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UNVEILING THE PRODUCTION, PURIFICATION, AND MULTIFACETED POTENTIAL OF LYSOZYMES: A REVIEW

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ABSTRACT

Lysozyme is a versatile enzyme that has garnered significant recognition due to its multitude of applications in diverse fields, such as pharmaceutical, healthcare, cosmetics, food, and agriculture industry. It is naturally derived from avian egg whites and subsequently produced using microbial systems such as bacteria, fungi, and yeast. Lysozyme is used in food preservation to inhibit the growth of microorganisms that cause food spoilage, thus extending shelf life and ensuring food safety. In therapeutics, it acts as an anticancer, antimicrobial and antiviral agent, with potential for combating life threatening diseases. Furthermore, lysozyme has also been used to improve gut health in livestock, thereby improving overall health of animals. Industrial production of lysozyme using microbial sources involving genetic engineering has modified the synthesis method, making it more stable, economical, and improved functionalities. Advanced purification techniques, involving precipitation, ion-exchange chromatography, and ultrafiltration yield meticulously refined product with high purity, and enzyme activity. Moreover, the advancement of lysozyme-based nanomaterial has enhanced antimicrobial effectiveness and facilitated targeted delivery in biomedical applications. This review article provides insights onto the lysozyme's sources, production, purification, and extensive applications, to encourage further research into its untapped potential.

Keywords: Lysozyme, Antimicrobial, Food preservation, Anti-cancer, Genetic engineering, Animal feed, Nanotechnology

INTRODUCTION

Lysozyme (E.C.3.2.17) is a naturally occurring enzyme with strong antimicrobial properties. It is found in tissues and secretions of humans, various animals, plants and bacteria and can be isolated from these different sources with high purity levels. Lysozyme was first noticed by Scottish scientist Alexander Fleming in 1922 while he was investigating nasal mucus (Zhao *et al.*, 2021).

Lysozyme is made up of a single polypeptide chain that creates a compact, globular structure. It consists of 129 amino acids, with a molecular weight of 14.4 kDa and a pH level of 10.9. Lysozyme is mainly found in egg white makes up 3.4% of the total protein.

The structure of lysozyme has two main parts: the N-domain, which is made of parallel β-sheets, and the C-domain, which contains four α-helices. There is a helix-loop-helix region between these two domains, located near the top of the enzyme's active site. Its active site is specifically designed to interact with glycosidic bonds found in peptidoglycan (Yao *et al.*, 2022). The secondary structure of is made up of 39% α-helix and 11% β-sheet and contains eight cysteine residues, which form bonds with each other. These bonds create a stable and compact tertiary structure (Naveed *et al.*, 2022). Due to the stable properties, it maintain its structure and activities under a wide range of environmental conditions i.e. temperature, pH etc (Liu *et al.*, 2020).

Nowadays, lysozyme has been utilized for number of applications in food, immunity, and other fields. Lysozyme plays a crucial role in immune responses by interacting with cells like macrophages and neutrophils, which helps enhance the clearance of pathogens (Ferraboschi *et al.*, 2021). This enzyme is found in human secretions and egg whites, serving as a primary defense mechanism (Chang *et al.*, 2021). Additionally, it is used in food preservation and therapeutic applications to combat bacterial infections.

SOURCES OF LYSOZYME

Lysozyme is a versatile enzyme that shows an extensive distribution across multitude of biological entities. It is found within the mucosal secretions and tissues of diverse animal species, including humans, as it serves an essential role in innate immunity by offering protection against pathogenic organisms such as fungi, viruses and bacteria (Nawaz *et al.*, 2022). The albumin of chicken eggs possesses a significant amount of lysozyme enzyme. Moreover, lysozyme is also present in the secretions of invertebrates, vertebrates, as well as in some plants. Additionally, it has been discovered that several viruses and bacteria produce lysozymes, which may be important for their interactions with host species (Wang *et al.*, 2023).

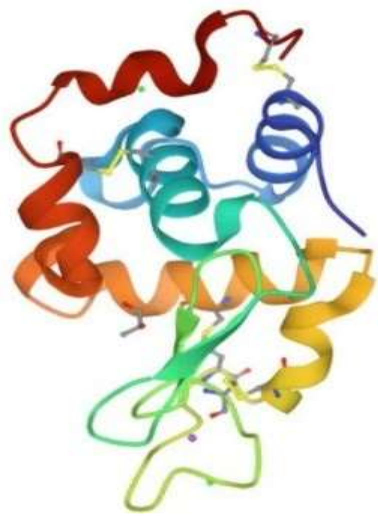

Fig. 1 Tertiary (3D) structure of hen egg white lysozyme (Bergamo and Sava, 2024).

Table 1. Shows the sources of Lysozyme enzyme

Sources	Organisms	References
Plants	*Hordeum vulgare*, *Triticum aestivum*, *Oryza sativa*	(Tanaka *et al.*, 2021)
Animals	*Gallus gallus domesticus*, *Homo sapiens* (tears, saliva, mucosal secretions, blood), *Bos taurus* (Cow milk),	(Pilevar *et al.*, 2022)
Microbes	*Bacillus subtilis*, *Bacillus licheniformis*, *Streptomyces coelicolor*, *Streptomyces griseus*, *Aspergillus* species	(Naveed *et al.*, 2022)
Marine Organisms	*Oncorhynchus mykiss*, *Salmo salar*	(Bastamy *et al.*, 2024)

PRODUCTION OF LYSOZYME

Lysozymes are frequently produced by using a range of microbial systems such as bacteria, fungi, and yeast. The predominant methodologies employed for the production of lysozyme encompass Solid-State Fermentation (SSF), Submerged Fermentation (SMF), and genetic engineering strategies, each presenting unique benefits concerning yield, economic viability, and scalability (Ferraboschi *et al.*, 2021).

Submerged Fermentation (SMF)/ Solid-State Fermentation (SSF) Method

Submerged Fermentation (SmF) and Solid-State Fermentation (SSF) are commonly employed techniques to cultivate such microbial strains that produces lysozyme enzyme naturally. SmF utilizes a liquid medium fortified with essential nutrients, alongside various minerals, conducted under meticulously controlled parameters of temperature, pH (7.0–8.0), and aeration, thereby resulting it particularly suitable for the production of lysozyme from bacterial and yeast sources (Gillani *et al.*, 2023). Conversely, SSF utilizes solid substrates, including wheat bran or rice husk, with moisture content to support microbial growth within trays or packed-bed bioreactors at optimized temperatures and a pH of 6.5 to 7.5, which is particularly advantageous for the growth of filamentous fungi (Vachher *et al.*, 2021).

Table 2. Shows the microbial sources, substrate, fermentation type, and parameters for lysozyme production

Microbial Source	Mode of Production	Substrates Used	Fermentation Parameters	References
Bacillus subtilis	Submerged Fermentation	Glucose, Yeast Extract	37°C, pH: 7.0–7.5, Aerobic	(Kim *et al.*, 2022)
Escherichia coli	Submerged Fermentation	Lactose, Peptone	37°C, pH: 7.0, Aerobic	(Roy *et al.*, 2024)
Saccharomyces cerevisiae	Submerged Fermentation	Glucose, Corn Steep Liquor	30°C, pH: 6.5–7.0, Aerobic	(Zhang *et al.*, 2020)
Aspergillus niger	Solid-State Fermentation	Wheat Bran, Rice Husk	30°C, pH: 6.5, Aerobic	(Yang *et al.*, 2021)
Streptomyces griseus	Submerged Fermentation	Starch, Soybean Meal	30°C, pH: 7.0–8.0, Aerobic	(Oh *et al.*, 2018)

Genetic Engineering method for lysozyme production

Genetic engineering has transformed lysozyme production by carrying out specific alterations for industrial and healthcare applications, helping to improve enzyme yield, stability, and activity (Kumar and Kutner, 2024). Recent breakthroughs in genetic engineering are centered on various kinds of approaches (Leśnierowski and Yang, 2021) to increase lysozyme production, which are discussed as follows:

Recombinant DNA technology

Through the use of recombinant DNA technology, genes that control the production of lysozyme may get integrated into the genome of *E. coli* or *Saccharomyces cerevisiae*, which will subsequently be cultivated under carefully monitored conditions to facilitate the expression of the lysozyme gene and the release of the enzyme into the medium (Maneira *et al.*, 2025). Moreover, expression vectors that contain potent promoters, for instance T7 and AOX1, contribute to substantial production of the enzyme.

Protein engineering for improved Stability and activity

One kind of genetic engineering approach is protein engineering that improves the stability of lysozyme via site-directed mutagenesis that alters crucial residues as well as introduces disulfide bonds for stability in structure. In addition, directed evolution produces variations with enhanced antibacterial activity that are resistant to heat and pH. Likewise, self-cleaving tags like intein do not require chemical cleavage, whereas fusion proteins like MBP, GST, and His-tag improve solubility and streamline purification (Ju *et al.*, 2025).

CRISPR-Cas9

CRISPR-Cas9 has also been employed to maximize the enzyme production by modifying regulatory elements, strengthening the metabolism of host, and increasing the secretion efficiency. Through the use of CRISPR interference (CRISPRi), strong promoter insertion, or repressor removal, scientists can manipulate gene expression to increase yield (D. G. Kim *et al.*, 2025). In microbial hosts, targeted protease deletions and changes in energy metabolism, reduces protein breakdown as well as metabolic stress while enhancing overall synthesis. In mammalian and plant-based systems, glycosylation modification using CRISPR-Cas9 enables accurate manipulation of glycan structures, thus enhancing lysozyme stability and biological activity for medicinal and food applications (Ju *et al.*, 2025).

Other modification techniques

Several other modification strategies have been used to increase the functional capabilities of lysozyme, such as adding lipophilic moieties, such as fatty acids, to lysozyme molecules to improve their bactericidal activity. For instance, Lysozyme treated with palmitic acid has demonstrated enhanced antibacterial action against *Edwardsiella tarda* and *E. coli*.

On the other side, lysozyme attaching with polymeric substances like dextran or chitosan may improve its stability, solubility, and antimicrobial effectiveness (Jia *et al.*, 2021). These changes have proven successful in combating infections like *E. coli* and *Staphylococcus aureus*. Furthermore, in medicinal applications, PEGylation—the attachment of polyethylene glycol (PEG) chains decreases immunogenicity and prolongs the half-life of lysozyme.

PURIFICATION TECHNIQUES

Lysozyme purification usually implies a number of steps in order to filter out the highly active and pure enzyme. The most frequently used approaches involve ammonium sulfate precipitation, ion-exchange chromatography, affinity chromatography, and gel filtration. The enzyme is first concentrated using ammonium sulfate precipitation after the crude extract has been clarified to eliminate particulate matter (Carrillo and Ramos, 2018). Proteins are separated by charge by employing ion-exchange chromatography, though affinity chromatography makes use of the unique binding interactions that lysozyme has with immobilised ligands (Anastas *et al.*, 2021). The enzyme is further refined by gel filtration, which differentiates proteins according to their molecular size. The usefulness of each technique is assessed by examining the activity of the enzyme, recovery yield, and protein concentration.

Table 3. Shows the Purification methods for different sources

Source	Chromatographic Technique	Precipitation Method	Ultrafiltration	References
Anas platyrhynchos domesticus	None	Ammonium Sulfate	Yes	(Huong *et al.*, 2020)
Gallus gallus domesticus	Ion-Exchange Chromatography	None	No	(Carrillo and Ramos, 2018)
Bacillus subtilis BSN314	Affinity Chromatography	Ammonium Sulfate	Yes	(Li *et al.*, 2022)
Pichia pastoris Strain NCY-2	Gel Filtration Chromatography	None	No	(Jia *et al.*, 2024)
Urechis unicinctus	Ion-Exchange Chromatography	None	Yes	(He *et al.*, 2022)
Escherichia coli	Affinity Chromatography	Ammonium Sulfate	No	(Tran *et al.*, 2024)
Aspergillus oryzae	Ion-Exchange Chromatography	None	Yes	(Chen *et al.*, 2022)

Table 4. Summarizing the purification process of lysozyme, including enzyme activity, recovery yield, purification fold, protein concentration, and specific activity:

Purification Step	Enzyme Activity (U)	Recovery Yield (%)	Purification Fold	Protein (mg/mL)	Specific Activity (U/mg)	References
Crude Extract	3000	100	1	30	100	(He *et al.*, 2022)
Ammonium Sulfate Precipitation	2400	80	3	10	240	(Liu *et al.*, 2020)
Ion-Exchange Chromatography	1440	48	14.5	1	1440	(Naveed *et al.*, 2022)
Gel Filtration	1000	33	40	0.5	2000	(Yao *et al.*, 2022)

LYSOZYME'S MECHANISM OF ACTION

Lysozyme has been studied a lot for its main role in enzymatic activity, but recent researches shows that it also involves in non-enzymatic activities like helping the immune system, fighting viruses, and defending against harmful microbes (Tanaka *et al.*, 2021).

Hydrolysis of peptidoglycan

Lysozyme breaks down the cell walls of bacteria by breaking the bonds between two important sugars, N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG), which are found in a substance called peptidoglycan. Peptidoglycan gives strength to bacterial cell walls. When lysozyme breaks these bonds, it weakens the cell wall, causing the bacteria to burst and die due to osmotic pressure (Tanaka *et al.*, 2021). This process works well against Gram-positive bacteria which have thicker peptidoglycan layers than Gram-negative bacteria.

Lipid bilayer interaction

Lysozyme can also break down microbial membranes by directly interacting with lipid bilayers. This is particularly important for Gram-negative bacteria, which are less affected by lysozyme's enzymatic activity because of their outer membrane. When lysozyme is partially unfolded or denatured, it can bind to bacterial membranes, causing damage and eventually breaking the membrane apart (Nawaz *et al.*, 2022). For example heat-denatured lysozyme can stick to membranes better and kill both Gram-positive and Gram-negative bacteria under certain pH conditions.

Controlling the immune system and reducing inflammation

Lysozyme has an important role in activating the immune response as well. Lysozyme breaks down the tough walls of bacterial cells. This process releases important parts, like peptidoglycan, that activate the immune system's pattern recognition receptors in cells. This activation triggers a strong immune response, which helps the body get rid of harmful bacteria and restore health (Du *et al.*, 2023).

Lysozyme has an interesting role in reducing inflammation. In the body's mucosal areas, it helps clear away dead cells and debris, which helps prevent too much inflammation. This shows how important lysozyme is for balancing immune responses and keeping tissues healthy during infections (Khorshidian *et al.*, 2022).

Antiviral activity

Recent studies showed that lysozyme can fight viruses apart from its enzymatic role. It can bind to viral genetic material, changing its structure and stops viruses from making new copies of themselves and from producing essential proteins. Therefore, lysozyme could be a useful option for antiviral treatments (Bergamo & Sava, 2024). Importantly, both whole lysozyme and heat-degraded lysozyme have these antiviral effects. This suggests that its cationic nature is responsible for these activities.

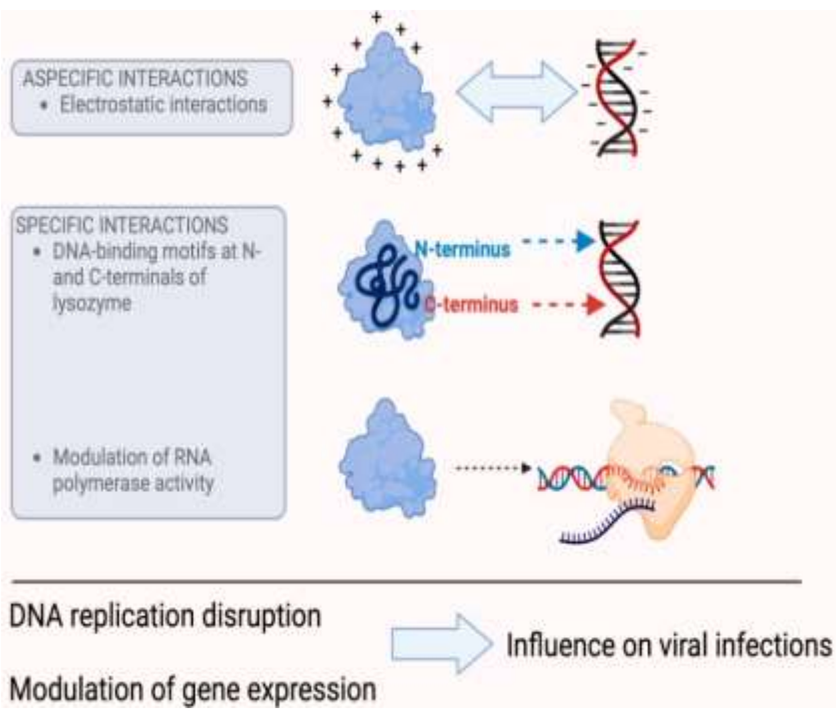

Fig. 2 Presumed mechanisms of the antiviral effect of lysozyme (Bergamo and Sava, 2024).

APPLICATIONS OF LYSOZYME

Food industry

Lysozyme offers a wide range of applications in the food industry as an antimicrobial agent, enhancing the quality and shelf life of the food product. Microbial activities cause food spoilage, thus reducing the quality and often induce foodborne illness. It has been used in the making process of different products such as cheese, beer, wine, etc to control spoilage bacteria (Iram *et al.*, 2023). It does not impact the fermenting activity of yeast and, therefore has potential use in fermented beverages for its antimicrobial properties, stabilizing the product for a long time (Zhang *et al.*, 2021). The first industrial use of lysozyme was in hard cheese making.

Furthermore, lysozyme is also a promising agent for preserving other food items like meat, seafood, processed food, fruits, vegetables, and bakery items. This contains high water content, proteins, oil and fats, favourable for microbial growth. Lysozyme plays a vital role in inhibiting microbial and various enzymatic activity that cause spoilage and protein degradation (Chen *et al.*, 2022). As a result of these applications, it has been utilized in antimicrobial packaging solutions.

Active packaging of food requires the components that stabilize the food quality and extend the shelf life without treating it with heat or radiation. To protect the product from contamination and microbial growth, antimicrobials are integrated into packing material. So being the active component of the packaging material, lysozyme would protect the food product from spoilage by inhibiting the microbial activities (Khorshidian *et al.*, 2022; Zhang *et al.*, 2021).

Medical and Pharmaceutical Industry

As anti-cancer agent

Various studies have shown the anti-tumor properties of lysozyme. It inhibits the proliferation of tumor cells by inducing immunological factors such as interleukin, interferon, lymphocytes etc. activating the host immunity against tumor (Jiang *et al.*, 2021). Hence, it has been used as an anti-tumor agent in clinical trials to devise novel cancer treatment. Moreover it is remarkable to be utilized as marker for cancer diagnosis.

As antimicrobial and antiviral agent

Lysozyme also has significant role in treating bacterial and viral diseases. Antiviral properties include the interaction with viral proteins, inhibiting pro-inflammatory cytokines, antioxidant activity while antibacterial nature destroys the structure of pathogenic bacteria involved in oral and respiratory infections (Beaussart *et al.*, 2021). Hence it has the potential to be used in antiviral and antibacterial drugs for the treatment of various infections.

Among different viruses, lysozyme is proven to be effective against COVID-19 in recent studies. The antimicrobial peptides interfere with viral entry by deactivating the virus envelops. Various studies have showed the promising antiviral nature of lysozyme is effective to combat pandemic causing viruses (Mann and Ndung'u, 2020).

Livestock sector

The gut health of animals determines their overall performance such as meat quality, food digestibility, growth, and milk or egg production. Antibiotics have been used as growth promoters in animal feed but are still not considered as reliable tool due to antibiotic resistance. Studies have shown that lysozyme-supplemented feed has improved the growth and is beneficial for gut microbiome (Vachher *et al.*, 2021). Although the detailed mechanism is still unknown, lysozyme can replace antibiotics in animal feed to avoid antibiotic resistance development.

Nanotechnology advancements in lysozyme applications

Recent advancements have used lysozyme in different nanostructured systems, broadening its uses in medicine, diagnostics, and many more. Recent studies showed that lysozyme conjugated with nanoparticles like Zinc Oxide (ZnO) and Graphene Oxide (GO) has shown better ability to fight bacteria. These nano-conjugates not only make lysozyme more stable but also stop bacterial growth more effectively than lysozyme alone (Yuan *et al.*, 2021). Scientists have created hydrogels and micro-carriers that use lysozyme to release antibiotics over a long time. These systems work well for healing wounds and fighting infections caused by bacteria that are resistant to drugs. Moreover lysozyme integrated bimetallic nanocomposites have been developed for cancer diagnosis and treatment. These composites like silver-gold and silver-platinum nanoparticles have anticancer properties and can be used for live-cell imaging and detecting hydrogen peroxide (Upadhyaya *et al.*, 2025). They are biocompatible and have multiple functions, making them valuable tools for cancer treatment.

Lysozyme-based nano-formulations can replace antibiotics in animal feed. When combined with safe polymers, these formulations are more effective at fighting germs and can help reduce antibiotic resistance in livestock and poultry (Aratboni *et al.*, 2024).

Lysozyme nanoparticle-encapsulated gold nano-clusters have been developed for ratio-metric fluorescent pH sensing. These nanoparticles display two emission bands allowing accurate pH assessment in enzymatic processes and living cells. Their stability and sensitivity make them valuable tools for diagnostic applications. A study showed that Molecularly Imprinted Polymers (MIPs) using lysozyme have been developed to recognize and separate lysozyme from complex biological samples selectively (Elshemey *et al.*, 2025). These imprinted nanoparticles like those made from titanium dioxide have a high capacity for adsorption and selectivity which makes them useful for diagnostic and analytical purposes such as protein analysis.

Moreover, a new hydrogel that holds lysozyme nanozymes has been created to help monitor wound infections (Upadhyaya *et al.*, 2025). This system fights bacteria and detects signs of infection using color changes, allowing real-time tracking and therapeutic capabilities in wound care.

CONCLUSION

The exceptional antimicrobial characteristics of lysozyme, alongside its extensive range of applications, make it an invaluable enzyme across various sectors. From its application in food preservation to its potential to fight drug-resistant infections and improve the health of livestock, lysozyme provides a sustainable and environmentally friendly substitute for traditional techniques. The synthesis of this enzyme has been transformed by its manufacturing using sophisticated microbial systems, which guarantee increased scalability and efficiency. As research on lysozyme keeps growing, its significance across industrial and therapeutic sectors will continue to expand, that contributes immensely to global health and sustainability initiatives.

FUTURE PERSPECTIVES

Expanding the therapeutic uses of lysozyme is a key target for future research, especially in the fight against diseases resistant to antibiotics. Continuous research in fermentation technology and genetic engineering hopes to improve its production, increasing its effectiveness and economy. There is also rising interest in lysozyme's potential applications in agriculture, including enhancing plant protection and animal health. Additionally, improvements in drug delivery methods may increase its efficacy in both clinical and agricultural fields.

Conflict of interest:

The authors declare no conflict of interest.

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