"Harnessing Nature's Toolbox: Protease Enzymes in Biotechnology and Industrial Applications"

Jeel Anand , Ronak S.Chaaya *

[a] Research Scholar, * Scientist

[a] jinalanand15@gmail.com , ronakcruxr2000@yahoo.com.in

Swaminarayan University, Ahmedabad – Mehsana Highway, At & Po-Saij , tal-kalol , Gandhinagar 382725 , Gujrat , India

Abstract

Natural resources play a vital role in maintaining human health and environmental balance. Beneficial microorganisms such as *Bacillus* and *Pseudomonas* sp. contribute to improving gut health, soil fertility, and ecological stability by aiding in organic matter decomposition, nutrient release, and pollutant detoxification. Throughout history, herbs, roots, and fungi have been utilized in medicine, leading to the development of pharmaceuticals like aspirin and morphine. Natural foods offer essential nutrients and antioxidants, while natural disinfectants and beauty products provide safe alternatives. The dynamic ecosystem of soil supports sustainable agriculture and bioremediation through various types such as sandy and clay, which help in promoting plant growth and disease resistance, ultimately contributing to overall well-being and environmental harmony. The preservation of natural resources is vital for maintaining the well-being of both humans and the environment. Beneficial microorganisms, such as *Bacillus* and Pseudomonas, play significant roles in improving gut health, soil fertility, and ecological stability. They achieve this by breaking down organic matter, releasing essential nutrients, and detoxifying pollutants. Throughout history, herbs, roots, and fungi have been integral to traditional medicine, leading to the development of pharmaceuticals like aspirin and morphine. Natural foods provide essential nutrients and antioxidants, while natural disinfectants and beauty products offer safe and effective alternatives. Studies have shown that exposure to nature reduces stress and improves mental well-being. Additionally, natural fibers provide sustainable options for textiles, highlighting the importance of natural sources in promoting overall wellness and environmental harmony.

Keywords: Enzyme , protease , application , soil **.**

1. Introduction

Natural sources play a crucial role in maintaining both human health and environmental stability. Beneficial bacteria found in soil, water, and plants, such as *Bacillus* and *Pseudomonas* sp , support various functions like gut health, immune system, and ecological balance. *Bacillus* bacteria aid in soil fertility by decomposing organic matter and releasing essential nutrients like nitrogen, phosphorus, and potassium, while also producing antibiotics that protect plants. On the other hand, Pseudomonas bacteria help detoxify pollutants and stimulate plant growth, contributing to overall soil health and disease prevention. **[Adriano, D.C. 1986].** Moreover, natural elements like herbs, roots, and fungi have been utilized for centuries in traditional medicine and modern pharmaceuticals, resulting in the development of drugs like aspirin and morphine. Consuming natural foods like fruits, vegetables, grains, nuts, and seeds provides essential nutrients, vitamins, and antioxidants necessary for maintaining good health and preventing diseases. **[Akerblom G, 1984].** The natural world also plays a vital role in preserving ecological balance by conserving biodiversity, regulating climate, and ensuring the availability of clean air, water, and soil. Natural disinfectants such as vinegar and essential oils offer safe and effective cleaning solutions. Additionally, immersing oneself in nature has been shown to reduce stress and improve mental health. Beauty products containing natural ingredients like coconut oil and aloe vera are preferred for their effectiveness and safety. Furthermore, natural fibers like cotton and wool offer sustainable and biodegradable options for textiles and clothing, emphasizing the importance of natural sources in promoting overall well-being and ecological harmony. [**Anderson, R.1980].**

1.1 Soil

Soil is not just a substrate for plants but a dynamic ecosystem with far-reaching implications for human health and well-being.

The number of bacteria in 1g of soil ranges from 1 million or less several billions. That their population needs to be expressed within such extremes is in part due to the great difference in population that do exist among and within soils. Somewhat more disconcerting is the fact that with existing methodology, that bacterial population cannot be determined with precision in any given soil sample.

Soil bacterial population as determined by cultural methods range from a few thousand cell per gram and podsols usually contain comparatively high bacteria population. Commonly for arable soils culture methods of examination reveal from 5-50 millions bacteria/g soil.

Soil is indispensable for human well-being as it nourishes through the flourishing of plants, harbors advantageous microorganisms that enhance well-being and alleviate stress, possesses innate disinfectants that combat the proliferation of detrimental pathogens, sustains biodiversity that enhances the purity of air, water, and food, and assists in bioremediation to restore contaminated surroundings. Therefore, tending to soil is paramount in upholding a pristine environment and fostering human vitality. **[A.Burges 1967]**

The opulent sandy soil, adorned with its grandiose large particles, possesses the remarkable ability to swiftly drain water. However, it tends to be deficient in nutrients, presenting a challenge in retaining precious moisture. The exquisite clay soil, with its delicate small particles, gracefully embraces water, holding it with utmost elegance. This soil, adorned with its richness in nutrients, exudes a regal aura. Yet, it can be rather sluggish in draining water and is susceptible to compaction. The refined silt soil, boasting its medium-sized particles, gracefully balances the art of holding moisture better than sandy soil, while still allowing water to flow with grace, albeit more swiftly than clay soil. **[Allen, B.L., Fanning, D.S., 1983]**. This soil, often blessed with fertility, is a splendid choice for agricultural pursuits. The majestic loam soil, a harmonious blend of sand, silt, and clay particles, epitomizes perfection. With its impeccable drainage system, it effortlessly retains moisture, showcasing its opulence. This soil, abundant in nutrients, is a haven for gardening and farming, radiating an aura of prosperity. Peat soil, a luxurious blend of organic matter, flourishes in waterlogged conditions where the slow process of decomposition takes place. Its acidity adds a touch of exclusivity, while its nutrient-poor nature demands careful attention. However, with meticulous management, this opulent soil can be transformed into a haven for horticulture. Chalky soil, reminiscent of a grand palace, boasts a lavish abundance of calcium carbonate, rendering it alkaline in pH. Its free-draining nature adds an air of sophistication, yet it may lack certain nutrients, necessitating the supplementation fit for regal plants.Black soil, known by its regal name of vertisols, showcases its opulence through its high clay content and remarkable ability to swell and shrink with moisture fluctuations. This fertile soil exudes an air of grandeur, but its propensity to crack in dry conditions poses a challenge to those who dare to cultivate it. **[American Geological Institute (AGI), 1996].**

The soil is a treasure trove of exquisite microorganisms, encompassing a splendid array of bacteria, fungi, and viruses, each possessing their own unique qualities. Amongst these remarkable beings, there are those that possess the extraordinary ability to create compounds with the power to combat harmful microbes. One such example is *Bacillus subtilis*, a bacterium that gracefully resides within the soil. This magnificent creature not only synthesizes enzymes but also produces antimicrobial peptides, which can be harnessed for the purpose of disinfection. **[American Geological Institute (AGI), 2002.**]. By harnessing the potential of these soil microorganisms, we can elevate the effectiveness and sustainability of disinfectant formulations, presenting a splendid alternative to synthetic chemicals that is both gentle on the environment and efficacious in its performance. The soil serves as a luxurious shield against pollution, showcasing its prowess in environmental protection. Toxins stemming from industrial activities, like heavy metals and organic compounds, may seep into the soil, where a harmonious blend of physical, chemical, and biological mechanisms collaborate to lessen their harm. Innovative methods such as phytoremediation harness the soil's knack for fostering plant development and microbial vitality, employing greenery and soil microbes to purify tainted areas, rejuvenating them back to their natural splendor. Within the realm of wastewater treatment, the soil assumes a paramount position in the refinement of effluent through the utilization of constructed wetlands and soil infiltration systems. The soil's microscopic organisms engage in the metabolic breakdown of organic substances and the eradication of harmful pathogens, thereby making a significant contribution to the treatment and reutilization of wastewater for both agricultural and industrial endeavors. These ingenious natural treatment systems ingeniously harness the soil's innate ability for biological decomposition, thereby presenting economically viable and ecologically sound resolutions for the management of wastewater**. [Hart, B., 1995].**

The soil, indeed, conceals a vast assortment of advantageous bacteria that assume pivotal roles in the well-being of the soil, the growth of plants, and the functioning of the ecosystem. Among these remarkable bacteria, one stands out: *Bacillus subtilis*

, a prevalent soil bacterium, is renowned for its multifaceted advantageous attributes. As a Gram-positive bacterium, it possesses the ability to form endospores, enabling it to endure even the harshest environmental conditions, such as drought and scorching heat. Extensive research and application in agriculture and biotechnology have been dedicated to *Bacillus subtilis*, owing to its remarkable capacity to stimulate plant growth and provide a shield against harmful pathogens. One of the ways in which *Bacillus subtilis* contributes to the enrichment of soil and the prosperity of plants is through the production of antimicrobial compounds. **[T. D. (1968)]**. It synthesizes antimicrobial peptides and enzymes that effectively impede the growth of plant pathogens, including fungi and bacteria, thereby safeguarding plants against diseases. Moreover, *Bacillus subtilis* possesses the ability to outcompete detrimental microbes for vital nutrients and space, thereby further augmenting the overall health of plants. Furthermore, *Bacillus subtilis* has been scientifically proven to enhance plant growth through a variety of mechanisms. This includes the production of plant growth-promoting hormones like auxins, cytokinins, and gibberellins, which play a crucial role in stimulating root development and improving nutrient absorption in plants. Moreover, Bacillus subtilis is capable of solubilizing phosphorus and fixing atmospheric nitrogen, thereby increasing the availability of these vital nutrients to plants. **[Augusto, L. (2016)].**

In the realm of agriculture, *Bacillus subtilis* is commonly utilized as a biocontrol agent and a key component in microbial-based fertilizers and soil enhancements. By leveraging the advantageous characteristics of *Bacillus subtilis*, farmers can diminish their dependence on chemical pesticides and fertilizers, thus fostering sustainable agricultural practices and environmental conservation. **[Biswas, T. D. (1968)].**

1.2 Bacillus subtilis

Bacillus subtilis, a remarkable Gram-positive bacterium, gracefully thrives in the bountiful realms of soil, air, and water. This exquisite microorganism, known for its benign nature towards both humans and the environment, has captivated the attention of scholars and scientists alike. Its prodigious talent for generating an array of enzymes, particularly proteases, has been meticulously explored and harnessed for industrial purposes. The innate presence of *Bacillus subtilis* in diverse habitats, coupled with its impeccable safety record, renders it an exquisite selection for a myriad of biotechnological and industrial endeavors. **[Trends Microbiol.2008].** Its unrivaled ability to produce proteases, meticulously documented and celebrated, further solidifies its position as a paragon of excellence in the realm of microorganisms. *Bacillus subtilis*, a ubiquitous microorganism, is commonly encountered in a plethora of natural settings, most notably within the fertile soil, where it assumes a pivotal role in the intricate process of organic matter decomposition. **[Harwood CR 1992**]. However, its presence is not confined solely to the soil, as it gracefully permeates other habitats such as the aqueous realm, the ethereal air, and even establishes harmonious associations with both flora and fauna. Within the soil, *Bacillus subtilis* thrives magnificently, owing to its remarkable capacity to generate tenacious spores that exhibit unparalleled resilience against the harshest of conditions. These extraordinary spores bestow upon the bacterium the ability to endure and persist within a myriad of environments until the opportune moment arises for growth and reproduction. **[Ogasawara N, Moszer I 1997].** Moreover, *Bacillus subtilis* has been meticulously extracted from an extensive array of sources, ranging from sedimentary deposits and decaying botanical matter to the fertile grounds of compost, and even the intricate gastrointestinal tracts of diverse animal species. *Bacillus subtilis* is a remarkably diverse bacterial species that is capable of growth within many environments. **[Barbe V 2009].** Recent microarray-based comparative genomic analyses have revealed that members of this species also exhibit considerable genomic diversity. The identification of strain-specific genes might explain how B. subtilis has become so broadly adapted. The goal of identifying ecologically adaptive genes could soon be realized with the imminent release of several new B. subtilis genome sequences. As we embark upon this exciting new era of B. subtilis comparative genomics we review what is currently known about the ecology and evolution of this species. **[Kobayashi K 2003].**

Bacillus subtilis, a distinguished microorganism, is widely recognized for its remarkable ability to produce protease enzymes, most notably subtilisin, a serine protease. These enzymes play a pivotal role in numerous industries, including detergents, food processing, and leather treatment. The cultivation of *Bacillus subtilis* involves sophisticated submerged fermentation processes, where the optimization of environmental factors is paramount to achieving optimal production levels. Moreover, the application of genetic engineering techniques further enhances the yields and stability of these invaluable proteases. **[Nicolas P 2012].**

What sets *Bacillus subtilis* apart is not only its exceptional enzymatic capabilities but also its non-pathogenic nature, earning it the esteemed GRAS status. Additionally, this extraordinary microbe exhibits robust growth under diverse conditions, making it an ideal candidate for a wide range of industrial and biotechnological applications. The proteases produced by *Bacillus subtilis* are highly regarded for their remarkable specificity, efficiency, and adaptability, underscoring their profound significance in both commercial and research settings. **[Büscher JM 2012].**

overview of the bacteria, detailing the types of proteases they produce, specific characteristics, and their applications across different industries.

Bacteria	Protease type	Protease Name	Characteristics	Applications
B.subtilis	Neutral, alkaline proteases	Subtilisin, nattokinase	High activity, stability under varied pH conditions	Industrial enzyme production, food processing
Bacillus licheniformis	Thermostable proteases	Subtilisin Carlsberg	High thermal stability, active in alkaline conditions	Detergents, leather processing, textile industry
Bacillus amyloliquefaciens	Neutral, alkaline proteases	Subtilisin BPN', protease N	Stability in wide pH and temperature ranges	Food processing, bioremediation, fermentation
Pseudomonas aeruginosa	Elastase, alkaline protease	LasB (elastase), alkaline protease A	Role in pathogenicity, biofilm formation, tissue degradation	Pathogenicity research, medical microbiology
Pseudomonas Fluorescens	Psychrotrophic proteases	Pseudolysin	Active at low temperatures, spoilage of refrigerated foods	Food spoilage studies, dairy industry
Streptomyces griseus	Trypsin-like proteases	Streptogrisin B, proteinase K	Highly specific cleavage, broad substrate specificity	Protein sequencing, molecular biology, pharmaceuticals
Streptomyces fradiae	Chymotrypsin-like proteases	Chymotrypsin-like protease	Effective protein degradation, broad substrate specificity	Biotechnology, enzyme research, pharmaceuticals
Lactococcus lactis	Casein hydrolysis proteases	Proteinase PrtP	Essential for casein breakdown, flavor development in cheese	Dairy fermentations, cheese ripening, flavor enhancement

Clostridium histolyticum	Collagenases, proteases	ColH, ColG	Degrades collagen, useful in tissue dissociation	Medical research, wound care, tissue engineering
Thermus aquaticus	Thermostable proteases	Taq protease	Stable at high temperatures, used in PCR-related processes	High-temperature industrial applications, biotechnology

1.3 Source of protease enzyme

A. Microbial sources

Bacteria: *Bacillus species*, such as Bacillus subtilis, are highly esteemed for their proficiency in generating proteases. Their remarkable capacity to release substantial quantities of enzymes into the surrounding environment, coupled with their ease of genetic modification and swift proliferation, make them a preferred choice in the realm of enzyme production. **[Jha, R.K., X. Zi-rong. 2004].**

Fungi: Renowned for their exquisite ability to produce both acid and neutral proteases, the Aspergillus and Penicillium species stand as remarkable examples in the fungal kingdom. These fungi possess the remarkable capability to thrive on a diverse range of substrates, making them effortlessly cultivable. **[Ahuja, S.K.; Ferreira 2004, Kumar, V 2014].**

Yeast: Yeasts such as Saccharomyces cerevisiae are known for their ability to produce specialized proteases that are highly advantageous in the realms of baking and brewing**. [Saleemuddin, M., 199].**

B. Plant Sources

Extracted from the succulent latex or luscious juice of papaya and pineapple, proteases like papain and bromelain exude an air of luxury. **[Ganesh kumar C 1999].** These exquisite enzymes find their purpose in the realm of culinary arts, where they gracefully tenderize meat and enhance the finesse of various food processing applications.

C. Animal Sources

Luxuriously crafted proteases such as trypsin, chymotrypsin, and pepsin are extracted from the intricate digestive systems of majestic creatures. These exquisite enzymes find their place of honor in the realms of both medical and pharmaceutical industries.

Microbial, plant, and animal-derived protease enzymes possess distinct characteristics ideal for various industrial uses. Microbial proteases are known for their adaptability and simplicity in manufacturing, plant proteases excel in food-related functions due to their efficacy and natural origins, while animal proteases are reserved for specialized medical and diagnostic purposes. Selecting the appropriate protease hinges on factors such as enzyme specificity, stability, cost-effectiveness, and compliance with regulatory standards tailored to the specific application at hand. **[P. Binod 2013].**

1.4 Production Method

Immersed and state-of-the-art methods of fermentation are elegantly employed for the synthesis of protease. Exquisite wild-type filamentous fungi or bacteria are meticulously utilized for the grand-scale manufacturing of protease using various cultivation methodologies.

1.4.1 Submerged fermentation

Submerged Fermentation (SmF) is a biotechnological process in which microorganisms are grown in a liquid nutrient medium inside bioreactors or fermenters. This method allows for precise control of environmental factors like pH, temperature, and oxygen levels, which are crucial for maximizing microbial growth and product output. The liquid medium ensures even distribution of nutrients, resulting in consistent microbial growth. SmF is scalable, making it ideal for large-scale industrial applications.The process starts with inoculation, where microorganisms are introduced into the liquid medium. [**Auld B.A. (1993)].** Throughout fermentation, these cultures are carefully monitored and controlled to support growth and product formation. Once finished, the culture broth is collected, and downstream processing is carried out to extract and purify the desired product.SmF finds various uses, such as in pharmaceuticals for manufacturing antibiotics and vaccines, in the food and beverage sector for fermenting beer, wine, and dairy products, and in the production of industrial enzymes used in detergents, textiles, and food processing**.[Shraddha, Shekher 2018]**. The advantages of SmF include enhanced productivity due to controlled environments, ease of management through automated systems, and consistent product quality. However, the method can be expensive due to the requirement for advanced equipment and control systems, making it a substantial investment for industrial purposes. **[Gnanadoss J. J. (2013)].**

1.4.2 Solid-State Fermentation (SSF)

Solid-State Fermentation (SSF) is a technique used to cultivate microorganisms on solid substrates without the presence of free-flowing water. The solid substrate acts as both a source of nutrients and a surface for microbial growth. This method is particularly effective for growing fungi and certain bacteria, as they thrive in low moisture conditions. SSF is a cost-effective approach that utilizes agricultural residues or other inexpensive materials, requiring simpler technology and less sophisticated equipment compared to Submerged Fermentation (SmF).

The SSF process involves several key steps. Initially, the solid substrate is prepared, which may involve chopping, soaking, or sterilization. The substrate is then inoculated with the desired microorganisms. The inoculated substrate is incubated in a controlled environment where temperature, humidity, and aeration are regulated to optimize microbial growth and product formation. Once the fermentation period is complete, the fermented product is collected. Subsequent processing includes extracting and purifying the desired product from the solid substrate**. [Nityanand C. (2011)].**

SSF has a wide range of applications, including enzyme synthesis, bioethanol production, and environmental cleanup. It is commonly used to produce fungal proteases, cellulases, and industrial enzymes. In bioethanol production, SSF converts lignocellulosic residues into bioethanol for use as fuel. [**D'Annibale et al.,**

2000]. Additionally, SSF aids in environmental cleanup by breaking down pollutants through microbial action on solid materials.
The advantages of SSF are significant. It is cost-effective due to lower energy and water requirements, as well as the use of inexpensive substrates. The process often results in higher product concentrations, simplifying downstream processing. Furthermore, SSF requires less complex technology and infrastructure, making it easier to implement.However, SSF does present challenges. Scaling up the process can be difficult due to issues with heat and mass transfer. Monitoring and controlling environmental parameters are crucial for successful SSF implementation. [**Krajewska, 2004**].

Comparison of SmF and SSF:

parameter	Submerged Fermentation (SmF)	Solid-State Fermentation (SSF)
Water Content	High	low
Equipment	Complex and automated bioreactors	Simpler and less automated
control	Easy control of pH, temperature, oxygen	Difficult to control and monitor
Substrate	Liquid nutrient medium	Solid substrate (e.g., agricultural residues)
Microorganisms	Suitable for a wide range of microorganisms	Best suited for fungi and certain bacteria
Cost	Higher due to sophisticated equipment	Lower due to use of inexpensive substrates
Scale up	Easier to scale up	More challenging due to heat and mass transfer
Product yield	Generally high	Can be high but varies with substrate

1.5 Downstream process of protease

Downstream processing in biotechnology involves the purification and separation of desired products from a mixture, typically after they have been produced by cells or organisms. **[Abraham LD, Breuil C (1996)].** Here is a comprehensive breakdown of the two steps you mentioned: cell disruption and purification.

Cell Disruption:
Cell disruption serves as the initial stage in downstream processing for intracellular proteases. The objective is to rupture the cells and release the proteases contained within them. There are several methods that can be employed to achieve this:

a. Mechanical Disruption: This method entails physically breaking open the cells using mechanical force. Techniques such as grinding with a mortar and pestle, homogenization using a blender or homogenizer, or high-pressure homogenization can be utilized. Mechanical disruption is suitable for larger-scale operations, but it may not be gentle enough for delicate cells or may not yield high purity.

b. Enzymatic Lysis: Enzymes like lysozyme or proteinase K can selectively break down components of the cell wall or membrane, facilitating cell lysis. This approach is often gentle and can be effective for specific cell types, but it may require optimization for different cell types and conditions. **[Bhattacharyya BC (2001)].**

a. Sonication:

Ultrasonic waves are applied to the cell suspension, leading to disruption through the formation and collapse of cavitation bubbles. This method is effective for smaller-scale operations and can be gentle on sensitive cells. However, it may generate heat and necessitate optimization to prevent protein denaturation.

2.Purification:

Purification involves isolating proteases from a crude mixture of cellular components to obtain a pure product. Various techniques can be used for this purpose:

a. Precipitation: Proteins can be selectively precipitated by adding agents like ammonium sulfate or organic solvents. Different proteins have different solubilities at varying agent concentrations, allowing for selective precipitation. While this method is simple and moderately effective, it may lack high selectivity. **[Anson ML (1938)].**

b. Chromatography:

a. Ion Exchange Chromatography: Proteins are separated based on their net charge at a specific pH. Proteases with different charges interact differently with charged chromatography resins, enabling separation.
b. Affinity Chromatography: This method utilizes specific interactions between the protease and a ligand immobilized on the chromatography matrix. Common ligands include antibodies, substrates, or inhibitors specific to the protease of interest.
c. Gel Filtration Chromatography (Size Exclusion Chromatography): Proteins are separated based on their size and shape. Proteases of varying molecular weights elute at different times, facilitating separation.
d. Ultrafiltration: This technique uses semipermeable membranes with defined pore sizes to separate proteins based on their molecular weight or size. Proteases can be concentrated and separated from smaller molecules and contaminants through selective permeation.

The method of purification relies on several factors including the characteristics of the protease, the level of purity desired, the size of the operation, and financial factors. Frequently, a blend of methods is employed in a purification procedure to attain the desired level of purity and output. **[Bhattacharyya BC (1993)].**

1.6 Optimization

Genetic Modification: Utilizing genetic engineering methods can improve protease production, stability, and specificity in microbial strains. This process entails modifying an organism's genetic material to introduce or enhance specific characteristics. In the case of protease production, genes responsible for proteases or enzymes involved in their synthesis can be targeted.

DNA Recombination Technology: DNA recombination technology facilitates the integration of protease genes into highly productive microbial hosts. This procedure involves identifying the gene of interest, such as those encoding proteases with favorable traits, and integrating them into the genome of a suitable host organism. Through this method, the host organism can generate the desired protease in significant quantities.

Fermentation Conditions:

pH: The pH of the fermentation medium is a critical factor in protease production. Different microbial strains have specific optimal pH ranges for synthesizing protease. For instance, *Bacillus* species typically achieve the highest protease yields at alkaline pH levels. The optimization process includes adjusting the pH of the fermentation medium to the range that promotes the growth and productivity of the chosen microbial strain.

Temperature: The temperature has a significant impact on microbial growth and enzyme activity. The optimal temperature for protease production varies depending on the microbial strain. *Bacillus* species often prefer moderate temperatures to maximize protease yield. Maintaining temperature control during fermentation is crucial to preserve enzyme stability and activity.

Aeration: Sufficient aeration is vital for microbial growth and metabolite production in fermentation. Proper oxygen supply enhances cell growth and protein synthesis, including protease production. Aeration rates and agitation speed are fine-tuned to ensure the microbial culture receives enough oxygen without experiencing excessive shear stress**. [J. T. Pronk (2015)].**

Nutrient Composition: The composition of the fermentation medium greatly influences protease production. Nutrients like carbon and nitrogen sources, vitamins, minerals, and trace elements impact microbial growth and protein synthesis. **[O.Levitan 2014].**Optimization involves selecting the right nutrient sources and concentrations to support robust microbial growth and achieve high protease yield. The enhancement of protease production in microbial strains can be significantly improved through the optimization of fermentation conditions and the utilization of genetic engineering techniques. This can result in increased yields, stability, and specificity of the target enzyme. It is crucial to continuously monitor and adjust these factors to ensure maximum efficiency in protease production within industrial bioprocesses. **[M. Grabsztunowicz 2014].**

1.7 Application of protease enzyme

Proteases are essential enzymes with significant functions across multiple industries, as they possess the capability to degrade proteins into smaller peptides or amino acids. Below is an extensive examination of their utilization in diverse industrial sectors. **[X.-G. Zhu 2010].**

Food industries:

Proteases play a significant role in the food industry, serving various purposes:

1. Cheese Making: Proteases are essential in cheese production as they assist in the coagulation of milk proteins, a crucial step in cheese formation. Specific proteases break down proteins, enhancing the flavor, texture, and ripening of cheese.

2. Meat Tenderization: Proteases are utilized to tenderize meat by breaking down tough connective tissue proteins like collagen. This process improves the tenderness of the meat, making it more enjoyable to eat.

3. Protein Hydrolysates Production: Proteases are employed to hydrolyze proteins into smaller peptides or amino acids, resulting in protein hydrolysates. These hydrolysates are used in various food products such as infant formulas, sports nutrition supplements, and medical nutrition products due to their enhanced digestibility and bioavailability. **[B. Bailleul 2010].**

Detergent industries:

Proteases play a crucial role in the detergent industry as essential ingredients in laundry detergents. Their primary function is to effectively eliminate protein-based stains like blood, egg, grass, and food stains. **[N. Alvarez 2010]**. By specifically targeting and breaking down the proteins found in stains, these enzymes greatly assist in the removal of such stains during the washing process. The inclusion of proteases significantly enhances the overall effectiveness of laundry detergents, particularly when it comes to tackling stubborn, proteinaceous stains. Proteases have various applications in surgical disinfectants and medical cleaning solutions**. [A.Y. Tamine 2008].** Here are some ways in which proteases can be utilized in this context:

Biofilm Disruption: Proteases can assist in disrupting biofilms, which are bacterial communities that can develop on medical instruments, surfaces, and wounds. By breaking down the extracellular matrix proteins that hold biofilms together, proteases enhance the effectiveness of disinfection procedures.

Instrument Cleaning: Proteases can be incorporated into cleaning solutions for decontaminating surgical instruments and medical equipment. By breaking down proteinaceous residues like blood, tissue, and other organic matter, proteases aid in the removal of contaminants from instruments, ensuring thorough cleaning and sterilization.

Wound Debridement: In certain situations, proteases may be used for enzymatic debridement of wounds. Chronic wounds often contain necrotic tissue and fibrin deposits, which can hinder healing and increase infection risks. Topical application of proteases can help remove this necrotic tissue, promoting wound healing and reducing complications.

Preventing Cross-Contamination: Proteases play a role in preventing cross-contamination in medical environments by effectively eliminating organic residues from surfaces, equipment, and textiles. This is especially crucial in surgical settings where maintaining a sterile environment is essential for preventing healthcare-associated infections.

Non-corrosive Cleaning: Unlike harsh chemical disinfectants, proteases are generally non-corrosive to metals and other materials commonly found in medical instruments and equipment. This characteristic makes protease-based cleaning solutions suitable for use on a wide range of surfaces without causing damage or deterioration.

Biocompatibility: Microbial-derived proteases can be modified to ensure biocompatibility and safety in medical applications. With the right formulation, protease-based disinfectants present minimal risks of adverse effects on patients, healthcare workers, and the environment. **[G. Ge´ san-Guiziou (2002)].**

Improving Effectiveness: Proteases have the ability to improve the effectiveness of other disinfectant agents by aiding in the elimination of organic contaminants that could hinder their antimicrobial activity. The combination of proteases with other disinfectants or antimicrobial agents can produce synergistic effects, resulting in enhanced disinfection outcomes.In the realm of surgical and medical settings, proteases reign supreme as a multifaceted and efficacious remedy for disinfection and cleansing. With their remarkable ability to zero in on proteinaceous remnants and biofilms, proteases play a pivotal role in upholding aseptic conditions, mitigating the peril of infections, and safeguarding the well-being of patients within healthcare domains**. [S.E. Kentish 2001].**

Pharmaceuticals industries:

Proteases, also referred to as proteolytic enzymes or peptidases, are essential enzymes that degrade proteins into smaller peptides or amino acids. They serve pivotal functions in various biological processes, such as digestion, immune response, and tissue remodeling. **[Bakke, OM. 1995].** In the realm of pharmaceuticals and medicine, proteases are utilized in diverse applications:

Wound Debridement:

Operational Mechanism: Proteases facilitate the elimination of necrotic tissue, slough, and debris from wounds by disintegrating proteins in dead or damaged tissue.

Varieties of Proteases Utilized: Enzymes commonly employed for wound debridement encompass collagenases, like Clostridial collagenase (e.g., Collagenase Santyl), which specifically target collagen, a fundamental element of the extracellular matrix in tissues.

Enzymatic Removal of Necrotic Tissue:

Objective: The presence of necrotic tissue can hinder the healing process of wounds and escalate the risk of infection. Utilizing proteases for enzymatic debridement presents a less invasive alternative to surgical debridement.

Utilizations: Enzymatic debridement can be applied not only in wound care but also in the treatment of chronic ulcers, burns, and traumatic injuries.

Products: Pharmaceutical formulations containing proteases for enzymatic debridement are available in a variety of luxurious forms, including ointments, creams, and gels**. [Comanor, W.S. (1986)],**

Formulations for Digestive Assistance:

Role in Digestion: Proteases play a crucial role in the breakdown of dietary proteins during the process of digestion. They meticulously dismantle proteins into peptides and amino acids, facilitating their absorption within the digestive tract.

Therapeutic Usage: Digestive aid formulations containing proteases are employed in cases where there is an inadequate production of endogenous digestive enzymes, such as pancreatic insufficiency or specific digestive disorders.

Prominent Proteases in Digestive Aids: Notable examples encompass pancreatic enzymes like pancrelipase, which encompasses an array of proteases (such as trypsin and chymotrypsin), lipases, and amylases. **[Leibowitz, A., (1985)],**

Additional Medical Applications:

Anti-inflammatory Properties: Certain proteases possess remarkable anti-inflammatory properties, thus warranting exploration for their potential therapeutic utilization in conditions characterized by inflammation.

Research and Advancement: Proteases are also extensively studied for their involvement in disease mechanisms, drug delivery systems, and as targets for therapeutic intervention in conditions like cancer and neurodegenerative diseases.

Proteases, with their multifaceted nature, find themselves indispensable in the realm of pharmaceuticals and medicine. Their applications span a wide spectrum, encompassing everything from tending to wounds to formulating aids for digestion. The sheer adaptability of these enzymes renders them invaluable instruments in a myriad of therapeutic and research settings. **[G. Frey, Chem. Soc. Rev. 2008].**

1.7 Conclusion:

Natural origins sustain health and ecological balance by harnessing the power of beneficial microorganisms, age-old remedies, and nourishing organic foods. The soil, teeming with life, is enriched by the presence of remarkable microorganisms like *Bacillus subtilis*, which not only enhance soil fertility but also purify it from harmful pollutants. Furthermore, the botanical wonders and fungi found in nature have made significant contributions to the advancement of modern medicine. In our quest for sustainability, we have embraced the use of eco-friendly sanitizers, cosmetics, and textiles. These conscientious choices not only promote a healthier planet but also ensure the longevity of our precious resources. By immersing ourselves in the embrace of nature, we find solace and tranquility, as it has been proven to alleviate stress and enhance our mental well-being. The protease enzymes, skillfully produced by the remarkable *Bacillus subtilis*, play a pivotal role in various industries such as food, detergents, and pharmaceuticals. Their remarkable versatility and efficiency have proven indispensable in the art of cheese making, tenderizing meat, and even aiding in wound care. These enzymes, with their remarkable capabilities, have become the cornerstone of industrial, medical, and biotechnological applications, championing sustainable practices and reducing our reliance on synthetic chemicals. In this age of enlightenment, we must cherish and nurture the natural origins that sustain us. By embracing the power of microorganisms, traditional remedies, and organic foods, we not only safeguard our health but also preserve the delicate ecological balance that is essential for our existence. Let us continue to tread lightly upon this Earth, guided by the wisdom of nature, as we forge a path towards a more sustainable future.

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