Peptide Arsenal: Unlocking Plant Disease Resistance with Antimicrobial Peptides

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Abstract

Antimicrobial peptides (AMPs) are small, typically positively charged polypeptides produced by plants as part of their innate defense mechanisms against a wide range of pathogens. These peptides play a pivotal role in plant disease management by directly targeting and inhibiting the growth of various microorganisms, including bacteria, fungi and viruses. Serving as a first line of defense, AMPs disrupt microbial membranes, inhibit cell wall synthesis and interfere with cell division while also modulating the plant's immune response. Found in diverse organisms such as microorganisms, insects, plants, animals and humans. plant-based AMPs can be categorized into distinct classes, including Thionins, Defensins, Hevein-like peptides, Knottins, α-hairpinins, Lipid transfer proteins, Snakins and Cyclotides, each characterized by unique structures and modes of action. Various factors influence the antimicrobial activities of AMPs against different microbial types, primarily through the interaction of cationic AMPs with anionic microbial membranes both extracellularly and intracellularly, thereby disrupting normal physiological activities of pathogens. The incorporation of plant AMPs into disease management strategies presents a promising avenue for sustainable agriculture, offering a targeted and environmentally friendly approach to combat pathogenic infections.

Introduction

Antimicrobial peptides (AMPs), also referred to as host defense peptides, are small molecular peptides composed of 12 to 50 amino acids that are ubiquitously present across various life forms, from bacteria and fungi to plants, insects and even humans. These peptides are integral components of innate immunity, serving as a frontline defense against microbial invasion. AMPs possess a positive net charge and an amphiphilic structure, enabling them to strongly interact with microbial membranes, leading to their disruption. Their broad-spectrum activity extends to bacteria, fungi, viruses and other pathogenic microorganisms, making them highly versatile in their antimicrobial action. One of the key characteristics of antimicrobial peptides (AMPs) is their capability to target intracellular components, thereby inhibiting essential processes like cell wall synthesis, nucleic acid replication and protein synthesis. Beyond their direct antimicrobial effects, AMPs also have the potential to modulate the host's immune response thereby strengthening the overall defense against pathogens. Due to their varied mechanisms of action, AMPs offer a promising approach to addressing the escalating challenge of antimicrobial resistance (AMR) observed in numerous pathogens. To date, a vast array of AMPs—3,425 peptides—have been cataloged in the Antimicrobial Peptide Database (APD3), reflecting the extensive research and interest in their potential applications in plant and human disease management (http://aps.unmc.edu/AP/, accessed June 30, 2022). These unique characteristics position AMPs as a crucial tool not only in natural immune responses but also in biotechnological and agricultural innovations aimed at sustainable disease control.

Characteristics of Antimicrobial Peptides

- Small Size: AMPs are composed of 12 to 50 amino acids.
- Length: They require at least 7–8 amino acids for effective activity.
- Net Charge: A higher positive charge enhances interactions with bacteria.
- Hydrophobicity: Greater hydrophobicity disrupts bacterial membranes.
- Amphipathic Nature: AMPs have both hydrophilic and hydrophobic regions.
- Amino Acid Composition: They often contain cationic amino acids like lysine and arginine.
- Broad-Spectrum Activity: AMPs target various microorganisms effectively.

- Diverse Structures: They include α-helices, β-sheets, and loop structures.
- Low Resistance Development: Pathogens are less likely to develop resistance to AMPs.

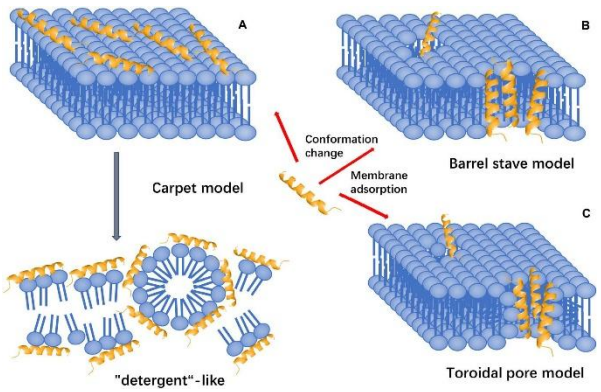
Mechanism of Action

The mechanism of action of antimicrobial peptides (AMPs) primarily revolves around their interaction with microbial cell membranes, leading to disruption, pore formation and ultimately cell death. There are three main models that explain how AMPs target and compromise these membranes: The Carpet Model, Barrel-Stave Model and Toroidal Pore Model. Each model outlines a distinct process of membrane interaction and disruption, depending on the structure and behaviour of the peptides.

In the **Carpet Model**, AMPs align parallel to the surface of the microbial membrane. The peptides' hydrophilic regions face outward, interacting with the surrounding aqueous environment, while their hydrophobic regions interact with the lipid bilayer of the membrane (Fig A). As the peptides accumulate on the membrane surface, they coat it like a carpet, which leads to membrane destabilization. Unlike other models that involve pore formation, the Carpet Model causes membrane disruption in a detergent-like manner, breaking the membrane apart at high concentrations. This mode of action is more prevalent with peptides that have a β-sheet structure and requires a critical peptide concentration to effectively damage the membrane. The membrane destabilization leads to leakage of intracellular contents, ultimately resulting in microbial cell death (Oren and Shai, 1998; Shenkarev *et al*., 2011; Corrêa *et al*., 2019).

The **Barrel-Stave Model** involves a different mechanism where AMPs penetrate the membrane in the form of multimers, assembling to form transmembrane pores. In this model, the hydrophobic regions of the peptides interact with the lipid bilayer, while the hydrophilic regions line the interior of the pore (Fig 2). The peptides behave like staves in a barrel, surrounding and forming a cylindrical pore through which ions and small molecules can pass. This pore formation results in the efflux of cytoplasmic contents and destabilizes the cell often leading to rapid cell death due to a loss of membrane integrity. An example of this is Alamethicin, a peptide that aggregates in the membrane to create stable channels, leading to cytoplasmic outflow. Protegrin-1, another AMP, has also been shown to form β-barrels in this manner, leading to similar pore formation and membrane destabilization (Lohner and Prossnigg, 2009; Lipkin and Lazaridis, 2015).

The **Toroidal Pore Model**, also referred to as the Wormhole Model, represents a hybrid mechanism where both peptides and lipids participate in the formation of a pore. In this model, AMPs embed themselves perpendicularly into the membrane and induce the bending of the lipid bilayer, forming a continuous pore (Fig C). The lipids curve inward along with the peptides, creating a pore with a ring-like structure. The diameter of these pores is generally between 1–2 nm, large enough to disrupt the cell's osmotic balance. This leads to the leakage of ions and other molecules from the cell, ultimately causing cell death. Unlike the Barrel-Stave Model, where the peptides alone form the pore, the Toroidal Pore Model integrates the lipid molecules of the membrane into the pore structure. AMPs such as Magainin 2, Lacticin Q and Arenicin are known to employ this mechanism, efficiently destabilizing microbial membranes and



leading to rapid cell death (Matsuzaki *et al*., 1996).

Classification of Antimicrobial peptides: AMPs are classified based on source, activity, structural characteristics and amino acid-rich species

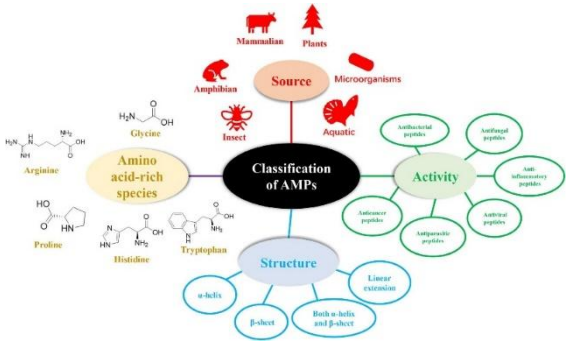


Table 1. Classification of Antimicrobial Peptides

S.no	Classification	Key features	Examples
1.	Based on Source	Antimicrobial peptides (AMPs) are naturally derived from different organisms like plants, animals, insects and microorganisms.	Plants: Thionins, Defensins Insects: Cecropin, Moricin Humans: LL-37, Defensins Microbes: Bacitracin, Gramicidin
2.	Based on Activity	AMPs exhibit a broad range of activities against specific pathogens: bacteria (gram-positive/negative), fungi, viruses and parasites.	Antibacterial: LL-37 (bacteria), Magainin Antifungal: Histatins (fungi), Plant defensins Antiviral: Lactoferrin, Defensins (HIV)
3.	Based on Mechanism of Action	AMPs kill or inhibit microbes *via* various mechanisms: membrane disruption, inhibition of nucleic acid/protein synthesis, and targeting intracellular components.	Membrane disruption: Melittin, Cecropin Inhibition of macromolecular synthesis: PR-39 (ribosome interaction) Intracellular targeting: Indolicidin
4.	Based on Structure		
4.1	Linear α-helical AMPs	Abundant in nature, these adopt amphipathic structures on membranes, forming pores through interaction between hydrophilic and hydrophobic regions.	Cecropin, Pleurocidin, Magainin, Melittin
4.2	β-sheet AMPs	Contain β-strands stabilized by disulfide bridges, exhibiting cationic and hydrophobic features critical for membrane targeting and antimicrobial activity.	Protegrin-1, Defensins (α and β), Tachyplesin
4.3	Mixed αβ AMPs	AMPs that contain both α-helices and β-sheets, usually involved in strong interactions with fungal and microbial membranes.	Human defensins, Protegrin
4.5	Non-αβ or Linear Extended AMPs	These peptides lack α-helical and β-sheet structures and are classified as tryptophan-, proline-, and glycine-rich peptides, often inhibiting intracellular processes.	Indolicidin, PR-39, Tritrpticin, Histatins
5	Based on Mode of Action	AMPs can either directly kill pathogens or modulate the host immune response by promoting inflammation, wound healing or immune cell recruitment.	Direct killing: Cathelicidins Immune modulation: LL-37, Defensins (wound healing)

Conclusion

The advent of genetic engineering and genome-targeting technologies has significantly advanced the ability to overexpress antimicrobial peptides (AMPs) in target plant species, thereby enhancing their intrinsic disease resistance mechanisms. Given their multifaceted roles in plant defense, AMPs emerge as promising alternatives to conventional chemical fungicides, offering a unique advantage through their ability to specifically target

pathogens without adversely affecting beneficial organisms. The diverse functional repertoire of AMPs, coupled with their regulatory dynamics and interactions with other defense signaling pathways, positions them as pivotal components of integrated plant disease management strategies. Furthermore, their potential for specificity, low environmental impact and reduced risk of resistance development makes AMPs an innovative solution to combat plant diseases in a sustainable manner.

Future Prospects

The genetic engineering of AMPs into crops holds immense promise for fortifying plants against a broad spectrum of bacterial, fungal and viral pathogens. By harnessing the specificity of AMPs, researchers can develop biopesticides that directly target pathogens, thereby decreasing reliance on traditional chemical pesticides that often pose environmental risks. Additionally, the formulation of AMPs into foliar sprays could serve as an effective preventive measure, providing an extra layer of protection against pathogenic infections. This targeted and environmentally friendly approach not only aligns with contemporary agricultural practices aimed at sustainability but also underscores the transformative potential of AMPs in safeguarding plant health and enhancing crop yield in an increasingly challenging climate.

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