage of the enzymes in bacteria [43]. Proteases may be employed to solubilize keratin compounds in order to transform waste items like feathers into protein concentrates that can be utilised in animal feeds. Streptomyces species have an alkaline protease with significant keratinolytic activity.

Production of Soy Sauce: Soybeans are a valuable source of food since they provide high-quality protein. Proteases used to make soy sauce and other soy products since ancient times. In the preparation of soy sauce, fungal-derived alkaline and neutral proteases play a crucial role. Soy proteins may benefit from proteolytic treatment to increase their functional characteristics. Soluble hydrolysates with excellent solubility, good protein output, and mild bitterness are produced by treating soy proteins with alkalase at pH 8. The hydrolysate is utilized in the production of protein-fortified soft beverages and dietetic meals.

Manipulation of the hydrolytic conditions for Bacillus alkaline protease to hydrolyze soy protein may result in the creation of different functional and fanciful qualities. The accompanying formation of a distinctive bitter taste in the protease-mediated generation of protein hydrolysates is well-known and is attributable to the presence of hydrophobic amino acids as well as proline within the peptides formed by proteolysis.

Although the bitterness is frequently ascribed to the peptides' terminal hydrophobic amino acids, nonterminal hydrophobic amino acids in small-to medium-sized oligopeptides may also have a bitter taste, but bigger peptides do not. A protease produced by Pseudomonas R098 has been blamed for bitter tastes noticed in several cheese-making procedures. As a result, it's critical to keep the hydrolysis process under control in order to avoid harsh flavours.

Brewing and Cereal Processing Sector: Proteases are widely used in the brewing industry. *Bacillus subtilis* protease is used to solubilize protein from barley adjuncts in the preparation of brewing wort, releasing peptides and amino acids that may meet the nitrogen supply demand. Chill proofing, a treatment that prevents the production of precipitates during cold storage, uses proteolytic enzymes. The presence of proteinaceous compounds in beer causes hazes, which also precipitate polyphenols and oligosaccharides.

The insoluble complex is prevented from aggregating by hydrolysis of the protein components. Proteinases are used in the brewing industry to breakdown proteins and keep beer from turning hazy as it cools. Native proteases are found in the process of grain malting, which is usually done with barley. They help clear up the wort and make it more useful as a food for yeast and as a finished food product. During the malting process and during mashing, the malt proteases normally

create the required quantity of amino nitrogen from the major proteins of barley, hordein, and glutelin. As the quantity of malt in cereals is reduced or removed due to the usage of unmalted cereals, industrial proteases, particularly neutral proteases from Bacillus and *Aspergillus species*, must be used to replace malt protease. Bacillus alkaline protease is ineffective.

These enzymes may be employed to make cereal extract concentrates, where filterable extract yield is more important than amino nitrogen, and papain can be utilised to increase proteolysis in this scenario. The temperature–time holds used during mashing are intended to match the activity needs of mashing enzymes such as protease, amylases, and glucanases. The lowest temperature held in mashing with malt enzymes is approximately 50°C, known as the 'proteolytic' stand, because malt proteases have temperature optima around this setting and swiftly denature as the temperature rises.

Some microbial proteases, including papain, have greater temperature optima between 55 and 65°C, allowing the mashing process to be carried out at higher temperatures that are better for starch gelatinization and betaglucan hydrolysis. Proteases may also be used to remove chill haze in the brewing process. When beer is held at low temperatures after final filtering, a haze may form owing to the limited solubility of residual proteoglycans in the finished beer. Papain does a great job of breaking down these polymers, which gets rid of the haze [61-63].

Cheese Making and the Dairy Industry: In the dairy sector, the most common use of proteases is in the production of cheese. Animal rennets, microbial milk coagulants, vegetable rennet, and genetically modified chymosin are the four primary types of milk-coagulating enzymes. Acid aspartate proteases include both animal and microbial milk coagulating proteases. The microbial enzymes had two key flaws- bitterness in the cheese after storage owing to high quantities of nonspecific; and heat-stable proteases, and a poor yield. After a lot of research, enzymes have been made that are completely inactive at typical pasteurisation temperatures and have very small amounts of non-specific proteases. Proteases' fundamental purpose in cheese production is to hydrolyze particular peptide bonds to produce paracasein and macropeptides. Because it is very specific to casein, chymosin is often used to make cheese, which is why it works so well [51, 64-65].

Because of proteases' capacity to coagulate milk proteins into curds and release whey, acidic aspartic proteases are utilized as milk-clotting enzymes in cheese production. Animal, microbial, and genetically modified calf rennet are the three forms of commercial milk-coagulating enzymes (chymosin). Rennet is a protease with anunusually high substrate specificity, hydrolyzing a single-specific peptide bond in the k-casein fraction of milk to create para-k-casein and a macropeptide while avoiding other caseins. The use of lactic acid-producing starter cultures to lower the pH of the milk (or cream) to about pH 4.6, where the major casein proteins approach their isoelectric pHs, making them more receptive to coagulation, is customary in the early stages of the cheese-making process.

Rhizomucor miehei developed the most significant native microbial cheese-making enzyme that was commercialized as a consequence of studies. In the late 1980s, recombinant chymosin was introduced. Whey is a by-product of cheese production high in proteins, some of which may become intractable due to heat denaturation. Trypsin and microbial proteases can break down both insoluble and soluble whey proteins into peptides and amino acids [66-67].

Milk-clotting proteases as exogenous enzymes in cheese production is the most well-known use of proteases in the dairy business. Heat labile variants of these enzymes have been developed to facilitate thermal inactivation and make them suitable for use in the milk industry, where whey serum is used as a by-product; the main application of these microbial coagulants is to prepare vegetarian cheese, thus replacing animal-derived chemises. The alteration of proteases or the microbial strains that produce them has received an even larger number of patents. Feijoo-siota *et al*. (2014) [68] found that lactic acid bacteria, in particular, can be used to speed up the ageing of cheese to make it taste better or give it new textures [69-70]. During the malting process and mashing, the malt proteases normally

create the required quantity of amino nitrogen from the major barley, hordein and glutelin proteins. As the quantity of malt in cereals is reduced or removed due to the usage of unmalted cereals, industrial proteases, particularly neutral proteases from Bacillus and Aspergillus species, must be used to replace malt protease. Bacillus alkaline protease is ineffective. In this scenario, these enzymes may be employed to make cereal extract concentrates, where filterable extract yield is more important than amino nitrogen, and papain can increase proteolysis.

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Photography Industry: In the photographic business, a substantial amount of silver is used in light-sensitive emulsions. This film is processed; the operation entails separating the silver-containing gelatin from the film base to extract the valuable silver.

Although the aqueous solution includes both gelatin and silver, the presence of protein prevents the silver from being separated. Proteolytic enzymes and a temperature of 50°C and a pH of 8.0 quickly disintegrate gelatin, allowing the silver particles to separate. Alkaline proteases from *B. subtilis*[62]and *B. coagulans* PB-77 were shown to dissolve the gelatinous coating on old X-ray films, making it possible to get the silver back.

Bioremediation of the Environment: The alkaline protease aids in waste disposal from various food processing companies. Proteases help reduce the biological oxygen demand of aquatic systems by solubilizing proteinaceous waste. If the hard keratin structure of the waste feathers is entirely dissolved, they make up around 5% of the body weight of a chicken, providing a rich protein source for food and feed. The keratinolytic protease is used to degrade waste keratinous material from chicken waste and as a depilatory agent to remove hair from drains in the food and feed sector [70].

A commercially available formulation including proteolytic enzymes from *B. subtilis*, *B. amyloliquefaciens*, and *Streptomyces* sp., as well as a disulfide-reducing agent (thioglycolate), improves hair breakdown and aids in the removal of hair-containing deposits clogging pipes. Sericin, which makes up roughly a quarter of the total weight of raw silk, coats the raw silk strands' perimeter and gives them a rough texture. Degumming silk sericin removes it from the inner core of fibroin using starch while shrink-proofing and twist-setting silk yarns [71].

An alternate technique is to use enzyme preparations such as protease before silk dyeing. An alkaline protease produced by Bacillus sp. RGR14 was tested for its ability to degum silk. Psychrotrophes are suitable for enzyme synthesis because they are active at low temperatures and are stable in alkaline environments. In the presence of oxidants and detergents, laundry additives are in high demand. Poychrotophs proteases are used in environmental bioremediation, food, and molecular biology [72-78].

Medical Field-pharmaceutical Industry: Proteolytic enzymes are most often used in the medical and pharmaceutical industries to aid digestion. Human tissue inflammation and discomfort are reduced by injecting certain foreign proteases into the body. Oral injection of proteases from *Aspergillus oryzae* has previously been utilised to treat lytic enzyme deficiency disorders.

In the treatment of burns and wounds, clostridial collagenase or subtilisin is used in conjunction with broad-spectrum antibiotics. Asparaginase derived from *E. coli* removes asparagine from the

circulation in certain lymphocytic leukemia. *Conidiobolus coronatus* alkaline protease was discovered to replace trypsin in animal cell cultures. Curcain, a plant protease isolated from *Jatropha curcus* latex, was discovered to be a wound healing agent. Proteolytic enzymes have been shown to alleviate the discomfort of breast engorgement in breastfeeding mothers, as well as pain, edoema and inflammation induced by sugary foods and injuries.

Conventional chemotherapy treats tumour cells with single anticancer agents or combinations, which primarily interfere with macromolecular synthesis processes such as DNA synthesis and mitosis, ultimately leading to the death of proliferating cells. The tumour cells frequently resist chemotherapeutic agents, resulting in high levels of chemo resistance. Inhibition of proteases is a potential condition for anticancer medicines.

They may be used as a potent medical weapon by combining them with NP-based delivery methods, which may assist in overcoming chemo resistance. This revolutionary therapy technique may significantly enhance drug absorption, extend circulation time, and accomplish precise treatment accumulation in tumour tissues. It was reported that in the long run, lowering the dose will help keep healthy tissues healthy, reduce side effects, and lead to better patient outcomes and a higher quality of life [80-81].

The fibrinolytic enzyme was extremely active and stable at a moderate pH and temperature. Clinical studies might further explore it as a viable candidate for thrombolytic treatment. A clot-busting enzyme gives cancer, stroke, and heart sufferer's fresh hope. The major pharmacological compounds that may be exploited to generate novel and efficient fibrinolytic medicines are enzymes from natural marine isolates. The results of the current study could lead to new ways to treat cardiovascular diseases [32].

One of the most important discoveries in biomedical research throughout the second half of the twentieth century was the biochemical characterization of the proteolytic pathways that make up blood coagulation. Understanding these pathways is critical for improving world health by using them to treat hemostasis and thrombosis diseases. The proteases of the coagulation cascade and their regulatory proteins play a critical role in the thrombo-inflammation paradigm. These ideas show how important the coagulation cascade is in cardiovascular pathology, not just for thrombotic processes but also for atherosclerotic processes and the response to ischemia-reperfusion damage [82-84].

Because of the relevance of proteases, several attempts have been made to screen proteases and their inhibitors as potential therapeutic compounds. HIV-1 protease inhibitors, for example, have been used to extend the lives of people with the human immunodeficiency virus (HIV). Because illnesses and proteases have such a tight link, there is a significant incentive to create sensitive, selective, and robust protease assays and sensors that can be used to find novel proteases and inhibitors.

Fluorescence-based approaches are the most common for homogeneous tests because of their excellent sensitivity and quantitative findings. Electrochemical techniques, surface spectroscopic techniques, and enzyme-linked peptide protease tests are among the most widely utilised. Hao, Ong, and Yang (2017) [85] reported about the latest developments in liquid crystal (LC)-based protease assays and how they can be used to find proteases and their inhibitors.

The chemical and biological constitution of various significant druggable proteases expressed by bacteria, viruses, fungi, eukaryotes, and prions were addressed. HIV protease and HCV NS3-4A protease are two of the enzymes that have been targeted to a therapeutically effective level. The molecular and pharmacological properties of several protease inhibitors were explored.

A total of 25 inhibitors are powerful and safe enough to be tested in people, and they are all in various stages of clinical development. Agbowuro *et al.* (2018) [86] looked at how protease inhibitors work and how well they work in the clinic against infectious pathogens. They also looked at how they were made and the next steps for using protease inhibitors as anti-infective drugs.

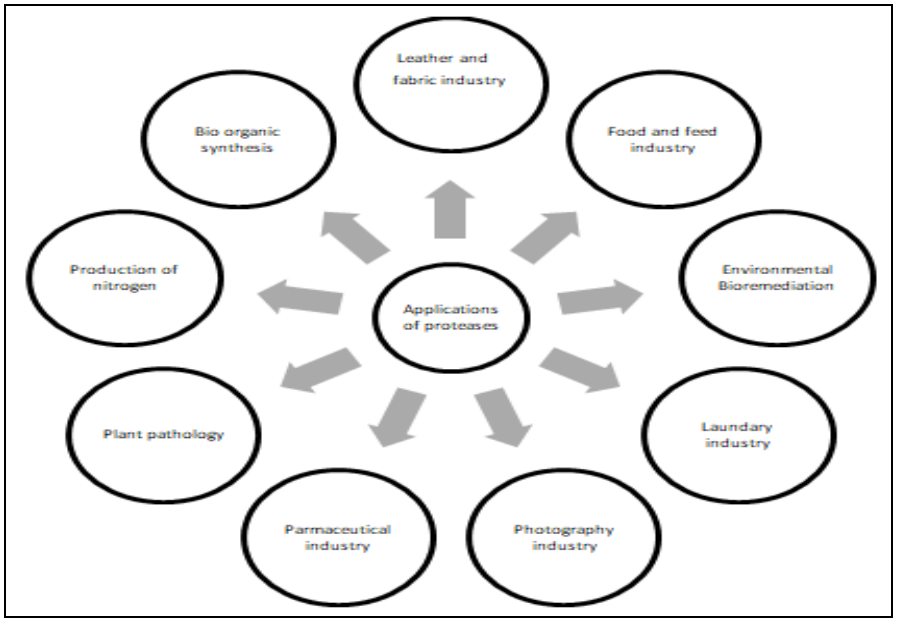

FIG. 2: APPLICATIONS OF PROTEASES ENZYME

Plant Pathology: A pathological way of life is inextricably tied to the continual need to confront diverse difficulties posed by the outside world (both within and outside the host). Pathogens have developed complex systems to battle the host's defensive mechanisms and to be able to tolerate harsh environmental conditions in order to colonise the host and establish infection. Proteases, as essential components of these systems, play a role in a number of infection-related activities. It was reported that there are important regulators of phytopathogenic bacteria because they change how many virulence proteins are made and how they work [87].

Production of Nitrogen: Amino acids, peptides, and proteases are organic nitrogen sources for microbial growth. While amino acids and small oligopeptides can be absorbed directly by microbial cells, long peptides and proteins must be degraded into amino acids and oligopeptides before they can be used as nitrogen sources for microbial growth. Some heterotrophic microbes may manufacture extracellular proteases (ex-proteases) to hydrolyze lengthy peptides/proteins into utilisable amino acids and oligopeptides, providing resources for themselves and perhaps other microorganisms that cannot produce ex-proteases. Because all organisms generate proteins and most of them are destroyed by microbial exproteases when released into the environment, there are a lot of proteases in nature. On Earth, microbial exproteases play an important role in spontaneous protein degradation and nitrogen recycling [88].

Proteases in Organic Synthesis: Hydrolytic enzymes are used to catalyse reactions using water as a reactant or product, in which another solvent partly or completely replaces the water concentration in the reaction media, moving the chemical equilibrium toward synthesis. Because water molecules may play a role in maintaining the conformational structure of proteins required for biocatalysis, a lot of research has gone into figuring out how water, and specifically water concentration, affects enzyme activity and stability, as well as the implications of implementing enzyme reactions in nonconventional organic media or biphasic aqueous–organic systems. Enzymes that are more stable in organic solvents have been discovered or created. Thermus strain Rt4A2 microbial protease is particularly stable in organic solvents. Covalent modification of the enzyme with chosen polymers or the use of covalent or non-covalent techniques to attach the enzyme to supports has made subtilisin more stable in organic solvents. Chen and Arnold [89] used directed evolution technologies and principles to generate subtilisin proteases that were active in the presence of organic solvents and therefore suited to employing proteases to catalyse peptide and related bond formation. PST-01, a *Pseudomonas aeruginosa* protease with considerable similarity to thermolysin, also has a disulfide link (one of two),

which contributes significantly to its solvent stability. The use of supercritical fluids as the reaction solvent is another way to replace water in the reaction media [90-91].

Bio-organic Synthesis: Toya Soda Company Japan made use of proteases' ability to produce di- and oligopeptides by linking peptide esters to N-protected amino acids, mediated by thermolysin from *B. thermoproteolyticus*, for aspartame production. The immobilised thermolysin speeds up the reaction between L-aspartyl-methyl ester and L-phenylalanine with a blocking agent for the amino group. This makes aspartame, which can then be removed chemically.

Different peptides were made with the help of *Flavobacterium meningo septum* prolyl-endopeptidase, a thermostable protease from *C. thermohydrosulfuricum*, and *Streptomyces griseuspronase*. Proteases like subtilisin and clostripain have been employed to synthesise glycoconjugates. The enzyme might be directly involved in carbohydrate acylation or making a peptide bond between a glycopeptide and a peptide.

Proteases' ability to mediate a variety of other reactions besides peptide bond cleavage, such as reactions involving esters and amides of carboxylic acids, makes them interesting and effective tools for enantioselective bond cleavage or formation to resolve pairs of enantiomers in racemic mixtures. Sulfurin, *A. oryzae* protease and serine alkaline protease from *Thermoactinomyces vulgaris* are examples of enzymes that have been used for this purpose.

P. putida ATCC 12633 leucine aminopeptidase has been cloned into *E. coli* K-12 and is being commercialised for the synthesis of optically pure L- and D-amino acids employing resolution techniques. In the preparation of Leu-and Met-enkephalin, subtilisin, thermolysin, chymotrypsin, and papain were used. Subtilisin was shown to be efficient in deacylating per acylated nucleosides in a regioselective manner. In the resolution of racemic amines, subtilisin also facilitated acylation reactions of dialkyl and diallyl carbonates. Subtilisin effectively resolved racemic amine mixtures in the creation of a precursor for the synthesis of rasagiline, which is used to treat depression and dementia, as well as the synthesis of oral calcimimetic medicines. In the dynamic kinetic resolution of secondary alcohols, subtilisin–ruthenium combinations proved successful [92]. Human insulin may be made from swine insulin by changing the amino acid No. 30 of the B insulin chain from alanine to threonine using a protease. Because lysine is amino acid No. 29 on the B insulin chain, a lysine-specific protease removes the terminal alanine. The des-B30-insulin product is then mixed with threoninyl-tert-butyl ester in an organic solvent with the help of a lysine-specific protease from Achromobacter sp. to make human insulin.

The tert-butyl ester-blocking agent is then taken away. The subetilisin family of alkaline serine proteases is extensively used in a variety of industries. Proteases derived from bacillus account for over 60% of all enzyme sales across the world. Proteases have particular applications in the purification of non-protein compounds from animal or plant extracts, such as carbohydrate gums and mucopolysaccharide extraction.

Proteases have a wide range of applications in various industries such as food, pharmaceuticals, and cosmetics, and have been widely commercialised by various companies around the world, despite the fact that the production of these enzymes has been significantly improved by the use of hyper-producing strains of fungi and bacteria and genetically modified microbes, and efforts are still being made to find newer sources of enzymes, better production techniques, and novel enzymes [93].

Proteases are also used to recover silver from traditional gelatin-containing photographic films, such as X-ray films, and in the liquefaction of industrial and domestic organic waste. Proteases are digestive enzymes that may be eaten by humans and animals.

The Effects of Recombinant Technology on the Production of Microbial Proteases: The genome of *B. subtilis* and its entire sequencing were accorded top attention among Gram-positive bacteria [94]. The discovery of genes related to Bacillus species' primary secretory pathway, which encode five types I and a type II signal peptidase, was thought to be crucial to these species' ability to

release significant quantities of extracellular enzymes. *Bacillus halodurans* 15's whole genome has also been sequenced, which is particularly significant considering this strain's ability to manufacture extracellular enzymes such as protease with high alkaline activity and stability.

Early recombinant research strategies involving the introduction of engineered plasmids in *B. subtilis* were problematic because recombination processes over many generations resulted in plasmid sequence deletions. This problem was partially resolved by the development of mutant strains lacking certain recombinant enzymes. The usage of native alpha-amylase native high enzyme-producing promoter in the production of high-level expression of *B. subtilis* genes has been noted in a few cases. Procedures involving direct insertion of recombinant DNA into the chromosome produced more stable clones, as did strategies involving gene copy number amplification [95].

The research focused on a variety of industrially significant Aspergillus species, such as *Aspergillus nidulans*, *Aspergillus niger* and *Aspergillus oryzae*. Aspergillus species have transcriptional, translational, and Post-translational constraints, and ways to enhance heterologous gene expression via gene fusion, protease-deficient mutants, and other approaches have been developed. Calf chymosin, generated by an Aspergillus species and used in cheese manufacturing, was the first recombinant heterologous product to be approved by the US FDA.

The precursor, preprochymosin, is generated initially, followed by the cleavage of the 16-amino-acid presequence during the secretion process. During further processing of the protein at a low pH, a 42-amino-acid prosequence is eliminated. A mix of techniques, including gene fusion, conventional mutation/selection, and the use of aspergillopepsin-fewer mutants, was used to improve chymosin production.

On the other hand, DSM uses *Kluyveromyces lactis* as the microbial host to manufacture Maxiren, a recombinant chymosin product. Rao *et al.* (1998) have detailed information on the cloning, sequencing, and expression of protease genes from a variety of microbial sources, including bacteria (*Bacillus, Lactococcus, Streptomyces, Serratia, Pseudomonas, Aeromonas, Vibrio* species, and *E. coli*), fungi (*Aspergillus, Fusarium, Rhizopus,* and *Mucor* species), and yeast [96-105].

CONCLUSION: Proteases are special enzymes yielded from microorganisms, plants, and mammals that break down peptide bonds. These peptidases can be categorized as endopeptidases or exopeptidases depending on where the cleavage site is located in the putative substrate. They are of enormous physiological and economic value. Proteases are employed in a number of fields, such as pharmaceutical, medicinal, and environmental bioremediation.

Microbes are the main source of enzymes because to their rapid growth, low cultivation needs, and ease of genetic manipulation. Microbial proteases have been used extensively in the food, dairy, and detergent industries since ancient times. As prospective targets for developing therapeutic medications to treat deadly diseases like cancer, malaria, and AIDS that are spreading at an alarming rate, proteases have drawn more attention. The capacity to clone the gene responsible for producing proteases in microorganisms has revolutionized genetic modification, providing new chances for introducing predesigned alterations that result in proteases with specific and desirable properties. Separating proteases from bacteria that can withstand extremely low temperatures is another goal.

Additionally, mucopolysaccharides and carbohydrate gums, non-protein substances generated from animal or plant extracts, can be extracted using proteases in more specialized ways. To create protein concentrates for animal diets, proteases can break down keratin-containing items, such as feather detritus. Alkaline proteases from Streptomyces species also exhibit strong keratinolytic activity. Meat may be softened using the plant proteases papain and bromelain and the neutral protease from *B. subtilis*. Proteases are used to recover silver from conventional gelatin-containing photographic film, including X-ray film, and to liquefy household and commercial organic waste. Proteases are digestive aids that both people and animals may consume.

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