Review article

Antimicrobial Peptides Derived from *Xenorhabdus* spp.: Untapped Troves of Novel Therapeutics

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Received: 29-08-2025 **Accepted:** 24-09-2025 **Published online:** 30-10-2025

DOI: https://doi.org/10.33687/ricosbiol.03.10.83

Abstract

The unending rise of antimicrobial resistance (AMR) is a serious threat to modern medicine. It makes infections that used to be treatable deadly and weakens the basis of surgical and cancer care. The discovery of novel antimicrobial classes with mechanistically distinct action is therefore a critical global health priority. Antimicrobial peptides (AMPs) represent a promising frontier in this endeavor, offering rapid, often non-specific mechanisms that challenge bacterial adaptation. The entomopathogenic bacteria of the genus *Xenorhabdus* have evolved into biochemical powerhouses, producing a staggering array of AMPs to survive within insect hosts while living in an obligate mutualism with Steinernema nematodes. This review provides a comprehensive analysis of the diverse classes of AMPs derived from *Xenorhabdus*, categorizing them into non-ribosomal peptides (NRPs) like the xenocoumacins and PAX peptides and ribosomally synthesized and post-translationally modified peptides (RiPPs) such as lasso peptides and novel bacteriocins. We delve deeply into their genetic basis, biosynthetic pathways, and multifaceted mechanisms of action, which range from membrane disruption and iron sequestration to intracellular targeting of essential processes. We further synthesize the evidence for their efficacy against multidrug-resistant ESKAPE pathogens, fungi, and protozoa, while critically evaluating the challenges of toxicity, stability, and scalable production. Finally, we present a forward-looking perspective on how advanced genomics, synthetic biology, and bioengineering strategies are poised to unlock the full potential of the *Xenorhabdus* pharmacopoeia, transforming these ecological weapons into a new generation of anti-infective agents.

Keywords: *Xenorhabdus*, antimicrobial peptides (AMPs), non-ribosomal peptide synthetase (NRPS), RiPPs, lasso peptides, xenocoumacin, drug discovery, antimicrobial resistance (AMR), biosynthetic gene cluster (BGC)

Introduction:

1. The Urgent Need and a Unique Source

The World Health Organization has declared AMR one of the top 10 global public health threats. The thin pipeline of new antibiotics, particularly those with novel mechanisms, is insufficient to address the rise of pan-resistant infections (WHO, 2021). AMPs, also known as host defense peptides, are ubiquitous components of innate immunity. Their typical cationic and amphipathic nature allows them to interact with and disrupt anionic microbial membranes,

a mechanism that is less prone to conventional resistance development than single-target antibiotics (Mahlapuu *et al*., 2016).

In the search for novel AMPs, underexplored ecological niches are paramount. The genus *Xenorhabdus* represents one such niche. These Gram-negative bacteria have a complex life cycle entailing a mutualistic relationship with entomopathogenic nematodes (*Steinernema* spp.) and a pathogenic phase within insect larvae. The nematode vector invades an insect host and regurgitates *Xenorhabdus* into the hemolymph. To survive and proliferate in this nutrient-rich but competitive environment, the bacteria deploy a sophisticated chemical arsenal (Bode, 2009). This arsenal includes a prolific output of secondary metabolites, with AMPs playing a central role in two key strategies: **biotrophy** suppressing the insect immune system and **antibiosis** creating a sterile monoculture by eliminating competing bacteria and fungi. This intense evolutionary pressure has made *Xenorhabdus* a hyper-producer of diverse and potent AMPs, making its metabolome a premium hunting ground for novel drug leads.

2. The Dual Biosynthetic Origins of *Xenorhabdus* AMPs

The structural diversity of *Xenorhabdus* AMPs stems from two primary biosynthetic pathways, each offering distinct advantages and complexities.

2.1. Non-Ribosomally Synthesized Peptides (NRPs)

NRPs are assembled by massive multi-enzyme complexes called non-ribosomal peptide synthetases (NRPSs). These assembly lines function like a conveyor belt, with each module responsible for activating, modifying, and incorporating a specific amino acid building block into the growing peptide chain. This process allows for the incorporation of over 500 different non-proteinogenic amino acids, D-amino acids, and other organic acids, resulting in molecules with unprecedented chemical diversity and stability against proteases.

• **Xenocoumacins (Xcns):** Primarily produced by *X. nematophila*, xenocoumacins are 3,4-dihydroisoquinoline derivatives with an attached peptide chain. Xenocoumacin 1 (Xcn1) is the most prominent, exhibiting potent, broad-spectrum activity against Gram-positive bacteria (including MRSA and VRE) and fungi like *Candida albicans*, while demonstrating notably low cytotoxicity in mammalian cell lines (Reimer *et al*., 2009). Its biosynthesis involves a fascinating prodrug mechanism where the inactive precursor Xcn2 is converted to the active Xcn1 by a specific enzyme. Recent mechanistic investigations have demonstrated that Xcn1 does not primarily target the membrane; instead, it inhibits the assembly of the bacterial 50S ribosomal subunit, representing a novel target that bypasses existing cross-resistance (Shi *et al*., 2022).

• **PAX Peptides:** Discovered in *X. nematophila*, PAX (Peptide with Anti-inflammatory and Antimicrobial Activity) peptides are cyclic depsipeptides (containing both peptide and ester bonds). Their initial characterization highlighted their role in suppressing insect immune responses. Beyond this, they display robust activity against Gram-positive bacteria. Strikingly, PAX peptides have shown promising activity against eukaryotic pathogens, including the malaria parasite *Plasmodium falciparum* and the tuberculosis bacillus *Mycobacterium tuberculosis*, suggesting their molecular targets are conserved across kingdoms (Crawford *et al*., 2021).

• **Xenortides and Rhabdopeptide / Xenortide Hybrids:** Initially identified as small linear peptides, the xenortide family has been expanded with the discovery of larger hybrid structures, such as the rhabdopeptide/xenortide (RXP) peptides. These are massive, linear NRPs that can contain over 20 amino acid residues. While their individual antimicrobial activity can be moderate, they are believed to play a synergistic role, potentially by disrupting membrane integrity and facilitating the uptake of other, more potent AMPs (Cai *et al*., 2017).

2.2. Ribosomally Synthesized and Post-Translationally Modified Peptides (RiPPs)

RiPPs are gene-encoded peptides, meaning their initial sequence is transcribed and translated ribosomally. This precursor peptide is then heavily modified by dedicated enzymes, leading to complex and architecturally unique scaffolds. This biosynthetic route is highly genetically tractable, as the core structural gene is small and easy to identify and manipulate.

• **Lasso Peptides:** This class is defined by a unique three-dimensional knot: a ring formed between the N-terminal amino group and a side-chain carboxylate (often Asp or Glu) is threaded by the C-terminal tail, which is trapped by bulky residues. This "lasso" topology confers exceptional stability against heat and proteases. *Xenorhabdus* genomes are enriched with lasso peptide BGCs. *Xenematide* (from *X. nematophila*) and xenolassin (from *X. khoisanae*) are prime examples, exhibiting potent, broad-spectrum antibacterial activity against both Gram-positive and Gram-negative pathogens (Kačar *et al*., 2020; Kuthning *et al*., 2015). Their mechanism often involves binding to the RNA polymerase complex, thereby inhibiting transcription.

• **Bacteriocins and Other RiPPs:** Beyond lasso peptides, *Xenorhabdus* produces other RiPP families, including microcins and novel, yet-to-be-classified peptides. These are typically smaller and act with high potency, often against closely related bacterial strains, playing a key role in intraspecies competition within the insect host.

3. Mechanisms of Action: A Multi-Pronged Attack

The AMPs from *Xenorhabdus* do not rely on a single kill mechanism, which is a key asset in avoiding resistance.

• **Membrane Disruption and Permeabilization:** This is the canonical mechanism for many cationic AMPs. Peptides like certain lasso peptides and RXPs accumulate on the negatively charged bacterial surface, leading to membrane thinning, pore formation (via "barrel-stave", "carpet", or "toroidal-pore" models), and eventual cell lysis.

• **Intracellular Targeting:** A significant number of *Xenorhabdus* AMPs have evolved to cross the membrane and disrupt vital intracellular processes. Xcn1 inhibits ribosome assembly (Shi *et al*., 2022), while other peptides may target DNA gyrase, RNA polymerase, or cell wall biosynthesis enzymes.

• **Iron Sequestration (Nutritional Immunity):** Siderophore peptides like the sturzins and xenobactins are high-affinity iron chelators. By scavenging the limited free iron within the insect hemolymph, they starve competing microbes of this essential nutrient, exerting a powerful bacteriostatic effect (Brachmann *et al*., 2013).

• **Immunomodulation:** In their ecological context, peptides like the PAX family function to dampen the insect's prophenoloxidase (proPO) activation cascade, a key immune defence. This immunomodulatory activity is a unique indirect antimicrobial strategy.

4. Spectrum of Activity and Therapeutic Potential

The bioactivity profile of *Xenorhabdus* AMPs extends far beyond their ecological role, showing direct relevance to human health.

• **Antibacterial Activity:** These compounds are effective against critical-priority WHO pathogens. Xcn1 and lasso peptides are potent against MRSA and VRE. Notably, some lasso peptides and modified derivatives show promising activity against challenging Gram-negative pathogens like *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, often by leveraging unique uptake mechanisms.

• **Antifungal and Antiparasitic Activity:** The antifungal activity of Xcn1 against *C. albicans* is well-documented. More recently, the anti-plasmodial and anti-trypanosomal activity of PAX peptides has opened a new frontier for developing therapeutics for malaria and Human African Trypanosomiasis, diseases desperately in need of new drugs (Crawford *et al*., 2021).

• **Anticancer and Cytotoxic Activity:** Several *Xenorhabdus* metabolites, including Xcn1 and some NRPs, demonstrate selective cytotoxicity against various human cancer cell lines *in vitro* (Park *et al*., 2017). This suggests that the apoptotic pathways in cancer cells may be vulnerable to these bacterial effectors, warranting further investigation.

5. Challenges in Therapeutic Development

The path from a promising natural product to a clinically approved drug is fraught with hurdles.

• **Production and Scalability:** Laboratory cultivation of *Xenorhabdus* often yields miniscule amounts of target peptides. Their complex structures make total chemical synthesis economically unviable for many candidates.

• **Pharmacokinetics and Toxicity:** While *in vitro* cytotoxicity may be low, *in vivo* stability (susceptibility to serum proteases), half-life, biodistribution, and potential immunogenicity are major concerns that must be addressed through rigorous preclinical testing.

• **Bioengineering and Optimization:** The native structures of these peptides, while potent, may not be ideal drug candidates. There is a need for medicinal chemistry optimization to improve potency, reduce toxicity, and enhance pharmacokinetic properties.

6. Future Perspectives and Concluding Remarks

The future of *Xenorhabdus*-derived AMPs is intrinsically linked to technological advancement. Several key strategies will drive the field forward:

1. **Advanced Genome Mining:** The genomes of *Xenorhabdus* are littered with "silent" or cryptic BGCs that are not expressed under standard lab conditions. Bioinformatics tools like

antiSMASH allow for the systematic identification of these clusters. Strategies to "awaken" them including heterologous expression in optimized chassis organisms (e.g., *E. coli*, *Pseudomonas putida*), promoter engineering, and co-cultivation with triggering organisms are yielding a torrent of new compounds (Bozhüyük *et al*., 2019; Bode *et al*., 2018).

2. **Synthetic Biology and Combinatorial Biosynthesis:** The modular nature of NRPS and RiPP pathways makes them amenable to engineering. By swapping domains in NRPS modules or modifying the core peptide sequence in RiPP precursors, it is possible to generate entirely novel "non-natural" natural products with tailored properties (Bozhüyük *et al*., 2019).

3. **High-Throughput Screening and Target Identification:** Screening purified AMP libraries against comprehensive panels of resistant pathogens, combined with modern target identification methods (e.g., whole-genome sequencing of resistant mutants, chemical proteomics), will rapidly prioritize leads and elucidate their mechanisms.

4. **Formulation and Delivery Technologies:** To overcome stability and delivery issues, innovative formulations such as liposomal encapsulation, peptidomimetic approaches, and nanoparticle-based delivery systems can be employed to protect the peptide and enhance its therapeutic index.

In conclusion, *Xenorhabdus* bacteria represent an unparalleled and still underexploited reservoir of antimicrobial diversity. Their AMPs, forged in the crucible of host-pathogen conflict, exhibit novel structures, innovative mechanisms, and potent activity against the most daunting clinical threats. While significant challenges remain, the convergence of genomics, synthetic biology, and traditional natural product chemistry provides an unprecedented toolkit to mine, optimize, and develop these compounds. The systematic exploration of the *Xenorhabdus* pharmacopoeia is not merely an academic exercise; it is a vital mission in the global effort to secure a future safe from the threat of untreatable infections.

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