Volume 3 Issue 4
www.westlandpublishers.com

ISSN: 3066-3822

Digital Journal of Science (DJS)

Review Article

Earthworm Immune Molecules as Novel Therapeutics

John Warren Reynolds[1,2*] and Wilma M Reynolds[1]

[1]Oligochaetology Laboratory, 9-1250 Weber Street East, Kitchener, N2A 4E1, Ontario Canada

[2]Research Associate, New Brunswick Museum, 277 Douglas Avenue, Saint John, E2K 1E5, New Brunswick, Canada

***Corresponding author:** John Warren Reynolds, Oligochaetology Laboratory, 9-1250 Weber Street East, Kitchener, N2A 4E1, Ontario, Canada.

Received Date: 04 June, 2026 ; **Published Date:** 17 July, 2026

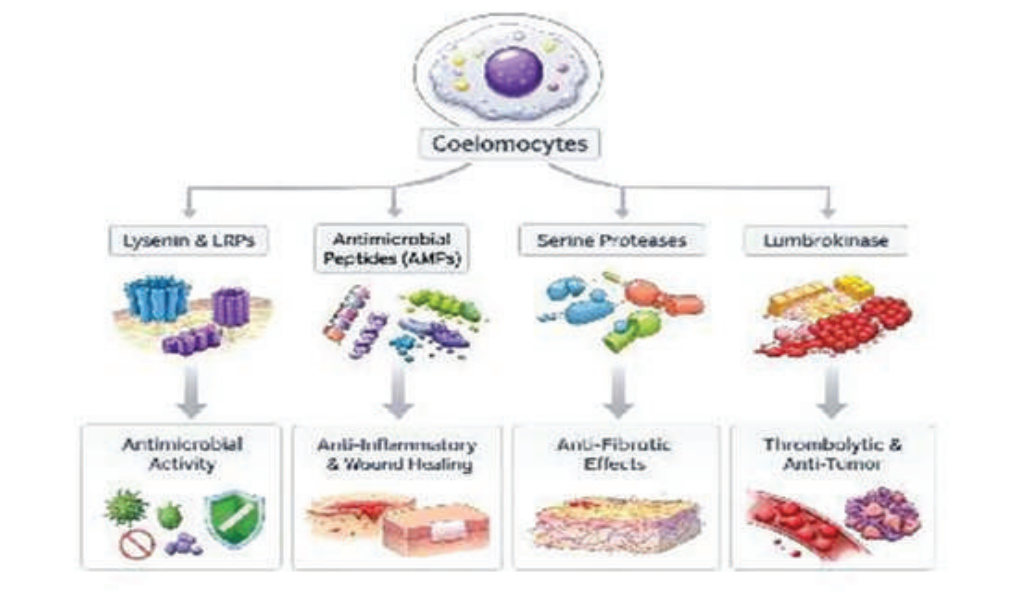
Keywords:
Earthworm Immunity; Innate Immunity; Coelomocytes; Lysenin; Lysenin-Related Proteins; Fetidin; Eiseniapore; Antimicrobial Peptides.

Citation:
Reynolds JW* and Reynolds WM (2026) Earthworm Immune Molecules as Novel Therapeutics. Digital J Sci 3(4): 165.

DOI: 10.63592/DJS/165

Graphical Abstract

Schematic representation of the major earthworm immune molecules and their therapeutic relevance. Coelomocytes serve as the central immune hub, producing four principal classes of effector molecules: lysenin and Lysenin-Related Proteins (LRPs), Antimicrobial Peptides (AMPs), serine proteases, and lumbrokinase. These molecules function synergistically to mediate antimicrobial defence, immune regulation, inflammation control, extracellular matrix remodelling, and fibrinolysis. Their diverse biological activities underpin emerging biomedical applications, including antimicrobial, anti-inflammatory, wound-healing, anti-fibrotic, thrombolytic, and anticancer therapies.



Abstract

Earthworms possess a highly developed innate immune system that produces a diverse repertoire of bioactive molecules with potent antimicrobial, cytolytic, fibrinolytic, and immunomodulatory activities. These immune effectors including pore-forming proteins such as lysenin, fetidin, and eiseniapore; Antimicrobial Peptides (AMPs); serine proteases; and fibrinolytic enzymes such as lumbrokinase—enable rapid recognition and elimination of bacteria, fungi, viruses, and parasites while simultaneously regulating inflammation, tissue repair, and physiological homeostasis. Increasing biochemical and pharmacological evidence demonstrates that these molecules possess broad therapeutic potential, including antibacterial, antifungal, antiviral, anti-inflammatory, wound-healing, anti-fibrotic, thrombolytic, and anticancer activities. Among these molecules, lumbrokinase has advanced furthest toward clinical application and is currently used in parts of Asia for the management of thrombotic disorders, with growing evidence supporting additional anti-inflammatory and anti-fibrotic effects. Despite these promising findings, many earthworm immune molecules remain incompletely characterized, and significant challenges related to molecular specificity, cytotoxicity, stability, targeted delivery, large-scale production, and regulatory approval continue to impede clinical translation. This review summarizes the current understanding of earthworm immune molecules, their mechanisms of action, and their emerging biomedical applications while highlighting the key scientific and translational challenges that must be addressed to facilitate the development of these evolutionarily unique molecules as next-generation therapeutic agents.

Introduction

Earthworms possess one of the most effective innate immune systems among soil invertebrates, having evolved sophisticated defence mechanisms to survive in environments rich in microorganisms, parasites, and other environmental challenges. Although they lack an adaptive immune system, earthworms rely on a coordinated network of cellular and humoral immune responses mediated by coelomocytes, pattern-recognition receptors, and a diverse repertoire of bioactive molecules. These immune components enable rapid recognition and elimination of invading pathogens while maintaining tissue integrity and physiological homeostasis [1, 2, 3]. Among the best-characterized earthworm immune molecules are pore-forming proteins, including lysenin and its related proteins, Antimicrobial Peptides (AMPs), serine proteases, and fibrinolytic enzymes such as lumbrokinase. Collectively, these molecules contribute not only to antimicrobial defence but also to inflammation control, extracellular matrix remodelling, tissue repair, and immune regulation. Their unique mechanisms of action and broad biological activities have attracted considerable attention as potential sources of novel therapeutic agents. In recent years, increasing experimental evidence has demonstrated that earthworm-derived immune molecules exhibit antibacterial, antifungal, antiviral, anti-inflammatory, wound-healing, anti-fibrotic, thrombolytic, and anticancer properties. Among these, lumbrokinase has progressed furthest toward clinical application, whereas other immune effectors continue to show promising results in preclinical investigations. These findings highlight the growing biomedical significance of earthworm immune molecules and their potential contribution to the development of innovative therapeutics. Despite these advances, many earthworm immune molecules remain only partially characterized, and important questions regarding their molecular mechanisms, therapeutic efficacy, safety, and clinical translation remain unresolved. This review therefore provides a comprehensive overview of the major classes of earthworm immune molecules, summarizes their mechanisms of action and biological functions, evaluates their emerging biomedical applications, and discusses the key challenges and future research directions required to facilitate their development as next-generation therapeutic agents.

Discussion

Earthworm immune molecules represent an evolutionarily conserved defence system with remarkable structural and functional diversity. Produced primarily by coelomocytes, these molecules function cooperatively to recognize pathogens, eliminate invading microorganisms, regulate inflammatory responses, promote tissue repair, and maintain physiological homeostasis. Their multifunctional nature has generated increasing interest not only in understanding innate immunity but also in exploring their potential as novel therapeutic agents. Although substantial progress has been made in identifying and characterizing several major classes of earthworm immune molecules, many aspects of their biological activity remain incompletely understood. Important challenges including incomplete mechanistic characterization, limited structural information, cytotoxicity, molecular stability, targeted delivery, large-scale production, and

regulatory considerations continue to restrict their clinical translation. Consequently, a comprehensive evaluation of current evidence is essential to consolidate existing knowledge, identify promising therapeutic applications, and define priorities for future research. The following sections examine the major components of the earthworm innate immune system and review the principal classes of earthworm immune molecules, their mechanisms of action, biological functions, and emerging biomedical applications. Particular emphasis is placed on their roles in antimicrobial defence, inflammation regulation, wound healing, fibrinolysis, tissue regeneration, and cancer therapy, together with the opportunities and challenges associated with their development as next-generation therapeutics.

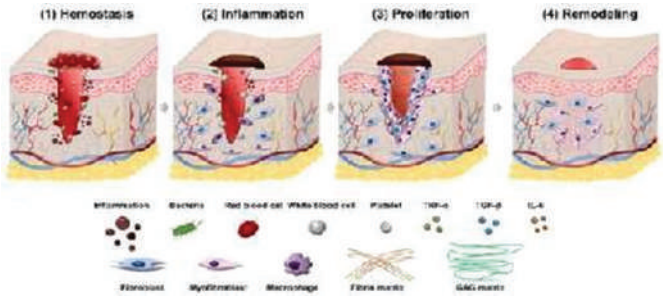


Figure 1: Illustration of four phases in the wound healing process [4].

Earthworm Innate Immune System

Earthworms possess a highly evolved innate immune system that enables them to survive in microbially rich soil environments. Although they lack adaptive immunity, they compensate through an efficient network of cellular and humoral defence mechanisms that provide rapid protection against bacteria, fungi, viruses, and parasites [1, 2]. This defence system is coordinated primarily by coelomocytes, which recognize invading pathogens, initiate immune signalling, and release a diverse range of bioactive molecules with antimicrobial, cytolytic, and immunomodulatory activities. Coelomocytes are the principal immune cells of annelids and function similarly to vertebrate macrophages, neutrophils, and natural killer-like cells (Figure 2). Two major populations are recognized: amoebocytes, which are responsible for phagocytosis, cytotoxicity, and pathogen encapsulation, and eleocytes (chloragocytes), which synthesize and secrete antimicrobial peptides, lectins, and other immune-related molecules. These cells circulate within the coelomic fluid and rapidly migrate to sites of injury or infection, where they eliminate pathogens through phagocytosis, encapsulation, Reactive Oxygen Species (ROS) production, and secretion of immune effectors such as lysenin, fetidin, Antimicrobial Peptides (AMPs), and serine proteases [1, 3].

Immune activation begins with the recognition of Pathogen-Associated Molecular Patterns (PAMPs) by Pattern-Recognition Receptors (PRRs). Earthworms possess lectins, Toll-like receptor-like signalling pathways, and the coelomic cytolytic factor (CCF), which function in a manner analogous to components of the vertebrate complement system [2, 3]. Activation of these receptors stimulates coelomocyte proliferation, secretion of cytolytic proteins, production of antimicrobial peptides, and activation of the phenoloxidase cascade, thereby establishing a rapid and coordinated defence response. In addition to cellular immunity, earthworms produce a diverse array of soluble immune molecules with important biological and therapeutic functions. These include pore-forming proteins such as lysenin and fetidin/eiseniapore, antimicrobial peptides including lumbricin, serine proteases involved in immune regulation and extracellular matrix remodelling, fibrinolytic enzymes such as lumbrokinase, phenoloxidase enzymes that participate in melanization, and antioxidants that protect host tissues from oxidative damage. Acting together, these molecules eliminate pathogens while maintaining tissue homeostasis and promoting recovery following infection.

The cellular immune response is characterized by several highly conserved defence mechanisms. Amoebocytes engulf bacteria, fungi, and other foreign particles through phagocytosis, whereas larger pathogens are immobilized by encapsulation and nodulation through the aggregation of multiple coelomocytes. Activated coelomocytes also release pore-forming proteins that directly lyse susceptible target cells, while oxidative burst responses generate reactive oxygen species that contribute to microbial killing and immune signalling. Concurrently, activation of the phenoloxidase system results in melanin deposition around invading organisms, cross-linking of extracellular proteins, and formation of physical barriers that limit pathogen dissemination and facilitate wound sealing. A defining characteristic of earthworm immunity is the close integration of cellular and humoral responses. Coelomocytes not only eliminate pathogens directly but also release antimicrobial peptides, cytolysins, proteases, and signalling molecules that amplify immune activity. Proteases activate downstream immune pathways and participate in tissue remodelling, whereas phenoloxidase products encapsulate invading organisms and antioxidants minimize collateral oxidative damage. This coordinated defence network provides effective protection against

diverse pathogens and forms the biological foundation for the therapeutic applications of earthworm-derived immune molecules discussed in the following sections.

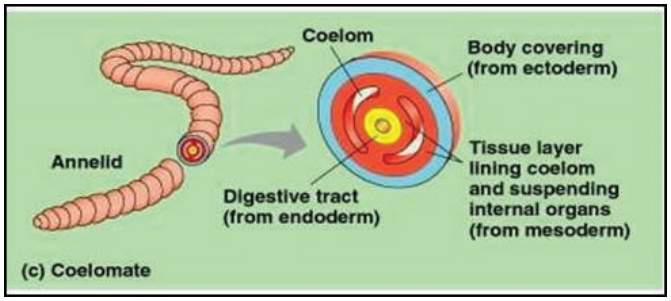


Figure 2: Coelomocytes' location in earthworms.

Major Earthworm Immune Molecules

Earthworm immunity relies on a diverse repertoire of bioactive molecules that function cooperatively to eliminate pathogens and maintain tissue homeostasis. Among these, pore-forming proteins, antimicrobial peptides, serine proteases, and fibrinolytic enzymes are the most extensively investigated because of their unique biological activities and increasing biomedical relevance. Although these molecules differ in structure and mechanism of action, they collectively contribute to antimicrobial defence, immune regulation, tissue repair, and extracellular matrix remodelling, making them attractive candidates for therapeutic development.

Lysenin and Lysenin-Related Proteins (LRPs)

Lysenin and its related proteins (LRPs) represent one of the best-characterized families of immune effector molecules in earthworms. First isolated from the coelomocytes of *Eisenia fetida*, lysenin is a sphingomyelin-specific pore-forming protein that plays a central role in innate immunity by inducing selective cytolysis of susceptible target cells [5, 6]. Members of the lysenin family, including LRP-1, LRP-2 (fetidin), and LRP-3, share conserved structural domains responsible for lipid recognition and pore formation and are rapidly mobilized during microbial challenge. Lysenin is a secreted protein of approximately 33 kDa that accumulates within coelomocyte granules before being released following immune stimulation. Its remarkable specificity for sphingomyelin distinguishes it from many other pore-forming toxins. After binding to sphingomyelin-rich membranes, lysenin undergoes oligomerization into a prepore complex, followed by conformational rearrangement and insertion into the membrane to generate pores approximately 3 nm in diameter [6, 7]. This highly regulated mechanism provides an excellent experimental model for investigating membrane organization, lipid-protein interactions, and targeted cytolysis. Within the earthworm immune system, lysenin and related proteins contribute to host defence through several complementary mechanisms. They directly lyse susceptible cells, agglutinate microorganisms, interact synergistically with oxidative and phenoloxidase pathways, and are rapidly secreted by activated coelomocytes during infection [1, 2]. LRP-2 (fetidin) and LRP-3 further broaden the antimicrobial capacity of the lysenin family through additional hemolytic and bactericidal activities, highlighting the functional diversity of these immune effectors.

The unique biochemical characteristics of lysenin have attracted considerable attention for biomedical applications. Its highly selective membrane-binding properties suggest potential utility as a template for novel antimicrobial agents, anti-biofilm therapeutics, and adjuncts to conventional antibiotic therapy. Variations in sphingomyelin distribution among mammalian cells also raise the possibility of engineering lysenin derivatives capable of selectively targeting tumour cells or diseased tissues with altered membrane lipid composition. Furthermore, the predictable pore size and membrane specificity of lysenin have stimulated research into nanopore biosensors, membrane-targeted drug delivery systems, and controlled molecular transport technologies [7]. Despite these promising applications, native lysenin exhibits significant cytotoxicity toward sphingomyelin-rich mammalian cells, limiting its direct therapeutic use. Consequently, current research focuses on engineering safer variants through site-directed mutagenesis, ligand-directed targeting, conditional activation strategies, and nanocarrier-based delivery systems that improve specificity while minimizing off-target toxicity. These approaches parallel those used successfully for other pore-forming proteins and represent an important step toward the clinical translation of lysenin-based therapeutics.

Fetidin and Eiseniapore

Fetidin and eiseniapore are important members of the Lysenin-Related Protein (LRP) family and constitute major cytolytic effectors of the earthworm innate immune system. Although lysenin has received greater research attention, these proteins possess distinct biochemical characteristics that broaden the immune repertoire of *Eisenia fetida* and related species. Both molecules exhibit hemolytic, antimicrobial, and cytolytic activities and are rapidly secreted by coelomocytes in response to microbial invasion, highlighting their essential role in early host defence [5, 1]. Fetidin was initially identified as a hemolytic protein in earthworm coelomic fluid and later recognized

as LRP-2 within the lysenin protein family. Eiseniapore, another structurally related pore-forming protein, shares conserved lipid-binding and pore-forming domains but differs in membrane specificity and antimicrobial activity [6]. Unlike lysenin, whose activity depends primarily on sphingomyelin recognition, fetidin and eiseniapore appear capable of interacting with a broader range of membrane lipids, thereby extending their cytolytic activity to a wider spectrum of microbial and eukaryotic target cells. The antimicrobial activity of these proteins is mediated through membrane binding, oligomerization, pore formation, and rapid osmotic lysis of susceptible cells. Experimental studies have demonstrated activity against Gram-positive and Gram-negative bacteria, fungi, and several protozoan pathogens [1, 8]. Their broader lipid recognition profile complements the strict sphingomyelin specificity of lysenin, suggesting that members of the LRP family function cooperatively to maximize antimicrobial protection within the earthworm immune system. Beyond their natural biological role, fetidin and eiseniapore possess considerable translational potential. Their broad antimicrobial spectrum makes them attractive candidates for the development of novel antibacterial and antifungal agents, particularly against multidrug-resistant microorganisms and biofilm-associated infections. Their membrane-disrupting properties may also facilitate penetration of microbial biofilms, enhancing the effectiveness of combination antimicrobial therapies. In addition, evidence that LRPs influence immune signalling pathways raises the possibility of developing these proteins as immunomodulatory agents capable of regulating host immune responses. However, therapeutic application remains challenging because of their inherent cytolytic activity toward mammalian cells. Future development will depend on engineering strategies that improve specificity while minimizing toxicity. Site-directed mutagenesis, targeted delivery systems, nanoparticle encapsulation, and conditional activation mechanisms represent promising approaches for overcoming these limitations and expanding the biomedical applications of this unique class of pore-forming proteins.

Antimicrobial Peptides

Antimicrobial peptides (AMPs) constitute one of the most important humoral defence mechanisms in earthworms and represent a highly diverse class of naturally occurring bioactive molecules. Produced primarily by coelomocytes and epithelial tissues, these peptides are rapidly synthesized following microbial challenge and provide broad-spectrum protection against bacteria, fungi, and viruses [9, 8, 1]. Their remarkable structural diversity reflects the evolutionary adaptations required for survival within the highly competitive microbial environment of soil ecosystems. Several antimicrobial peptides have been identified in earthworms, particularly in *Eisenia fetida* and *Lumbricus terrestris*. Among the best characterized is Lumbricin I, a proline-rich peptide with potent antibacterial and antifungal activity [9]. Additional peptides include lumbricin-related variants, lysenin-derived peptide fragments, and coelomic cytolytic factors, which participate not only in antimicrobial defence but also in immune recognition and signalling. Despite differences in amino acid sequence and molecular structure, these peptides share common physicochemical features, including positive charge, amphipathicity, and strong affinity for microbial membranes. Earthworm AMPs eliminate pathogens through multiple complementary mechanisms. Many peptides disrupt microbial membranes by forming pores or destabilizing lipid bilayers, leading to rapid leakage of intracellular contents and cell death. Others penetrate microbial cells and inhibit essential intracellular processes such as nucleic acid synthesis, protein translation, or enzyme function. Beyond their direct antimicrobial activity, several AMPs also modulate immune responses by stimulating coelomocyte activation, promoting cytokine-like signalling, and facilitating wound healing and tissue regeneration [10, 8]. These multifunctional properties closely resemble those of vertebrate defensins and cathelicidins, emphasizing the evolutionary conservation of innate immune strategies.

Both purified AMPs and crude earthworm extracts have demonstrated broad antimicrobial activity against Gram-positive and Gram-negative bacteria, including antibiotic-resistant strains, as well as pathogenic fungi and certain viruses [11, 12]. These findings support their potential application as topical antimicrobial agents for wound infections, dermatological diseases, and biofilm-associated conditions. Their ability to reduce microbial burden while simultaneously promoting tissue repair further enhances their value as wound-healing therapeutics. Although systemic therapeutic applications remain largely experimental, advances in peptide engineering offer promising opportunities for future clinical translation. Strategies such as peptide cyclization, incorporation of D-amino acids, PEGylation, and nanoparticle-based delivery systems have the potential to improve stability, reduce proteolytic degradation, and enhance tissue specificity. Continued optimization of these approaches may ultimately enable the use of earthworm-derived AMPs in the treatment of systemic bacterial, fungal, and viral infections [13, 14].

Serine Proteases

Serine proteases represent another important class of immune-associated molecules in earthworms, contributing to antimicrobial defence, extracellular matrix remodelling, inflammation regulation, and tissue repair. Although these enzymes have been studied less extensively than pore-forming proteins or antimicrobial peptides, available evidence indicates that they perform diverse biological functions that extend beyond simple proteolysis [15, 16]. Earthworm serine protease (ESP), first purified from *Eisenia fetida*, is a chymotrypsin-like enzyme with a molecular mass of approximately 28–30 kDa and exhibits high catalytic efficiency toward peptide substrates over a broad pH range [15]. Its biochemical stability and regulated activity suggest that it is well adapted to the dynamic physicochemical conditions of the coelomic cavity. Within the innate immune system, serine proteases participate in several complementary defence mechanisms. They contribute directly to pathogen elimination through degradation of microbial surface proteins while simultaneously activating downstream immune pathways, including phenoloxidase cascades and coelomocyte signalling networks [2]. In addition, these enzymes regulate inflammatory responses by processing cytokine-like molecules, removing damaged tissue components, and promoting extracellular matrix remodelling. Such functions parallel the roles of serine proteases in arthropods and vertebrates, highlighting the remarkable evolutionary conservation of protease-mediated immunity.

Beyond host defence, earthworm serine proteases have demonstrated anti-inflammatory and anti-tumour activities in experimental systems. Proposed mechanisms include degradation of pro-inflammatory mediators, regulation of extracellular matrix turnover, inhibition of tumour cell adhesion and migration, and synergistic interactions with antimicrobial peptides and pore-forming proteins [16]. These observations suggest that serine proteases may influence both immune regulation and tissue homeostasis while offering opportunities for therapeutic intervention. The therapeutic potential of these enzymes extends to several biomedical applications. Their ability to degrade extracellular matrix components makes them attractive candidates for anti-fibrotic therapies and scar reduction. Similarly, their capacity to remove necrotic tissue and stimulate cellular migration supports their potential use in wound healing and regenerative medicine. Interest has also emerged in exploiting serine proteases for modulation of tumour microenvironments and the development of engineered proteolytic drugs with improved substrate specificity. Despite these promising applications, several challenges remain before clinical translation can be achieved. These include preventing off-target proteolysis, maintaining enzymatic stability under physiological conditions, reducing immunogenicity, and developing efficient delivery systems. Future studies should therefore focus on detailed structural characterization, identification of additional proteases from diverse earthworm species, recombinant protein engineering, and advanced drug-delivery platforms capable of maximizing therapeutic efficacy while minimizing adverse effects.

Lumbrokinase

Lumbrokinase is the most clinically advanced earthworm-derived bioactive molecule and has attracted considerable attention because of its potent fibrinolytic and thrombolytic properties. Isolated primarily from *Lumbricus rubellus*, lumbrokinase comprises a group of serine proteases that selectively degrade fibrin and activate plasminogen, thereby promoting dissolution of blood clots while causing relatively limited systemic fibrinolysis [17, 18]. This high degree of fibrin specificity distinguishes lumbrokinase from conventional thrombolytic agents and contributes to its favourable safety profile. Biochemically, lumbrokinase consists of multiple isoenzymes with molecular weights ranging from approximately 20 to 35 kDa. These enzymes exhibit remarkable stability under physiological conditions and retain activity following oral administration, making them particularly attractive for therapeutic development. Their dual mechanism of action, involving both direct fibrin degradation and indirect activation of endogenous fibrinolytic pathways, enhances clot dissolution while reducing excessive degradation of circulating coagulation factors [19]. Within the earthworm, lumbrokinase is believed to contribute to extracellular matrix remodelling, tissue repair, and regulation of coelomic fluid homeostasis. Although its precise physiological function remains incompletely understood, these enzymes likely participate in maintaining tissue integrity following injury while preventing excessive fibrin accumulation. Their coordinated activity with other immune effectors highlights the multifunctional nature of earthworm innate immunity.

Clinically, lumbrokinase has been used in several Asian countries for the management of thrombotic disorders, including ischemic stroke, deep vein thrombosis, myocardial infarction, and peripheral vascular disease [17, 19]. Numerous experimental and clinical studies have demonstrated improvements in blood viscosity, microcirculation, and vascular perfusion, while adverse bleeding events appear less frequent than those associated with conventional thrombolytic

therapies. These observations have generated increasing international interest in the broader clinical application of lumbrokinase. Emerging evidence also suggests that the therapeutic potential of lumbrokinase extends beyond cardiovascular medicine. Experimental studies indicate anti-inflammatory activity through modulation of inflammatory mediators, promotion of wound healing by improving tissue perfusion, and possible anti-fibrotic effects resulting from degradation of excessive extracellular fibrin deposition [20]. Additional investigations have proposed roles in reducing tumour-associated fibrin matrices, thereby limiting cancer cell invasion and metastasis, although these applications remain largely preclinical. Despite encouraging clinical experience, several challenges continue to limit the widespread adoption of lumbrokinase. Natural preparations contain multiple enzyme isoforms whose composition varies according to species and extraction methods, complicating product standardization and regulatory approval. Furthermore, large-scale recombinant production, detailed pharmacokinetic characterization, and multicentre clinical trials outside Asia remain necessary before lumbrokinase can be fully integrated into global therapeutic practice. Continued advances in protein purification, recombinant expression systems, and pharmaceutical formulation are expected to facilitate its future development as a safe and effective fibrinolytic agent.

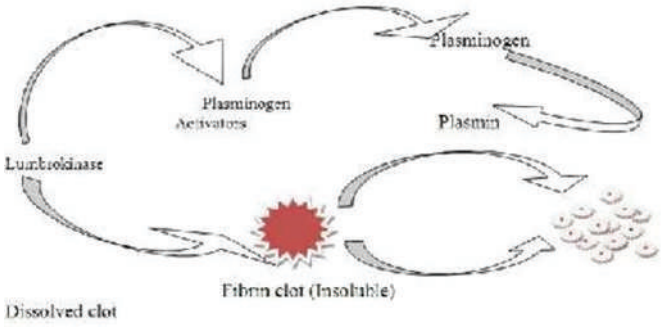


Figure 3: Diagrammatic view of Lumbrokinase Mediated Thrombolysis. Hence, Lumbrokinase is not only plasminogen activators, but also fibrin specific thrombolytic agent [20].

Bioactive Earthworm Extracts

Although considerable attention has focused on individual immune molecules, many therapeutic effects attributed to earthworms arise from complex mixtures of bioactive compounds present in coelomic fluid and tissue extracts. These preparations contain diverse combinations of pore-forming proteins, antimicrobial peptides, serine proteases, fibrinolytic enzymes, antioxidants, growth-promoting factors, and immunomodulatory molecules that function synergistically to promote host defence and tissue repair [11, 12]. Consequently, whole extracts often demonstrate broader biological activity than isolated components. Experimental studies have consistently reported antimicrobial effects against a wide range of bacterial and fungal pathogens, including antibiotic-resistant organisms. The combined activity of antimicrobial peptides, cytolytic proteins, and proteases contributes to disruption of microbial membranes, degradation of virulence factors, inhibition of biofilm formation, and enhancement of pathogen clearance. These observations support the potential application of earthworm-derived preparations as topical antimicrobial agents for infected wounds and chronic skin diseases. Beyond antimicrobial activity, earthworm extracts possess significant anti-inflammatory and regenerative properties. Experimental wound models have demonstrated accelerated tissue repair, increased fibroblast proliferation, enhanced collagen deposition, improved angiogenesis, and reduced inflammatory cell infiltration following treatment with earthworm extracts [12, 10]. These effects appear to result from the coordinated action of growth-promoting molecules, extracellular matrix-remodelling enzymes, and immunomodulatory peptides that collectively create a favourable environment for tissue regeneration.

Earthworm extracts have also demonstrated antioxidant activity by reducing oxidative stress associated with inflammation and tissue injury. Limiting reactive oxygen species not only protects healthy tissues from secondary damage but also facilitates more efficient wound healing and restoration of normal tissue architecture. These antioxidant effects complement the antimicrobial and anti-inflammatory properties of the extracts, further enhancing their therapeutic potential. In addition to regenerative medicine, preliminary investigations suggest possible applications in oncology and metabolic disorders. Certain extract components exhibit antiproliferative activity against tumour cells, whereas others influence immune regulation, extracellular matrix remodelling, and fibrin degradation. Although these findings remain largely experimental, they indicate that earthworm-derived preparations may provide multiple complementary mechanisms for managing complex diseases involving inflammation, fibrosis, impaired tissue repair, and abnormal cell proliferation. A major limitation of crude earthworm extracts is their inherent biological variability. The composition of bioactive molecules differs according to species, habitat, environmental conditions, and extraction methodology, making standardization difficult. Furthermore, undefined mixtures complicate mechanistic studies and regulatory approval. Future therapeutic development should therefore focus on identifying the

active components responsible for specific biological effects, establishing standardized manufacturing protocols, and developing recombinant or synthetic alternatives capable of reproducing the beneficial properties of natural extracts while ensuring consistent quality and safety.

Therapeutic Opportunities and Translational Challenges

Earthworm immune molecules constitute a diverse and largely untapped reservoir of bioactive compounds with considerable therapeutic potential. Collectively, pore-forming proteins, antimicrobial peptides, serine proteases, fibrinolytic enzymes, and complex coelomic extracts exhibit antimicrobial, anti-inflammatory, regenerative, thrombolytic, and immunomodulatory activities that address several major challenges in modern medicine. Unlike many conventional therapeutics, these molecules have evolved through millions of years of host–pathogen interactions, providing unique mechanisms of action that may complement or overcome limitations associated with existing drug classes [1, 2]. One of the most promising applications of earthworm immune molecules lies in the treatment of infectious diseases. The increasing prevalence of multidrug-resistant bacteria has created an urgent need for alternative antimicrobial agents with novel mechanisms of action. Pore-forming proteins, including lysenin and its related proteins, directly disrupt microbial membranes, whereas antimicrobial peptides destabilize bacterial and fungal cell envelopes and interfere with essential intracellular processes [9, 11]. Their rapid membrane-targeting activity reduces the likelihood of resistance development and makes them attractive candidates for topical wound therapies, chronic biofilm-associated infections, and combination treatments designed to enhance the efficacy of conventional antibiotics. In addition, preliminary studies suggest antiviral properties through inhibition of viral replication and modulation of innate immune responses, although these observations require further experimental validation.

The multifunctional nature of earthworm immune molecules also presents significant opportunities in regenerative medicine. Wound healing is a highly coordinated process involving microbial control, regulation of inflammation, extracellular matrix remodelling, angiogenesis, and tissue regeneration. Earthworm-derived extracts naturally integrate these biological activities through the combined action of antimicrobial peptides, serine proteases, growth-promoting factors, and anti-inflammatory molecules. Experimental studies have consistently demonstrated accelerated wound closure, enhanced fibroblast proliferation, increased collagen synthesis, improved vascularization, and reduced inflammatory responses following treatment with earthworm-derived preparations [12, 10]. These findings support their potential development as topical therapeutics for chronic wounds, diabetic ulcers, burn injuries, and post-surgical tissue repair. Cardiovascular medicine represents another area in which earthworm-derived therapeutics have already demonstrated clinical relevance. Lumbrokinase is currently used in several Asian countries for the management of thrombotic disorders because of its fibrin-specific thrombolytic activity and relatively favourable safety profile [17, 19]. Unlike conventional thrombolytic agents that often produce widespread fibrinolysis and increase bleeding risk, lumbrokinase preferentially targets fibrin-rich thrombi while preserving normal haemostatic function. Beyond thrombosis, accumulating evidence suggests possible applications in reducing tissue fibrosis, improving microvascular circulation, and promoting recovery following ischemic injury, although these indications require larger clinical studies. The unique biological properties of earthworm immune molecules have also generated interest in oncology. Tumour progression is closely associated with alterations in membrane lipid composition, chronic inflammation, extracellular matrix remodelling, and fibrin deposition, all of which represent potential therapeutic targets for earthworm-derived proteins. Lysenin and related pore-forming proteins may selectively recognize tumour cells with altered sphingomyelin distribution, while serine proteases and lumbrokinase may interfere with tumour invasion by degrading extracellular fibrin matrices and modifying the tumour microenvironment [6, 21]. Although these applications remain largely experimental, they highlight the versatility of earthworm immune molecules as potential components of future anticancer strategies.

Despite these encouraging findings, several challenges continue to limit clinical translation. Many native earthworm proteins exhibit cytotoxicity, limited stability under physiological conditions, or insufficient target specificity. In addition, natural extracts display considerable variability arising from differences in species, environmental conditions, extraction procedures, and protein composition, making standardization difficult. Regulatory approval also requires detailed characterization of molecular structure, pharmacokinetics, immunogenicity, manufacturing consistency, and long-term safety. Advances in protein engineering and pharmaceutical technology provide promising solutions to these limitations. Recombinant protein production can generate highly purified molecules with consistent biological activity while reducing dependence on natural harvesting. Site-directed

mutagenesis and rational protein engineering may improve target specificity and reduce off-target toxicity. Similarly, nanotechnology-based delivery systems, liposomes, polymeric nanoparticles, hydrogels, and stimulus-responsive formulations offer opportunities to enhance bioavailability, protect proteins from degradation, and enable localized drug release. Together, these approaches have the potential to transform naturally occurring earthworm immune molecules into safe, standardized, and clinically effective therapeutic agents. Overall, earthworm immune molecules represent an emerging platform for the development of next-generation biologics. Their broad spectrum of biological activities, combined with evolutionary distinct mechanisms of action, provides a strong foundation for therapeutic innovation across infectious diseases, regenerative medicine, cardiovascular disorders, chronic inflammatory conditions, and oncology. Continued interdisciplinary research integrating molecular biology, immunology, structural biology, protein engineering, and translational medicine will be essential to realize their full clinical potential.

Future Perspectives

Although substantial progress has been made in characterizing earthworm immune molecules, their translation into clinically useful therapeutics remains at an early stage. Future research should therefore focus on addressing the biological, technological, and regulatory challenges that currently limit their development. A comprehensive understanding of molecular structure, mechanisms of action, pharmacological behaviour, and long-term safety will be essential for transforming these naturally occurring molecules into reliable therapeutic agents. One important priority is the identification and characterization of additional immune molecules from the vast diversity of earthworm species. To date, most studies have concentrated on *Eisenia fetida* and *Lumbricus terrestris*, despite the existence of more than 7,000 known earthworm species occupying diverse ecological environments [22]. Comparative genomics, transcriptomics, proteomics, and metabolomics offer valuable opportunities to discover novel bioactive compounds with unique antimicrobial, immunomodulatory, or regenerative properties. Such studies will also improve understanding of the evolutionary diversification of innate immune systems across annelids. Detailed structural and mechanistic investigations remain equally important. High-resolution techniques such as cryo-electron microscopy, X-ray crystallography, and molecular dynamics simulations can provide insight into protein architecture, membrane recognition, and structure–function relationships. These data will facilitate rational protein engineering aimed at improving specificity, reducing cytotoxicity, and enhancing therapeutic efficacy. Protein engineering and recombinant biotechnology are expected to play central roles in future therapeutic development. Native earthworm proteins often exhibit limitations including instability, immunogenicity, and undesirable cytotoxicity. Recombinant expression systems, site-directed mutagenesis, domain engineering, and fusion-protein technologies may overcome these challenges by generating safer molecules with improved pharmacological properties and greater production consistency. Recombinant production would also reduce dependence on large-scale harvesting of earthworms, thereby addressing ethical and environmental concerns associated with natural extraction.

Advances in drug-delivery technologies will further support clinical translation. Nanoparticle-based carriers, liposomes, polymeric delivery systems, hydrogels, and microneedle platforms may improve bioavailability, protect proteins from enzymatic degradation, and enable targeted or controlled release. These approaches have the potential to increase therapeutic efficacy while minimizing systemic toxicity and off-target effects. Future investigations should also move beyond individual molecules to examine the coordinated interactions among multiple immune effectors. In vivo, pore-forming proteins, antimicrobial peptides, serine proteases, fibrinolytic enzymes, and other soluble factors function as integrated networks rather than isolated entities. Systems biology approaches capable of analysing these complex interactions may reveal synergistic mechanisms that cannot be appreciated through studies of single proteins alone. Such knowledge could facilitate the development of multi-component biologics inspired by the natural composition of earthworm coelomic fluid. Despite encouraging experimental findings, clinical evidence remains limited. Well-designed preclinical studies are required to establish pharmacokinetic profiles, tissue distribution, immunogenicity, toxicity, and long-term safety. Subsequently, multicentre randomized clinical trials will be necessary to evaluate efficacy across diverse patient populations and to support regulatory approval. Standardized manufacturing procedures, quality-control measures, and Good Manufacturing Practice (GMP) production will also be essential for ensuring reproducibility and clinical acceptance. Overall, continued collaboration among immunologists, structural biologists, pharmacologists, bioengineers, and clinicians will be critical for advancing earthworm immune molecules from experimental observations to practical biomedical applications. Such interdisciplinary efforts are likely to accelerate the development of innovative therapeutics capable of addressing several major global health challenges.

Conclusion

Earthworm immune molecules constitute one of the most diverse and underexplored sources of naturally occurring bioactive compounds with significant biomedical potential. Pore-forming proteins, antimicrobial peptides, serine proteases, fibrinolytic enzymes, and complex coelomic extracts collectively provide a sophisticated innate defence system that combines antimicrobial activity, immune regulation, tissue repair, extracellular matrix remodelling, and fibrinolysis. These complementary functions closely correspond to current therapeutic priorities, including the management of antimicrobial resistance, chronic inflammation, thrombosis, impaired wound healing, fibrosis, and cancer. A defining feature of earthworm immunity is the coordinated interaction among multiple immune effectors. Rather than acting independently, these molecules function synergistically to eliminate pathogens, regulate inflammatory responses, promote tissue regeneration, and maintain physiological homeostasis. This integrated defence strategy provides a valuable model for developing multifunctional biologics capable of addressing complex diseases through complementary mechanisms of action. Among the molecules reviewed, lumbrokinase has already demonstrated clinical utility in thrombotic disorders, illustrating the translational potential of earthworm-derived therapeutics. Likewise, lysenin, antimicrobial peptides, serine proteases, and bioactive coelomic extracts have shown promising antimicrobial, anti-inflammatory, regenerative, and anticancer activities in experimental studies. However, broader clinical application will depend on overcoming important challenges related to molecular characterization, protein engineering, standardized production, targeted delivery, and comprehensive safety evaluation. Advances in structural biology, recombinant biotechnology, nanomedicine, and systems immunology provide an encouraging foundation for future progress. As these technologies continue to evolve, earthworm immune molecules are expected to emerge not only as valuable therapeutic candidates but also as important models for understanding the evolution and functional diversity of innate immunity. Continued interdisciplinary research will be essential to unlock their full biomedical potential and facilitate the development of safe, effective, and innovative therapies for a wide range of human diseases.

Conflict of Interest: Nill

Endnotes: Nill

References

1. Bilej M, Procházková P, Šilerová M, Josková R (2010) Earthworm Immunity. In: Söderhäll K (ed.) Invertebrate Immunity. Advances in Experimental Medicine and Biology, vol 708. Springer, Boston, USA.
2. Cooper EL, Roch P (2003) Earthworm immunity: A model of immune competence. Pedobiologia 47(5-6): 676-688.
3. Engelmann P (2011) Earthworm immunity. Invertebrate Survival Journal 8: 67-75.
4. Trinh X-T, Long N-V, Van Anh LT, Nga PT, Giang NN, et al. (2022) A Comprehensive Review of Natural Compounds for Wound Healing: Targeting Bioactivity Perspective. International Journal of Molecular Science 23(17): 9573.
5. Sekizawa Y, Kubo T, Kobayashi H, Nakajima T, Natori S (1997) Molecular cloning of cDNA for lysenin, a novel protein in the earthworm *Eisenia foetida* that causes contraction of rat vascular smooth muscle. Gene 191(1): 97-102.
6. Shogomori H, Kobayashi T (2008) Lysenin: A sphingomyelin specific pore-forming toxin. Biochimica et Biophysica Acta 1780(3): 612-618.
7. Anderluh G, Lakey JH (2008) Disparate proteins use similar architectures to damage membranes. Trends Biochem Sci 33(10): 482-490.
8. Venkatachalam S, Christyraj JRSS, Bosco RBD, Yesudhason BV (2025) Antimicrobial peptides from earthworms: Emerging candidates for novel therapeutic applications. Toxicon 2264: 108458.
9. Cho JH, Park CB, Yoon YG, Kim SC (1998) Lumbricin I, a novel proline-rich antimicrobial peptide from the earthworm: purification, cDNA cloning and molecular characterization. Biochim Biophys Acta 1408(1): 67-76.
10. Wang D, Ruan Z, Zhang R, Wang X, Wang R, et al. (2021) Effect of Earthworm on Wound Healing: A Systematic Review and Meta-Analysis. Frontiers in Pharmacology 12: 691742.
11. Cooper EL, Hrzenjak TM, Grdiša M (2004) Alternative sources of fibrinolytic, anticoagulative, antimicrobial and anticancer molecules. International Journal of Immunopathology and Pharmacology 17(3): 237-244.
12. Grdiša M, Popović M, Hrženjak T (2004) Stimulation of growth factor synthesis in skin wounds using tissue extract (G-90) from the earthworm *Eissenia* (*sic*) *foetida*. Cell Biochemistry & Function 22(6): 373-378.

13. Nyberg KG, Conte MA, Kostyun JL, Forde A, Bely AE (2012) Transcriptome characterization via 454 pyrosequencing of the annelid *Pristina leidyi*, an emerging model for studying the evolution of regeneration. BMC Genomics 13: 287.
14. Reynolds JW, Reynolds WM (2026) Earthworms as Models for Regenerative Medicine. Journal of Clinical Medicine and Medical Research 3(6): 1-15.
15. Nakajima N, Sugimoto M, Ishihara K (2003) Earthworm-serine protease: Characterization, molecular cloning, and application of the catalytic functions. Journal of Molecular Catalysis B Enzymatic 23(2-6): 191-212.
16. Cooper EL (1996) Earthworm Immunity. Progress in Molecular and Subcellular Biology 15: 10–45.
17. Mihara H, Sumi H, Yoneta T, Mizumoto H, Ikeda R, et al. (1991) A novel fibrinolytic enzyme extracted from the earthworm, *Lumbricus rubellus*. The Japanese Journal of Physiology 41(3): 461-472.
18. Verma MK, Pulicherla KK (2011) Lumbrokinase – A Potent and Stable Fibrin–Specific Plasminogen Activator. International Journal of Bio-Science and Bio-Technology 3(2): 57-70.
19. Jin L, Jin H, Zhang G, Xu G, Wang R (2018) Lumbrokinase: A review of its pharmacological properties and clinical applications. Frontiers in Pharmacology 9: 549.
20. Zhang Z, Wang C, Li X, Wang Z, Li P (2011) Earthworm fibrinolytic enzyme: Anti-inflammatory and wound healing properties. Journal of Ethnopharmacology 134(3): 1008-1013. DOI: 10.1016/j.jep.2011.01.010
21. Jin M, Chen W, Huang W, Rong L, Gao Z (2013) Preparation of pegylated lumbrokinase and an evaluation of its thrombolytic activity both *in vitro* and *in vivo*. Acta Pharmaceutica Sinica B 3(2): 123-129.
22. Reynolds JW, Wetzel MJ (2026) Nomenclatura Oligochaetologica – A catalogue of names, descriptions and type specimens of the Oligochaeta. Editio Secunda.