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Systematic approach & Application in relevance to Economic & Therapeutic actions of Proteolytic Enzyme: A Review

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Abstract

Proteolytic enzyme is an enzyme that increases the degradation of proteins into smaller polypeptides or single amino acids. Enzymes are highly complex, their roles and behaviour are determined by a variety of factors. Proteolytic enzyme is present in a variety of foods as well as naturally occurring supplements such as pineapple, papaya, ginger, kiwi, and others. Animals, bacteria, viruses, and fungi also contain protease. Having both synthetic and degradative properties, proteases exist in the group of unique class of enzymes. Proteases are broadly categorised as Endopeptidase and Exopeptidase enzymes based on the place of action on their substrate. Because of their significant position in biological processes, they have a high medicinal and medical profile. They not only have certain physiological functions and responsibilities in living organisms, but they are also very important in many sectors and have a lot of economic benefits. A large number of biological sources of proteases described in this review clearly demonstrate the importance of plants and their applied and industrial uses. The aim of this paper is to provide an overview of proteases, their classification, and the various applications of proteases in several fields, clinical studies and the future scope of proteolytic enzymes.

Keywords: Enzymes, Protease, Papain, Bromeliad, Inflammation.

Introduction

The development of enzyme technology was divided into four stages: the Empirical phase, the Descriptive phase, the Descriptive and Quantitative phase, and the Planned Application phase. During the empirical process, goods such as beer, wine, leather, and bread were mass-produced on a wide scale without the knowledge of enzymes. During the descriptive time, the enzyme activity initiated the proof by perceiving the extracts of cereals, baker's yeast, and mammalian stomachs demonstrating the capacity to digest sucrose (invertase activity), starch (amylase activity), and beef (proteolysis activity). Besides the descriptive and quantitative phase, the discovery of new enzymes occurred and for the enzymologists main goal was the math modeling of the enzyme activity. With the passage of time, the protein nature of enzymes became more apparent, and new enzymes were created and categorised. In the expected implementation process, industrial

applications of enzymes increased due to improved knowledge of molecular structure, catalysis mechanism, quantification of action, immobilisation, and refinement of analytical techniques and equipment (1).

Enzymes, also known as biocatalysts, are stable proteins or nucleic acids. Enzymes can facilitate the result of biochemical reactions at the concentrations that are optimal for the normal functioning, development, and proliferation of any living organism, whether unicellular or multicellular, plants or animals (2-4). Enzymes epitomize the main community of proteins that plays critical roles in a variety of progressions such as gene expression, cell division, metabolism, and immune system responses (5).

Enzymes are highly complex, and their roles and behaviour are determined by a variety of factors, together with their sequence, three-dimensional structure, interaction with other molecules, and stability (6). Because of differences in substrate and reaction specificities, enzymes have a wide range of activities and applications. Enzyme-catalyzed reactions are extremely effective. They arise as a result of environmental factors such as temperature, pH, and strain. Enzymes may often be found in free or immobilised ways (as entire cells or isolated enzymes immobilised on a suitable support) (7, 8).

The first enzyme nomenclature scheme was formed in Enzyme Commission (EC) in Brussels at The International Congress of Biochemistry. Previously, the first edition was released in 1961. In 1992, the international Union of Biochemistry and Molecular Biology (IUBMB) published the sixth edition, which included 3196 separate enzymes, with an electronic supplement. Enzymes are categorised based on the catalysed reactions, and in the terms of a single enzyme protein, but it can also be accompanying to a group of proteins with the same catalytic property (9, 10).

This review addresses biological characteristics, distribution, and classification of the proteolytic enzymes. Also the review emphasizes the economic and therapeutic uses of the proteolytic enzymes, and also the clinical research findings of the proteolytic enzymes have been reported on the various activities.

Biological characteristics of Proteolytic enzyme (Proteases)

Proteolytic enzymes are a class of enzymes whose catalytic property is to hydrolyze peptide bond of proteins. Proteases and proteinases are other names for them. Proteases are a broad cluster of enzymes that belong to the family of hydrolases (11). Proteases are ubiquitous in nature and play a significant role in both commercial and physiological arenas. Protease enzymes are used in all living organisms such as microorganisms, plants and animals. These enzymes constitute approximately 2% of the gene codes in higher species (12). Proteolytic enzymes have traditionally been assumed as degradative enzymes which are efficient for the cleaving of protein foods. Small peptides and amino acids, which are vital to the organism, are liberated by the protease enzyme. The protease enzymes participate in the cross over of the cellular protein. Hence, Proteinases shows outstanding properties such as lysosome enzymes are Cathepsin B and Cathepsin D, mammalian digestive are pepsin, trypsin and chymotrypsin. They can perform selective protein modification through restricted cleavage, for example; Blood clotting, Lysis of fibrin clots, and processing and activation of zymogenic types of enzymes (13) along with transport and processing of secretory proteins across membranes (14).

Distribution of Proteolytic enzymes

Proteases are widely spread in the biological source. As a result, it is a significant subtype of the digestive enzyme (10). The plant kingdom takes first place in the protease verdict, followed by bacteria, fungi, animals, algae, and viruses. About 27 and 67% of isolated proteases are of biological origin, regardless of whether they are mammal, microbial, or plant proteases, with the remainder being poorly examined (14). Plants contain 34.92% of cysteine proteases. Microbes secrete 13.21% of serine and 8.81% of aspartic proteases. In 2010 Shamkant & Raghunath TM reported that bacteria are also a source of glutamic acid. Plant proteases can be originated in any part of the plant, including the latex, gum, seed, fruit, vine, leaf, stem, and root (13). Latex from the plant source is the most abundant form of protease. Approximately 43.91% of plant proteases remain unclassified. Asparaginyl

protease is a very uncommon discovery. Plants, in contrast, habitually had serine and cysteine endoproteases. Plants rarely contain Amino peptidases and Aspartic protease (14). Table 1 demonstrates the distribution of the Proteases based on the source.

Table 1. Distribution of the Proteases

Source	Percentage (%)
Plants	43.85
Bacteria	18.09
Fungi	15.08
Animal	11.15
Algae	7.42
Virus	4.41

Classification of Proteolytic enzymes

Proteases' biochemical role is critical for all living organisms, from bacteria to humans. Enzymes are categorised according to their source: microbial, plant, animal, and human enzymes (15).

Classification of the protease enzyme based on their site of action on their substrate:-

Exopeptidase:

Exopeptidase cleaves the peptide bond proximal to the amino or carboxyl terminal of substrate: they are further graded as aminopeptidase and carboxypeptidase according to their site of action at the N and C terminals (16). Aminopeptidase functions at the polypeptide chains free N terminus to releases as an amino acid (AA) peptide residue, an AA dipeptide residue, or an AA tripeptide residue. Aminopeptidase are renowned for eliminating the N-terminal, which can be present in heterologously expressing proteins but absent in generally found matured proteins. They are found in many different microbial groups, including fungi and bacteria. Caboxypeptidases function at the polypeptide chain's C termini to release an AA peptide residue or AA dipeptide residue. Serine carboxypeptidase, Metallo carboxypeptidase, and Cysteine carboxypeptidase are the three main classes according to the composition of the AA residues at the active site of the enzyme (17).

Endopeptidase:

Endopeptidase is distinguished by its preference for peptide bonds in the polypeptide chain's inner regions, away from the N and C terminal. The occurrence of a free amino or carboxyl group inhibits enzyme activity. Endopeptidase is a family of hydrolases that catalyze the hydrolysis of peptidic linkages and, as a result, the hydrolysis of proteins. These enzymes are also highly chemo and enantioselective, which are active at low pH (pH 6–8), and easy to handle biocatalysts that do not require the presence of cofactors (23).

Endopeptidase is classified into four subgroups based on their catalytic mechanism:-

Serine Proteases:

Serine proteases hydrolyze amino acids or peptide fragments in a two-step process that results in a covalently bonded enzyme-peptide intermediate and the loss of the amino acid or peptide fragment (18). This is followed by a deacylation phase, which involves a nucleophilic assault by water on the intermediate, culminating in peptide hydrolysis. Serine endopeptidase is divided into three categories based on their preferred primary substrate: trypsin-like cleavage occurs after positively charged residues; chymotrypsin-like cleavages occur after big hydrophobic residues; and elastase-like cleavage occurs after tiny hydrophobic residues. Recent research based on protease 3D structures and amino acid sequences comparisons near the primary substrate-binding site in viral and bacterial trypsin- like proteases suggests a putative general substrate binding scheme for proteases with glutamic acid specificity involving a histidine residue and a hydroxyl function (19).

Aspartic Proteases:

The catalytic activity of aspartic endopeptidase is dependent on aspartic acid residues. The enzymes of the pepsin family are bi-lobed molecules with the active-site cleft positioned between the lobes and each lobe providing one of the pair of aspartic acid residues required for catalytic activity, according to crystallographic analyses (20). The process, according to the structural and kinetic investigations, combines general acid-base catalysis with a lytic water molecule that directly participates in the reactions. The crystal structures of different aspartic protease- inhibitor complexes and thiol inhibitors that resemble a tetrahedral intermediate produced after the attack by the lytic water molecule (21).

Metalloproteases:

These enzymes are inactivated by dialysis or the addition of chelating chemicals because they rely on the presence of bound divalent cations. According to X-ray investigations of the thermolysin complex with a hydroxamic acid inhibitor, Glu143 aids the nucleophilic assault of a water molecule on the carbonyl carbon of the scissile peptide link, which is polarized by Zn 2+ ion (22). The Hi-Glu-Xaa-Xaa-His (HEXXH) motif, by X-ray crystallography to comprise part of the site for binding of the metal, generally zinc, is found in most metalloproteases (22).

Cysteine Proteases:

Cysteine proteases catalyze carboxylic acid derivative hydrolysis via double-displacement mechanism that includes general acid-base generation and the hydrolysis of an acyl-thiol intermediate. Cysteine proteases have a mode of action that is quite similar to serine proteases. This enzyme belongs to the cysteine peptidase family and is an excellent model for this group of enzymes (23).

Serine proteases are found in eukaryotes, viruses, and bacteria, implying that they are important to the species. Exopeptidase, endopeptidase, oligopeptidase, and omega peptidase are all serine proteases. Acid proteases are another name for aspartic protease. Their catalytic activity is dependent on two highly conserved aspartic acid residues (23).

Prokaryotes and eukaryotes both have cysteine proteases. Approximately 20 cysteine protease families have been recognized. Both cysteine proteases depend on a catalytic dyad of cysteine and histidine to work. Metallo proteases are the most complex forms of catalytic protease. They are distinguished by the fact that they need divalent metal ions to function. Enzymes from various sources, like hemorrhagic toxins from snake venoms, thermolysin from bacteria, collagenases from higher species are used (24).

Classification of proteolytic enzymes based on source

Physiologically for the entire living organism, proteases are very important. They are originated from different types of forms, including viruses, algae, fungi, bacteria, plants and animals.

Plant Proteases:

The most prominent plant proteases are papain, bromelain, keratinases, and ficin (25, 26). As conventional theme proteases from the plant source, papain has an extensive history. It is derived from the Carica papaya fruit latex, which is cultivated in central and west part of subtropical areas in Africa and also in India (24). Because of its variety in 148 proteinase and peptidase isozymes, crude preparation of papain enzyme has a broader application. Bromelain is extracted from both stem and juice of pineapples. Bromelain enzyme is classified as a cysteine protease (23).

Bacterial protease:

The proteases which are majorly commercially available are acidic or alkaline in nature and are formed by species of the genus Bacillus (24). Pseudomonas is a gram-negative bacterium that is mainly accountable for the development of proteolytic enzymes. Alkaline proteases are the most common proteases secreted by this bacterium. Pseudomonas aeruginosa has a variety of proteases that have been isolated and classified from various strains (24).

Animal Protease:

Pepsin, renin, chymotrypsin, and pancreatic trypsin are the most well-known proteases derived from animals. The most important intestinal digestive enzyme is Trypsin which is liable for the food protein hydrolysis (23). Peptide bonds with carboxyl groups which are abetted by Arginine and lysine residues are hydrolyzed by Serine Proteases. Chymotrypsin is found in the extract of animal pancreas. Pure chymotrypsin is an expensive enzyme that is only used for medical and analytical purposes. From the stomach of all the vertebrates, an acidic protease called Pepsin is found (24). In the existence of HCl, auto catalysts free the active enzyme from its zymogen, pepsinogen. Pepsin-like protease called Rennin is originated from the stomach of all nursing mammals as an inactive precursor called pro-rennin. By the action of pepsin, it is altered to active rennin.

Viral Proteases:

Viral protease has gained more consciousness due to their well-designed role in the production of viral proteins that cause lethal diseases for example; cancer and AIDS. Several viruses

include Aspartic, Cysteine, and Serine peptidases. Retroviral aspartyl proteases are homodimers that are articulated as part of the polyprotein precursor and are necessary for viral assembly and replication. The mature protease is activated after the precursor is autolyzed (17).

Economic and Therapeutic applications of Proteases in various fields

Proteases are a common agent present in all living things, including plants, animals, and microbes. They are one of the classes of industrial enzymes, and their global demand is rapidly expanding it account for 20% of the 60% of enzymes market globally. These are widely used enzymes in a variety of industries and biotechnology via Food manufacturing, pharmaceuticals, the leather industry, bakery; meat tendering, detergent, industry, brewing etc. are a few examples (16, 17 & 28).

Leather industry:

Proteases are used in the tanning, unhairing, and bating procedures in the leather industry. Because of its elastolytic and keratinolytic function, alkaline protease is increasingly being used in emerging leather industries. The bating system is important for the enzymatic activities of pancreatic proteases. Microbial alkaline proteases have grown in popularity in the leather industry (27, 29). Proteases, lipases and keratinases are the most widely used enzymes in the leather industry (30).

Nematode control:

Proteases enzymes play a vital part in bacteria-nematode-plant environment interactions and they function as a chief nematode balance factors in the soil. By understanding the mode of action of Bacillus spp against nematodes it theoretically upsurge the value of these organisms as an effective nematode control agents and helps to the development of new biological control strategies (31).

Brewing industry:

Proteases are often used in the brewing industry to eliminate the haziness. Because of the inclusion of proteins in the beer, it becomes hazy at freezing temperatures which effects on the beer's shelf life. As a result, removing these proteins from the beer makes it appear smoother. During the fermentation phase, protease enzymes are applied, and Bacillus subtilisprotease is used to solubilize protein from barley adjunct. When held at a cold temperature, the addition of proteases enzyme also aids in the prevention of precipitate development (32).

Chemical industry:

The latest studies have been reported that there is a great deal of attention in the enzymatic synthesis of peptides. Proteases derived from bacteria, fungi, plants, and animals have been successfully used to synthesise a variety of small peptides (dipeptides, and tripeptides). Proteases may be used to synthesise peptide bonds in either a thermodynamically or kinetically mediated manner (33). Due to their bioactive nature as well as a greater understanding of their biological roles and properties, the discovery of new methods suitable for the large-scale synthesis of biologically active peptides has been aggressively sought over the last decade. Cysteine protease (papain, bromelain), Serine protease (α-chymotrypsin, proteinase, and subtilisin) and esterase (lipase) are the some protease example which is involved in the chemo enzymatic peptide synthesis (34).

Detergent industry:

Enzymes are an environmentally safe alternative that is used to increase the washing effectiveness of traditional detergents. They are widely used in detergent formulations in developing countries. Enzymes are present in the majority of detergents currently on the market (39). Proteases are used in the detergent industry to remove the proteins stains such as grass, blood, egg, and human sweat which have a tendency to adhere strongly to textile fibers. Amylases are used to remove the residues of starch-based foods like potatoes, spaghetti, custards, gravies, and chocolate. Lipases are capable of removing fatty stains such as fats, butter, salad oil, sauces, and tough stains on collars and cuffs. Cotton and cotton blends contain cellulases, which are modified cellulose fibre structures. When it is added to detergent, it results in; colour brightening, softening, and soil removal in the textiles (40).

Food industry:

Since antiquity, proteases have been used in food production they are important in the

tenderization of meat, especially beef. Proteases like papain, bromelain, and ficin have been extensively investigated as tenderization of meat (35). Because of their capacity to hydrolyze connective tissue proteins as well as muscle fiber proteins, a thermophilic alkaline and alkaline elastase protease have proven to be active and auspicious enzymes for meat tenderizing (16). They are used in improving Bread quality in bakery, (36) Papain and bromelain are also used in the production of various sauces and dry-cured ham (37).

Dairy industry:

Another significant use of proteases is in the dairy industry. Proteases that exist naturally add greatly to the flavour characteristics of cheese. They are used to speed up the ripening of cheese, to change the functional properties of milk, and to reduce the allergenic properties of milk products. Proteases are also used in cheese production to hydrolyse the basic peptide bond, resulting in Para casein and macro peptides (36).

Proteases that are used as a drug in the market:

Wound healing- it is a vital process after skin damage, and it is linked to the presence of protease-activated receptors and inflammation. Protease-activated receptors are a type of receptor that is activated by protease cleavage or chemical agonists (41).

Digestive aid:

Aspergillus oryzae proteases have been rummage-scale as a digestive aid to treat some lytic syndromes of enzyme deficiency.

Anti-inflammatory properties:

Secretory leukocyte protease and Serine protease enzymes obtained from bacteria were used as a substitute for serrapeptase in dietary supplements for anti-inflammatory, cardiovascular effects against respiratory inflammation, and mouse colitis (38).

Cancer:

Proteases have the potential to be therapeutic in the treatment of cancer. Several research groups are investigating the use of proteases involved in apoptosis administered through gene therapy to selectively destroy cancer cells (38). The FDA approved enzyme as listed in table 2.

Table 2. Food & Drug Administration (FDA) approved enzymes list (41)

Type of protease	EC number	Source of Protein	Applications	Targeted pathway	Approved year by FDA
Serine	3.4.21	Extracted from urine or from primary kidney cell culture	Thrombolysis	Converts plasminogen into plasmin	1978
		Recombinant in Chinese hamster ovary (CHO) cells		Plasminogen activator	1987 (AMI) 1996 (Stroke) 2002 (Catheter clearing)
		Recombinant in E. coli		Plasminogen activator	1996
		Recombinant in CHO cells	Procoagulant	Plasminogen activator	2000
		Human plasma		FX activator	1990
		Recombinant in CHO cells		FX activator	1997
		Recombinant in BHK cells		FX and FIX activator	1999
		Bovine		Fibrinogen activator	2006
		Recombinant in CHO cells		Fibrinogen activator	2008
		Recombinant in human cell line	Sepsis	Plasminogen activator	2001
Zinc	3.4.17.14	Bacterial (*C. botulinum*)	Neuromuscular	Syntaxin and SNAP-25 deactivator	1989
		Bacterial (*C. botulinum*)		Synaptobrevin deactivator	2000
Serine	3.4.21	Porcine pancreatic extract	Digestion	Aids digestion of protein	2009

Clinical research Applications of Proteolytic enzymes

Protease supplementation has been proven to improve a variety of clinically linked disorders, including anti-inflammatory response and anti-angiogenic benefit as skeletal muscle function, antitumor effects, and so on.

Bromelain Proteases Reduce Human Platelet Aggregation in vitro, Adhesion to Bovine Endothelial Cells, and Thrombus Formation in Rat Vessels in vivo were carried out by (42). They reported that the thiol protease, bromelain, an extract from pineapple stern, was suggested to have antithrombotic and anticoagulant activities in vivo. The effects of bromelain on cell size distribution of isolated human platelets invitro by Coulter Counter measurements were studied. Bromelain, administered orally at 60mg/kg body weight, reduced thrombus development in a time-dependent manner. Intravenously administered bromelain at 30mg/kg was marginally more active in decreasing thrombus formation, suggesting that orally administered bromelain is more effective.

Miller and co-workers carried out a research for the Protease supplementation effects on the Skeletal Muscle function and DOMS following downhill running. The research was carried out by selecting 10 volunteers sprinted 30 minutes at 80 percent of their estimated peak heart rate. Two protease pills or a placebo were given 4 times a day for 4 days, starting one day before the exercise and ending 4 days. When compared to the placebo group, the study group revealed higher contractile function regeneration and lower symptoms of delayed-onset muscle soreness when downhill riding. According to

their findings, consuming protease supplements can aid lessen muscular discomfort after downhill jogging. Protease supplementation can also aid in muscle recovery and the regeneration of contractile function following vigorous exercise (43).

L P Hale et al reported that bromelain decreases the inflammation by the oral treatment in the murine model of inflammatory bowel disease called IL-10-deficient model. The clinical and histologic severity of inflammatory bowel disease (IBD) was assessed in-vivo by using IL-10 mice where bromelain is given orally. Consistent oral treatment of bromelain was found to reduce the severity of spontaneous colitis in C57BL/6 IL-10 mice. So they conclude that bromelain with proteolytic activity was critical for anti-inflammatory benefits (44).

A study about the possible pathways underlying bromelain's anti-inflammatory activity was conducted by (45). Bromelain has been found in vitro to eliminate a number of cell surface molecules critical for leukocyte trafficking, together with CD128a/CXCR1 and CD128b/CXCR2, which operate as receptors for the neutrophil chemo attractant IL-8 and its murine homologues. Using an in vitro chemotaxis experiment, they discovered that bromelain-treated human neutrophils moved 40% slower in response to rhIL-8 than sham-treated neutrophils, while in vivo bromelain therapy reduced neutrophil migration by 50–85%. These findings demonstrated that bromelain may efficiently inhibit neutrophil movement to locations of acute inflammation and support the elimination of the CD128 chemokine receptor as a possibility.

Al-Khateeb et al reported a research to see whether serrapeptase, a proteolytic enzyme, could minimise postoperative swelling, discomfort, and trismus later third molar surgery. 24 stable persons with symmetrically impacted mandibular third molars who had surgical removal participated in a prospective, intra-individual, randomised, double-blind, cross-over study. At either the first or second surgery, according to the randomization protocol serrapeptase 5mg or placebo pills and 1000mg of paracetamol tablets were given to both patients. Cheek breadth, pain, and interincisal gap were assessed preoperatively, as well as on the first, second, third, and seventh postoperative days. The study indicated that administering the proteolytic enzyme serrapeptase postoperatively greatly decreased the presence of post-surgical complications, but there was no postoperative trismus effect (46).

Inflammation in the ulcerative colitis (UC) was reduced by the Oral treatment of bromelain. Bromelain has been found in vitro to inhibit the expression of mRNAs encoding pro-inflammatory cytokines by human leukocytes. Endoscopic colon samples from patients with UC, Crohn's disease (CD), and non-inflammatory bowel disease (IBD) controls were treated in vitro with bromelain or medium and cultured to see if bromelain affected mucosal cytokine production in IBD. It was assessed how much pro-inflammatory cytokines and chemokines were released. In the media from actively inflamed regions of UC and CD, significant alterations in granulocyte colony-stimulating factor (G-CSF), interferon (IFN)-, interleukin (IL)-1, IL-6, and tumour necrosis factor (TNF) were seen when compared to non-inflamed IBD tissue and non-IBD controls. After in vitro bromelain therapy, IBD-inflamed tissue released less G-CSF, granulocyte–macrophage colony-stimulating factor (GM-CSF), IFN-, CCL4/macrophage inhibitory protein (MIP)-1, and TNF. According to the findings, bromelain might be a potential therapy for inflammatory bowel disease (47).

Papain’s Anti Angiogenic effects on the human umbilical vein endothelial cells (HUVEC) in vitro was demonstrated by (48). Using viable cell labelling and a lactate dehydrogenase release test, the viability of cells after long papain treatment was studied. With the aim to evaluate the angiogenic activation against phosphorylated proteins AKT, MEK1/2, ERK1/2, SAPK/JNK and p38-MAPK, ELISA, was used. Cell migration was determined using an MTT test and growth inhibition was determined using a scratch test. In (Vascular endothelial growth factor) VEGF-activated HUVEC, papain had a significant anti-angiogenic effect. The effect may be attributed to interaction with the phosphorylation of AKT, MEK1/2, and SAPK/JNK. Cysteine proteases which are derived by plants are inhibited HUVEC cell growth and tube formation in a related manner and suggested that the plant proteolytic enzymes have the ability to be used as precautionary and therapeutic agents for angiogenesis-related human diseases.

The cytotoxic effects of bromelain in human gastrointestinal cancer cell lines were investigated by (49). Gastric cancer cell lines (KATO-III and MKN45) and two chemo resistant subpopulations of the HT29 colon adenocarcinoma cell line (HT29-5M21 and HT29-5F12) were treated with bromelain at various dosages, as well as cisplatin as a positive regulator. A sulforhodamine B test was used to determine the effect of bromelain on the growth and proliferation of cancer cells after 72 hours of treatment. The expression of apoptosis-associated proteins in MKN45 cells treated with bromelain was examined using Western blotting. Bromelain was found to be cytotoxic in a panel of human stomach and colon cancer cells. In a sample of MKN45 cells, several pathways were engaged in bromelain-induced cell death. Bromelain decreased cancer cell viability by inhibiting the Akt pathway and suppressing the oncoproteins Bcl2 and MUC1 while promoting apoptosis via the caspase system and extra nuclear p53.

A comparative analysis of anticancer effects of bromelain and papain in human cholangiocarcinoma cell lines was reported by (50). Cell proliferation, wound healing, invasion, and apoptosis tests, as well as western blotting, were used to study the effects of bromelain and papain on human CC Cholangiocarcinoma (CC cell generation, migration, invasion, and epithelial plasticity). Surprisingly, bromelain suppressed CC more efficiently than papain in general. The siRNA-mediated suppression of NFB on CC cells indicated that bromelain and papain are cytotoxic to human CC cell lines and that bromelain and, to a lesser extent, papain prevent tumour development via NFB/AMPK signalling. Bromelain, in particular, has the potential to emerge as an interesting, novel therapeutic solution that might lead to new discoveries in the treatment of human CC.

A research was carried out on bromelain inhibits the ability of colorectal cancer cells to proliferate via activation of ROS production and autophagy (51). They assessed the role of bromelain in inducing reactive oxygen species, superoxide, autophagosomes, lysosomes and induction of apoptosis and reported that bromelain inhibits the growth of colorectal cancer cells both in vitro and in vivo by triggering caspase-dependent and caspase-independent apoptosis and causing programmed autophagic cell death.

The antibiofilm activity of papain enzyme against pathogenic Klebsiella pneumonia was a study published by (52). The effect of papain enzyme on Klebsiella pneumonia planktonic cells, as well as the development, eradication and cell survival of biofilm, was investigated. It has been claimed that the papain enzyme has an antibiofilm action against drug-resistant K. pneumonia, but no antibacterial action was found, indicating that it may be used as an antibiofilm agent in conjunction with conventional antibacterial treatments.

Future prospective

Proteases are a diverse family of industrially significant enzymes that participate in a wide range of physiological and cellular activities. Over the last two decades, the usage of proteases in manufacturing and medicines has grown at an exponential rate. As novel protein engineering ideas and techniques are developed, the commercial protease markets will expand. Proteases are found in all animals, plants, and microorganisms because they are physiologically necessary. Because of this enzyme's economic potential, researchers and engineers are looking for strong and creative bacterial enzymes. Protein engineering will be crucial in the future creation of new proteases. Researchers are interested in proteolytic processes such as proteases for a number of reasons. Proteases have reignited interest as potential targets for developing therapeutic drugs to combat quickly scattering lethal illnesses such as AIDS, malaria & cancer. Using proteases' proteolytic activity in sick tissues can also give a new method for site-specific medication targeting and tumour imaging. To generate better protease-producing strains, researchers are employing sophisticated approaches protein/genetic engineering, molecular biology, and computational biology. Modern biotechnology has advanced fast from a laboratory snooping to a profitable business in a few of years (16, 27).

CONCLUSION

Enzymes are powerful biocatalysts that are being carefully and continuously explored due to their numerous and diverse applications in

biotechnology as well as human and animal health. Enzymes have also been utilised in medicine, such as enzyme therapy for the treatment of metabolic abnormalities and hereditary diseases such Gaucher's disease, Parkinson's disease, Phenylketonuria (PKU), and others. Cancer therapy and the treatment of infectious disorders where antibiotics are no longer effective are two of the most recent applications of enzymes (due to the emergence of antibiotic resistance in bacteria). For example; proteases, lipases, and amylases are also known as digestive aids which can be used alone or in drinks to treat digestive issues. This study focuses on the general aspects of proteases, with a particular emphasis on industrial applications, and future prospects. Proteases are a distinct class of enzymes due to their enormous physiological and economic significance. They are both degradative and synthetic in nature. Proteases are important in the detergent, medicinal, leather, fruit, and agricultural industries. The advancement of science has aided in the increased use of proteolytic enzymes for a variety of uses, and the application areas are expanding with the assistance of protein engineering techniques and chemical modification of the enzymes. Proteases' future commercial uses are increasingly expanding as new technical developments produce proteases with novel properties and substrate specificities. Biotechnology advancements provide a favourable environment for the synthesis of proteases and will help to promote their uses in order to deliver a maintainable source of income.

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ETHICAL ISSUES

Not applicable.

CONFLICT OF INTEREST

Authors declare no conflict of interest in this study.

LIST OF ABBREVIATIONS

A A: Amino Acid, DOMS: Delayed-onset Muscle Soreness, IBD: Inflammatory Bowel Disease, UC: Ulcerative colitis, TNF: Tumour Necrosis Factor, HUVEC: Human Umbilical Vein Endothelial Cells.

References

[1] Vitolo M, 2020. Brief review on enzyme activity. World J Pharm Res. 9(2), p. 60-76.
[2] Nelson DL, Lehninger AL, Cox MM. Lehninger, et al, 2008. Principles of Biochemistry. 5th Ed. New York, W.H. Freeman.
[3] Mitchell JB, 2017. Enzyme function and its evolution. Curr Opin Struct Biol. 47, p.151-156.
[4] Brasil, BdSAF, Siqueira FG, et al, 2017. Microalgae and cyanobacteria as enzyme biofactories. Algal Res. 25, p. 76-89.
[5] Almonacid DE, Babbit PC, 2011. Toward mechanistic classification of enzyme functions. Curr Opin Chem Biol. 15(3), p. 435-442.
[6] Schomburg I, Jeske L, Ulbrich M, et al, 2017. The Brenda enzyme information system-From a database to an expert system. J. Biotechnol. 261, p.194-206.
[7] Shilin Cao, Pei Xu, Yongzheng Ma, et al, 2016. Recent advances in immobilized enzymes on Nano carriers. Chin J Catal. 37, p.1814–1823.
[8] Meller K, Szumski M, Buszewski B, et al, 2017. Microfluidic reactors with immobilized enzymes—Characterization, dividing, perspectives. Sensors Actuat B Chem. 244, p.84–106.
[9] McDonald AG, Boyce S, Tipton KF, et al, 2009. ExplorEnz: The primary source of the IUBMB enzyme list. Nucleic Acids Res. 37, p.D593-D597.
[10] McDonald AG, Tipton KF, 2014. Fifty-five years of enzyme classification: advances and difficulties. FEBSJ. 281, p.583-592.
[11] Ryan CA, 1973. Proteolytic enzymes and their inhibitors in plants. Annu. Rev. Plant Physiology. 24, p.173-196.
[12] Barrett AJ, Rawlinga ND, Brien EA, et al, 2001. The MEROPS database as protease information system. J Struct Bio., 14, p.95-102.

[13] Chitre AS, 1994. Ph.D. Thesis, Nagpur University, Nagpur.
[14] Shamkant BB, Raghunath TM, 2010. Biological aspects of proteolytic enzymes: A Review. J Pharm Res. 3(9), p.2048-2068.
[15] Motyan JA, Toth F, Tozser J, et al, 2013. Research Application of proteolytic enzymes in molecular biology. Biomolecules. 3(8), p.923-942.
[16] Jabalia N, Mishra PC, Chaudhary N, et al, 2014. Applications, Challenges and Future Prospects of Proteases: An Overview. JANRM. 1(3), p. 5.
[17] Rani K, Rana R, Datt S, et al, 2012. Review on latest overview of proteases. Int J Cur Lif Sci. 2(1), p.12-18.
[18] Fastrez J, Fersht AR, 1973. Demonstration of the acyl-enzyme mechanism for the hydrolysis of peptides and anilides by chymotrypsin. Biochemistry. 12, p.2025–2034
[19] Barett AJ, 1994. Proteolytic enzymes: serine and cysteine peptidases. Methods Enzymol. 244, p.115.
[20] Blundell TL, Cooper JB, Sali A, et al, 1991. Comparisons of the sequences, 3-D structures and mechanisms of pepsin-like and retroviral aspartic proteinases. Adv. Exp. Med. Biol. 306, p.443–453
[21] Sielecki AR, Fujinaga M, Read RJ, et al, 1991. Refined structure of porcine pepsinogen at 1.8A resolution. J. Mol. Biol. 219, p.671–692.
[22] Holmes MA, Matthews BW, 1981. Binding of hydroxamic acid inhibitors to crystalline thermo lysine suggests a penta coordinate zinc intermediate in catalysis. Biochemistry. 20, p.6912–6920.
[23] Rao MB, Tanksale AM, Ghatge MS, et al, 1998. Molecular and Biotechnological Aspects of Microbial Proteases. Microbial Mol Biol Rev. 62(3), p.597–635.
[24] Gupta R, Beg QK, Lorenz P, et al, 2002. “Bacterial alkaline proteases: molecular approaches and industrial applications”. Appl Microbiol Biotechnol. (59), p.15–3.
[25] Shankar S, More SV, Laxman RS, et al, 2010. Recovery of silver from waste x-ray film by alkaline protease from Conidiobolus Coronatus. Indian J Biotechnol. 6(1), p.60-69.
[26] Kalpana DM, Rasheedha BA, Gnanaprabhal GR, et al, 2008. “Purification, characterization of alkaline protease enzyme from native isolate Aspergillus niger and its compatibility with commercial detergents”. Indian J Sci and Technol. 1(7), p.1-6.
[27] Razzaq A, Shamsi S, Ali A, et al, 2019. Microbial Proteases Applications. Front Bioeng Biotechnol. 12(7), p.110.
[28] Motyan J, Toth F, Tozser J, et al, 2013. Research Applications of Proteolytic Enzymes in Molecular Biology. Biomolecules. 3(4), p.923–42.
[29] Foroughi F, Keshavarz T, Evans CS, et al, 2006. Specificities of proteases for use in leather manufacture. J Chem Technol Biotechnol. 81(3), p.257–61.
[30] Souza FR De, Gutterres M, 2012. Application of enzymes in leather processing: a comparison between chemical and coenzymatic processes. Brazilian Journal of Chemical Engineering. 29(3), p.473-482.
[31] Lian LH, Tian BY, Xiong R, et al, 2007. Proteases from Bacillus: a new insight into the mechanism of action for rhizobacterial suppression of nematode populations. Lett Appl Microbiol. 45(3), p.262–9.
[32] Thakur N, Goyal M, Sharma S, et al, 2018. Proteases: Industrial Applications and Approaches used in Strain Improvement. Biological Forum – An International Journal. 10(1), p.158-167.
[33] Kumar D, Bhalla TC, 2005. Microbial proteases in peptide synthesis: Approaches and applications. Appl Microbiol Biotechnol, 68(6), p.726–36.
[34] Mehta A, 2010. Microbial proteases and their applications, 3rd Edn, IK International Publishing House Pvt Ltd, New Delhi. 199-226.
[35] Bekhit AA, Hopkins DL, Geesink G, et al, 2014. Exogenous proteases for meat tenderization. Crit Rev Food Sci Nutr. 54(8), p.1012–1031.
[36] Raveendran S, Parameswaran B, Ummalyma SB, et al, 2018. Applications of Microbial Enzymes in Food Industry. Food Technol Biotechnol. 56(1), p.16-30.
[37] Christopher N, Kumbalwar M, 2015. Enzymes used in Food Industry A Systematic Review. Int J Inno Res Sci Eng Technol. 4(10), p.7.
[38] Aladdin A, Alsaheb RAA, Pareek A, et al, 2017. Biotechnological Aspects and Pharmaceutical Applications of Bacterial

Proteases. Der Pharmacia Lettre. 9(2), p.9-20.
[39] Singh S, Bajaj BK, 2017. Potential application spectrum of microbial proteases for clean and green industrial production. Energy Ecol Environ. 2(6), p.370–86.
[40] Hasan F, Shah AA, Javed S, et al, 2010. Enzymes used in detergents: Lipases. African Journal of Biotechnology. 9(31), p.4836-4844.
[41] Craik CS, Page MJ, Madison EL, et al, 2011. Proteases as therapeutics. Biochem J. 435(1), p.1–16.
[42] Metzig C, Grabowska E, Eckert K, et al, 1999. Maurer, Bromelain proteases reduce human platelet aggregation in vitro, adhesion tobovine endothelial cells and thrombus formation in rat vessels in vivo. In Vivo. 13, p.7–12
[43] Miller PC, Bailey SP, Barnes ME, et al, 2004. The effects of protease supplementation on skeletal muscle function and DOMS following downhill running. J Sports Sci. 22(4), p.365–372.
[44] Hale LP, Greer PK, Trinh CT, et al, 2005. Treatment with oral bromelain decreases colonic inflammation in the IL-10-deficient murine model of inflammatory bowel disease. Clin Immunol. 116(2), p.135– 142.
[45] Fitzhugh DJ, Shan S, Dewhirst MW, et al, 2008. Bromelain treatment decreases neutrophil migration to sites of inflammation. Clin Immunol. 128(1), p.66–74.
[46] Al-Khateeb TH, Nusair Y, 2008. Effect of the proteolytic enzyme serrapeptase on swelling, pain and trismus after surgical extraction of mandibular third molars. Int J Oral Maxillofac Surg. 37(3), p.264–268.
[47] Onken JE, Greer PK, Calingaert B, et al, 2008. Bromelain treatment decreases secretion of pro-inflammatory cytokines and chemokines by colon biopsies in vitro. Clin Immunol. 126(3), p. 345–352.
[48] Mohr T, Desser L, 2013. Plant proteolytic enzyme papain abrogates angiogenic activation of human umbilical vein endothelial cells (HUVEC) in vitro. BMC Complement Altern Med. 13, p.231.
[49] Amini A, Ehteda A, Masoumi Moghaddam S, et al, 2013. Cytotoxic effects of bromelain in human gastrointestinal carcinoma cell lines (MKN45, KATO-III, HT29-5F12, and HT29-5M21). Onco Targets and Ther. 6, p.403–409.
[50] Müller A, Barat S, Chen X, et al, 2016. Comparative study of antitumor effects of bromelain and papain in human cholangiocarcinoma cell lines. Int J Oncol. 48, p.2025-2034.
[51] Chang TC, Wei PL, Makondi PT, et al, 2019. Bromelain inhibits the ability of colorectal cancer cells to proliferate via activation of ROS production and autophagy. PLoS One. 14(1), e0210274.
[52] Mohamed SH, Mohamed MSM, Khalil MS, et al, 2018. Antibiofilm activity of papain enzyme against pathogenic Klebsiella pneumonia. J App Pharm Sci. 8(6), p.163-168.