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Review article

Antimicrobial Enzymes and Proteins: Modes of Action Against Microbes and Microbial Biofilms

Olowonibi Olabisi Oloruntoba [1,*], Adegoke Sunday Adetunji [1], Omoyajowo Olaniyi [1] and Ohimai Anthony [2]

[1] Department of Microbiology, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria
[2] Department of Science Laboratory Technology, Auchi Polytechnic, Auchi, Edo State, Nigeria

*Correspondence: bisi4greatness08@gmail.com

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Abstract: Antimicrobial enzymes are naturally occurring or engineered biocatalysts that play a crucial role in innate immunity by inhibiting, disrupting, or eliminating pathogenic microorganisms, including bacteria, fungi, and viruses. These enzymes have emerged as promising alternatives to conventional antibiotics because they are broad-spectrum, biodegradable, specific, and less likely to induce resistance. This review explores major classes of antimicrobial enzymes, including lysozyme, lactoperoxidase, lactoferrin, and defensins, and highlights their structures, biological significance, mechanisms of action, and practical applications. Lysozyme hydrolyzes peptidoglycan in bacterial cell walls, while lactoperoxidase generates hypothiocyanite with potent antimicrobial activity. Lactoferrin exerts antimicrobial effects by sequestering iron and disrupting membranes, whereas defensins destroy pathogens by forming pores in microbial membranes and modulating host immunity. The review also examines the diverse mechanisms by which these enzymes combat microorganisms, including cell wall degradation, membrane disruption, biofilm degradation, nucleic acid destruction, nutrient deprivation, and induction of oxidative stress. The review particularly emphasizes their role in overcoming antimicrobial resistance, as antimicrobial enzymes can target resistant pathogens, disrupt biofilms, and enhance the efficacy of conventional antibiotics. The review also discusses their applications in healthcare, food preservation, agriculture, environmental management, industrial processing, and biotechnology. Advances in protein engineering, nanotechnology, synthetic biology, and enzyme formulation have expanded the potential of antimicrobial enzymes and improved their stability and effectiveness. Despite challenges related to production costs, stability, and large-scale implementation, continued research and technological innovation are expected to overcome these limitations. Overall, antimicrobial enzymes are versatile, sustainable antimicrobial agents with significant potential to address emerging microbial threats and contribute to the global fight against antimicrobial resistance.

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Introduction

Antimicrobial enzymes are found throughout nature and are important components of the defense systems of living organisms against infection. Numerous organisms, including bacteria, fungi, plants, and animals, synthesize these specialized proteins. Antimicrobial enzymes are a class of proteins capable of suppressing or killing microorganisms, including bacteria, fungi, and viruses. Acting as biochemical tools that degrade or arrest the growth of harmful microbes, they are essential components of the innate immune system. Many act by hydrolyzing key structural components of bacterial or fungal cell walls, while others sequester essential nutrients, generate

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oxidizing species, or permeabilize microbial membranes [1].

Antimicrobial enzymes are natural or engineered proteins that inhibit or destroy microorganisms. These enzymes are pivotal in various sectors such as healthcare, food preservation, agriculture, and biotechnology due to their ability to combat microbial infections. This detailed overview examines the types, mechanisms of action, applications, recent advancements, challenges, and prospects of antimicrobial enzymes [2].

Antimicrobial enzymes can exhibit various modes of action against microbes and microbial biofilms [3]. A population of bacteria embedded in extracellular polymeric materials is called a biofilm. Organisms preferentially adopt the biofilm mode of growth because it offers numerous advantages over the planktonic state [4]. Microbes first adhere to a surface and then undergo a maturation stage regulated by quorum sensing (QS). When compared to their planktonic state, organisms in biofilm can be 10–1000 times more resistant to antibiotics. This may result from specific phenotypic alterations, the slower growth of organisms within the biofilm, or limited penetration of antibiotics through the biofilm matrix. The capacity of microorganisms to form biofilms poses problems for industry, agriculture, and human health. In the fight against biofilms, natural compounds such as plant extracts and quorum sensing inhibitors appear to be viable substitutes for traditional antibiotics [5]. This review describes examples of antimicrobial enzymes, modes of action, and their applications in various industries.

Examples of antimicrobial enzymes and proteins

Lysozyme

This enzyme can cleave the peptidoglycan layer in the cell walls of certain bacteria, leading to their lysis and death. Lysozymes are a type of antimicrobial enzyme recognized for their ability to break down bacterial cell walls. They are ubiquitous in nature, found in numerous organisms including humans, animals, plants, and bacteria. Lysozymes play a crucial role in the innate immune system, serving as a first line of defense against bacterial infections [6, 7].

The structure of lysozyme includes a cleft or groove that serves as the active site, where the catalytic process occurs. The bacterial cell wall's peptidoglycan, a complex polymer made up of sugars and amino acids, is the main substrate that this active site binds to [8].

Lysozyme's catalytic mechanism is based on the hydrolysis of β-1,4-glycosidic bonds between N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) in the peptidoglycan layer, which weakens the cell wall and causes the bacterial cell to lyse and die. The enzyme works through an acid-base catalysis mechanism, where important amino acids in the active site, like glutamic acid and aspartic acid, are essential to the catalytic process [9].

Lysozymes are widely distributed in nature, occurring in various tissues and body fluids of humans and animals. In humans, lysozyme is present in high concentrations in secretions such as saliva, tears, mucus, and breast milk. It is also found in immune cells like neutrophils and macrophages, where it is stored in granules and released upon infection [9]. Plants produce lysozymes as part of their defense mechanisms against microbial pathogens. Even some bacteria produce lysozyme-like enzymes, which they use to remodel their own cell walls or to attack competing bacterial species [9].

Biological Significance: The main biological function of lysozyme is the protection of the host from bacterial infections. By breaking down the cell walls of invading bacteria, lysozyme helps to prevent the establishment and spread of infections. This antibacterial action is particularly important in mucosal surfaces, where the enzyme acts as a protective barrier against pathogens [10].

Lysozyme is also involved in modulating the immune response. It can enhance the activity of other antimicrobial agents and stimulate the production of cytokines, which are signaling molecules involved in the immune response. Additionally, lysozyme can act as an anti-inflammatory agent, reducing tissue damage and promoting healing during infections [11].

Clinical and Industrial Applications: Due to its potent antibacterial properties, lysozyme has numerous clinical and industrial applications. For instance, in medicine, lysozyme is used as a therapeutic agent for treating bacterial infections and as a component of wound dressings to prevent infection. It is also added to infant formulas to mimic the protective effects of breast milk [12].

Also, in the food industry, lysozyme is used as a natural preservative to extend the shelf life of various products. It is commonly added to cheese to prevent spoilage and to wine to control lactic acid bacteria. The enzyme's ability to lyse bacterial cells without affecting

human cells makes it an attractive alternative to chemical preservatives [8].

Lysozyme is also used in the biotechnology industry for the extraction of recombinant proteins. By lysing bacterial cells, lysozyme facilitates the release of intracellular proteins, which can then be purified for different applications. This use of lysozyme is particularly important in the production of biopharmaceuticals and other high-value proteins [10, 11].

Lactoperoxidase

This enzyme catalyses the oxidation of thiocyanate to hypothiocyanite, which has antimicrobial activity against bacteria and fungi. Lactoperoxidase (LPO) is a heme-containing enzyme that is part of the peroxidase family. It is predominantly found in milk, tears, saliva, and other mucosal secretions. LPO plays a crucial role in the body's defense mechanisms, particularly in the oral cavity and mammary gland, by exhibiting potent antimicrobial properties. This enzyme catalyzes the oxidation of various substrates, using hydrogen peroxide as the oxidising substrate, to produce compounds that are lethal to a broad spectrum of microorganisms, including bacteria, fungi, and viruses [13]. The molecular weight of lactoperoxidase, a glycoprotein, is roughly 78 kDa. It has a heme prosthetic group at its active site and is made up of a single polypeptide chain that has been folded into the distinctive peroxidase structure. The catalytic activity of the enzyme depends on the heme group, which is made up of an iron ion enclosed in a porphyrin ring [14-16]. The principal function of lactoperoxidase is to catalyse the oxidation of thiocyanate ions (SCN−) to hypothiocyanite ions (OSCN−) in the presence of hydrogen peroxide (H2O2). The reaction can be summarized as follows:

$H_2O_2 + SCN^- \rightarrow OSCN^- + H_2O$

Hypothiocyanite is a potent antimicrobial agent that inhibits the growth and activity of a wide range of pathogens by interfering with their metabolic processes, damaging cell walls, and disrupting cellular functions. OSCN− is active against both Gram-positive and Gram-negative bacteria, fungi, and some viruses [17].

Biological Significance: Lactoperoxidase plays an important role in the innate immune system, particularly in mucosal secretions where it helps to maintain microbial balance and prevent infections. In the oral cavity, LPO is part of the salivary defense system, helping to control the growth of oral bacteria and thereby contributing to oral health. It helps prevent dental caries, gingivitis, and other oral infections by maintaining a stable oral microbiome [10].

In the mammary gland, lactoperoxidase is crucial for protecting the glandular tissue and the milk itself from microbial contamination. This is particularly important for neonates who rely on breast milk as their primary source of nutrition and immune protection. The presence of LPO in breast milk provides infants with a critical line of defense against pathogens during the early stages of life [15].

The mode of action of lactoperoxidase involves a series of oxidation-reduction reactions. When lactoperoxidase encounters hydrogen peroxide, the enzyme undergoes a two-electron oxidation to form compound I, an oxyferryl heme intermediate. Compound I then oxidises thiocyanate in a single two-electron transfer, yielding hypothiocyanite and regenerating the native enzyme, thereby completing the catalytic cycle [15].

The antimicrobial activity of OSCN− is primarily due to its ability to oxidize essential sulfhydryl (SH) groups in microbial proteins, leading to the disruption of vital cellular functions and ultimately cell death. Additionally, OSCN− can interfere with the microbial electron transport chain and nutrient uptake systems, further inhibiting microbial growth [16].

Industrial and Clinical Applications: Due to its antimicrobial properties, lactoperoxidase has several industrial and clinical applications. In the dairy industry, LPO prolongs the shelf life of milk and dairy products by inhibiting the growth of spoilage bacteria. The lactoperoxidase system (LPS) is a natural preservation method that enhances the safety and quality of dairy products without the need for artificial preservatives [17].

In the pharmaceutical and healthcare sectors, lactoperoxidase is utilized in various formulations, including toothpaste, mouthwash, and wound care products. In oral care products, LPO helps reduce plaque formation, prevent dental caries, and control oral infections. In wound care, lactoperoxidase-containing products aid in preventing infections and promoting healing by maintaining a microbe-free environment [18].

Lactoperoxidase is also being explored for its potential in treating infections and inflammations in the respiratory tract, given its presence and activity in mucosal secretions. Moreover, the enzyme's broad-spectrum antimicrobial activity makes it a candidate

for inclusion in formulations designed to manage infections in immunocompromised patients [10].

Lactoferrin

This iron-binding protein can damage bacterial cell membranes and thereby inhibit microbial growth. Although lactoferrin is considered here alongside the antimicrobial enzymes, it is strictly an antimicrobial protein rather than a catalytic enzyme, since its activity arises from iron sequestration and direct membrane interaction rather than from substrate turnover. A multipurpose glycoprotein that is a member of the transferrin family, lactoferrin is well-known for its capacity to bind and move iron. It is found in different secretory fluids including milk, saliva, tears, and nasal secretions, and is also present in secondary granules of neutrophils. Lactoferrin has a variety of biological activities, such as antibacterial, antiviral, antifungal, anti-inflammatory, and immunomodulatory qualities, which contribute significantly to the body's defense systems [18].

A single polypeptide chain comprising roughly 690 amino acids makes up lactoferrin, forming a bilobed structure with two homologous lobes (N-lobe and C-lobe), each capable of binding one ferric ion (Fe^{3+}). The iron-binding sites are highly conserved and enable lactoferrin to tightly bind iron with high affinity. This iron-binding property is central to many of its biological functions [17].

Biological significance: The primary function of lactoferrin is its ability to bind iron. By sequestering iron, lactoferrin deprives bacteria and other pathogens of the iron necessary for their growth and proliferation. This mechanism, known as iron withholding, is a critical aspect of the innate immune response. Iron-binding also inhibits the formation of free radicals, which can cause tissue damage during inflammation [17].

Lactoferrin's antimicrobial properties are among its most studied functions. Numerous Gram-positive and Gram-negative bacteria are susceptible to its bacteriostatic and bactericidal actions. Lactoferrin's antibacterial properties involve several mechanisms:

- By binding free iron, lactoferrin limits the availability of this essential nutrient to bacteria, inhibiting their growth.
- Lactoferrin can bind to bacterial membranes, directly damaging the cell wall and membrane and leading to cell lysis.
- Lactoferrin can enhance the effectiveness of other antimicrobial agents, including lysozyme and antibiotics, through synergistic interactions.

In addition to its antibacterial activity, lactoferrin exhibits antifungal, antiviral, and antiparasitic properties. It has been demonstrated to prevent *Candida albicans* and other pathogenic fungi from growing, interfere with viral entry and replication for viruses like hepatitis C and herpes simplex, and inhibit parasitic infections by affecting the lifecycle of parasites like *Plasmodium falciparum* [10].

In human colostrum, the first milk produced after childbirth, lactoferrin is especially prevalent and serves a vital role in defending newborns against illnesses and promoting the growth of their immune systems. Human milk contains a considerably higher concentration of lactoferrin than cow's milk, indicating how important it is for the health of newborns [10]. Lactoferrin helps protect infants from gastrointestinal and respiratory infections by inhibiting the growth of pathogens and promoting the growth of beneficial microbiota. It supports the maturation of the neonatal immune system, enhancing both innate and adaptive immunity. Beyond its antimicrobial properties, lactoferrin contributes to the overall health and development of infants by promoting iron absorption and intestinal health.

Clinical Applications and Therapeutic Potential: Given its wide range of biological activities, lactoferrin has significant potential for therapeutic applications. It is being explored for use in various medical and health-related fields [15].

Infectious Diseases: Lactoferrin supplements are being investigated as adjunct treatments for bacterial, viral, and fungal infections, leveraging its antimicrobial and immunomodulatory properties.

Inflammatory Diseases: Its anti-inflammatory effects make lactoferrin a candidate for treating inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease (IBD).

Cancer: Emerging research suggests that lactoferrin may have anticancer properties, potentially through its ability to modulate the immune response, inhibit tumor growth, and induce apoptosis in cancer cells.

Nutritional Supplements: Lactoferrin is used in dietary supplements to enhance immune function, particularly in populations at higher risk of infections, such as infants, the elderly, and immunocompromised individuals.

Defensins

These are small, cationic peptides that can disrupt microbial cell membranes, causing cell lysis and death. Defensins are a class of small, cationic, antimicrobial peptides that play a critical role in the innate immune system of many organisms, including humans. They are found across a wide range of species from plants to animals and serve as a first line of defense against a broad spectrum of pathogens, including bacteria, fungi, and viruses. Defensins are characterized by their ability to disrupt microbial membranes, leading to cell death, and they also possess immunomodulatory functions that help orchestrate the host's immune response [14]. As with lactoferrin, defensins are antimicrobial peptides rather than enzymes in the catalytic sense; they are included here because they act alongside antimicrobial enzymes within the innate immune system.

Biological Significance: The primary antimicrobial mechanism of defensins involves their interaction with microbial membranes. Due to their cationic nature, defensins are attracted to the negatively charged components of microbial cell membranes. Upon binding, they insert themselves into the lipid bilayer, disrupting membrane integrity and leading to cell lysis. This membrane-disruptive action is rapid and effective against a broad range of pathogens [14].

- Defensins form pores in microbial membranes, causing leakage of cellular contents and subsequent cell death.
- By disrupting membrane integrity, defensins interfere with essential microbial processes, such as nutrient uptake and energy metabolism.
- In some cases, defensins can penetrate the microbial cell and interact with intracellular components, further disrupting cellular functions.

Beyond their direct antimicrobial activities, defensins play crucial roles in modulating the host immune response. They can act as signaling molecules, influencing both innate and adaptive immunity [16].

Clinical Significance and Therapeutic Potential: Given their broad-spectrum antimicrobial activity and immunomodulatory functions, defensins have significant clinical implications [15]. They are being explored for various therapeutic applications, including:

Infectious Diseases: Defensins have potential as therapeutic agents for treating bacterial, fungal, and viral infections. Their ability to target multiple pathogens reduces the likelihood of resistance development.

Inflammatory Diseases: Due to their immunomodulatory effects, defensins could be used to manage inflammatory and autoimmune conditions by modulating immune responses.

Cancer: Emerging research suggests that defensins might have antitumor properties, possibly through their ability to modulate the immune system and directly kill tumor cells.

Wound Healing: Defensins can promote wound healing by enhancing cell migration and proliferation, making them potential agents for treating chronic wounds and burns.

Modes of action of antimicrobial enzymes

The specific mode of action of an antimicrobial enzyme depends on its structure, catalytic activity, and the target microorganism or biofilm. The combination of these various mechanisms contributes to the broad-spectrum antimicrobial efficacy of these enzymes [11].

The primary function of antimicrobial enzymes is to neutralize harmful microorganisms through various biochemical mechanisms. One key mechanism is the hydrolysis of cell wall components. Enzymes such as lysozyme hydrolyse the peptidoglycan layer of the bacterial cell wall, resulting in cell lysis and death. This mechanism is particularly effective against Gram-positive bacteria, which possess a thick peptidoglycan layer.

By compromising the structural integrity of the cell wall, these enzymes induce osmotic imbalance, leading to the rupture of microbial cells [18, 19]. Another important mechanism involves the disruption of cell membrane integrity. Enzymes like phospholipases target the lipid bilayers of microbial membranes, hydrolyzing phospholipids and causing membrane destabilization. This destabilisation causes leakage of cellular contents and subsequent cell death. Because they target membrane lipids, these enzymes are active against a range of organisms, including bacteria, fungi, and protozoa. Lactoferrin and defensins, although not catalytic enzymes, similarly disrupt microbial cell membranes, causing leakage of cellular contents and cell death [16]. Dispersin B hydrolyses poly-N-acetylglucosamine, a key constituent of the extracellular polymeric substances (EPS) that hold biofilm cells together, thereby disrupting the biofilm structure. Some antimicrobial enzymes can also directly inhibit the activity of essential microbial

enzymes, disrupt crucial metabolic pathways and lead to cell death [19, 20].

Additionally, some antimicrobial enzymes act by degrading genetic material. Nucleases such as DNases and RNases break down the DNA and RNA of microorganisms, hindering their ability to replicate and transcribe essential genetic information. This degradation of genetic material prevents the microorganisms from reproducing and carrying out vital functions, effectively neutralizing the microbial threat. DNase can degrade the extracellular DNA, which is a vital constituent of the biofilm matrix, thereby weakening the biofilm's integrity [18].

Other common mechanisms of action include Interference with nutrient acquisition. Enzymes like lactoferrin can bind and sequester iron, an essential nutrient for microbial growth, thereby limiting the availability of this nutrient [21]. Also**,** lactoperoxidase can oxidize thiocyanate to produce hypothiocyanite, which can interfere with the uptake of essential nutrients by microbes [22]. In addition, certain antimicrobial enzymes can generate reactive oxygen species (ROS) or other oxidative species, which can cause damage to microbial cellular components, leading to cell death. Finally, some antimicrobial enzymes modulate host immune responses, acting synergistically with the immune system against biofilm-associated infections [23].

Regulation and specificity

The production and activity of antimicrobial enzymes are tightly regulated to ensure that they target pathogens without damaging the host's own tissues. Gene regulation plays an important role in this process. The genes encoding antimicrobial enzymes are often inducible, meaning they are expressed in response to specific stimuli such as the presence of pathogens. This inducible expression ensures that these enzymes are produced only when needed, conserving the host's energy and resources [10].

Post-translational modifications also play a significant role in regulating enzyme activity. These modifications, which include processes such as phosphorylation, glycosylation, and proteolytic cleavage, can alter the enzyme's activity, stability, and localization within the organism. By fine-tuning these modifications, the host can precisely control the function of antimicrobial enzymes [10].

Moreover, the activity of these enzymes can be modulated by specific inhibitors and activators. Inhibitors bind to the enzyme and prevent it from interacting with its substrate, thereby inhibiting its activity. Activators, on the other hand, enhance enzyme activity by inducing conformational changes that increase substrate affinity or catalytic efficiency. This regulatory mechanism ensures that antimicrobial enzymes act in a controlled and targeted manner [10].

Antimicrobial enzymes' action on antimicrobial resistance

Antimicrobial enzymes have garnered significant attention in clinical settings due to their potential to address the growing challenge of antimicrobial resistance (AMR). Antimicrobial enzymes can selectively disrupt bacterial cell walls or degrade specific microbial structures and signaling molecules. This specificity reduces the risk of harming the beneficial microbial flora.

Antimicrobial enzymes that target the breakdown of bacterial cellular machinery and biofilm development are crucial given the rising incidence of antibiotic resistance. Numerous products based on enzymes have already been brought to market for use in the food, biological, and healthcare sectors. Multi-enzyme formulations that comprise enzymes that can break down microbial DNA, polysaccharides, proteins, and quorum-sensing molecules are necessary for the successful removal of complex biofilms. By adding anti-quorum-sensing enzymes, biofilm reformation is avoided. In order to address the issues related to biofilm formation in manufacturing, environmental protection, and healthcare settings, it is imperative that effective complex enzyme formulations be developed. However, developments in whole-genome sequencing, enzyme engineering, and synthetic biology hold enormous promise for enabling the creation of stronger antibacterial and anti-biofilm enzymes [6].

Certain enzymes can penetrate and disrupt biofilms, making bacterial colonies more susceptible to treatment. This is particularly important in chronic infections where biofilms contribute to resistance. Enzymes like lysozyme and various proteolytic enzymes can be incorporated into topical treatments to enhance healing in infected wounds by reducing bacterial load.

Enzymatic formulations can be used in hand sanitizers and disinfectants, providing an additional mode of action against resistant pathogens. Enzymes can be used alongside conventional antibiotics to enhance efficacy. For example, cell wall-degrading enzymes such as lysostaphin and bacteriophage endolysins can increase the susceptibility of resistant staphylococci to β-lactam agents. A conceptually

related strategy combines β-lactam antibiotics with β-lactamase inhibitors such as clavulanic acid or avibactam, although these inhibitors are small molecules rather than enzymes.

Due to their unique mechanisms of action, antimicrobial enzymes may lead to slower rates of resistance development compared to conventional antibiotics. Some enzymes show activity against a wide range of pathogens, including Gram-positive and Gram-negative bacteria. This broad-spectrum potential is crucial in treating polymicrobial infections. Recent studies have demonstrated the effectiveness of various antimicrobial enzymes against resistant strains, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).

Application of antimicrobial enzymes

This special class of enzymes known as antimicrobial enzymes has garnered significant attention for their potential use in a wide array of applications, spanning from medicine to agriculture. Here, we delve into the various applications of antimicrobial enzymes, highlighting their significance and the advancements made in their utilization [7].

Application of antimicrobial enzymes in Infection Control and Treatment

Wound Care: Antimicrobial enzymes such as lysozyme and bacteriolytic enzymes are frequently incorporated into wound dressings to prevent infections and promote healing. They help to eliminate bacterial contamination in wounds and reduce the risk of biofilm formation [11].

Burn Treatment: Enzyme-based formulations are used in the treatment of burns to prevent infection and facilitate the removal of necrotic tissue. These formulations often include proteolytic enzymes that can break down dead tissue and reduce microbial load [14].

Oral Hygiene Products: Enzymes such as lysozyme, lactoferrin, and peroxidases are included in toothpastes and mouthwashes to combat oral pathogens. These enzymes help in reducing plaque formation, controlling bad breath, and preventing periodontal diseases [10].

Dental Implants: Antimicrobial enzymes are being investigated for coating dental implants to prevent peri-implantitis, a condition caused by bacterial infection around the implant [10].

Antibiotic Potentiation: Certain enzymes can degrade bacterial cell walls, enhancing the efficacy of conventional antibiotics. This combination can be particularly effective against antibiotic-resistant bacteria [10].

Antiviral Treatments: Enzymes that degrade viral components are being explored as antiviral agents. For example, proteases can inhibit the replication of viruses by targeting viral proteins [10].

Application of antimicrobial enzymes in Food and Beverage Industry

Food Preservation

Shelf-Life Extension: Lysozyme is used as a natural preservative to prolong the shelf life of a variety of food products, and is frequently applied together with bacteriocins such as nisin, which although not an enzyme acts by a complementary mechanism. These agents inhibit the growth of spoilage microorganisms and foodborne pathogens, thereby contributing to food safety [10].

Dairy Products: Lysozyme is commonly added to cheese to inhibit the growth of *Clostridium tyrobutyricum*, which causes late blowing, spoilage, and off-flavours [10].

Food Safety

Surface Decontamination: Enzyme-based solutions are used to sanitize food contact surfaces and equipment in food processing facilities. These solutions are effective in removing biofilms and reducing microbial contamination [12].

Packaging: Active packaging technologies incorporate antimicrobial enzymes into packaging materials to maintain food safety and quality during storage and transport [14].

Application of antimicrobial enzymes in Agriculture

Crop Protection

Biocontrol Agents: Enzymes such as chitinases and glucanases are used in agriculture to control fungal diseases in crops. These enzymes degrade the cell walls of pathogenic fungi, providing an environmentally friendly alternative to chemical fungicides [11].

Seed Treatment: Coating seeds with antimicrobial enzymes can protect them from soil-borne pathogens, improving germination rates and seedling health [11].

Animal Husbandry

Feed Additives: Enzymes are added to animal feed to improve gut health and reduce the incidence of infections in livestock. This not only promotes animal growth and productivity but also reduces the need for antibiotics [7].

Aquaculture: In fish farming, antimicrobial enzymes are used to control bacterial infections in water and on fish surfaces, enhancing fish health and reducing mortality rates [16].

Environmental Applications of antimicrobial enzymes

Waste Management

Bioremediation: Enzymes greatly assist the breakdown of organic contaminants in polluted soils and wastewater. Lytic enzymes degrade complex chemical molecules into simpler, less hazardous products [16].

Odor Control: Enzymatic treatments are used to manage odors in waste treatment facilities. These enzymes degrade malodorous compounds, improving air quality [16].

Pathogen Control: Antimicrobial enzymes are employed in water treatment processes to eliminate pathogenic microorganisms, ensuring safe drinking water. Enzymes like proteases and lipases can degrade biofilms in water distribution systems [8].

Application of antimicrobial enzymes in Industry

Textile Industry

Fabric Treatment: Enzymes are used in the textile industry to treat fabrics, preventing microbial degradation and odor formation. Antimicrobial finishes on textiles can provide long-lasting protection against microbial contamination [13].

Biofilm Control

Industrial Equipment: Biofilms can form on industrial equipment, leading to reduced efficiency and increased maintenance costs. Enzymatic treatments are effective in removing biofilms and preventing their reformation, ensuring smooth operation of industrial processes [17].

Application of antimicrobial enzymes in research and development

Genetic Engineering

Engineered Enzymes: Advances in genetic engineering and protein design have enabled the development of enzymes with enhanced antimicrobial properties. These engineered enzymes are tailored to target specific pathogens or environmental conditions [19].

Synthetic Biology: Synthetic biology approaches are being used to create novel antimicrobial enzymes with unique mechanisms of action, broadening the scope of their applications [17].

Diagnostic Tools

Biosensors: Enzyme-based biosensors are developed for the rapid detection of pathogens in clinical, food, and environmental samples. These biosensors offer high sensitivity and specificity, facilitating early intervention and treatment [24].

Conclusion

Antimicrobial enzymes hold considerable potential across applications ranging from healthcare to agriculture. Their capacity to target and eliminate pathogens, together with their biodegradability and low environmental impact, makes them attractive alternatives to conventional antimicrobial agents, particularly as antibiotic resistance continues to spread. Understanding their modes of action is therefore crucial to developing effective strategies against microbial infections and biofilm-related problems in medicine, the food industry, and environmental remediation. Advances in biotechnology, nanotechnology, and protein engineering are opening the way to more effective solutions, although continued research and development remain essential to overcome the practical difficulties of using these enzymes and to realize their full potential.

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