

Plant Immunity Boosted by Rhamnolipids and Lipopeptide Biosurfactants: A New Era in Plant Disease Management

Nazia Manzar* and Abhijeet Shankar Kashyap

Plant Pathology Lab, ICAR-National Bureau of Agriculturally Important Microorganisms, Maunath Bhanjan, India

Corresponding Author: naziamanزار786@gmail.com

Lipopeptide biosurfactants (LPs) are composed of a lipid tail connected to a short oligopeptide, either linear or cyclic. Predominantly produced in their cyclic form by fungi and various bacterial genera, LPs have gained significant recognition for their antibacterial, cytotoxic, anticancer, immunosuppressive, and surfactant properties (Raaijmakers et al., 2010). Cyclic lipopeptides (CLPs) constitute a category of biosurfactants extensively synthesized by diverse bacterial species known as plant-beneficial bacteria (D'aes et al., 2010). *Bacillus* and *Pseudomonas* are utilized as biocontrol agents and are renowned for producing a variety of structurally diverse and multifunctional cyclic lipopeptides (CLPs) with significant biological activities pertinent to plant protection (Raaijmakers et al., 2010). Bacterial CLPs are potent biosurfactants that exhibit significant destabilizing effects on biological membranes. Their antibacterial efficacy is thoroughly demonstrated in the realm of biocontrol through direct suppression of phytopathogens. In vitro assays utilizing purified cyclic lipopeptides (CLPs), with or without loss-of-function mutants of natural producers, have demonstrated the extensive spectrum of fungal and oomycete plant pathogens influenced by bacterial CLPs such as fengycins and iturins (refer to recent reviews by Rabbee et al. (2019) for *Bacillus*, and Geudens and Martins (2018) and Götze and Stallforth (2020) for *Pseudomonas* CLPs, respectively). Numerous studies demonstrate that CLP activity correlates with its ability to destabilize fungal cell membranes, leading to cytoplasmic leakage and subsequent hyphal death or inhibition of spore germination (Qian et al., 2016). The molecular basis of antifungal activity may be intricate, and, similar to rhamnolipids, the lipid content of the targeted cell membrane could significantly influence microbicidal activity (Wise et al., 2014). Similar to other antimicrobial peptides, CLPs not only disrupt membranes but can also directly or indirectly influence intracellular targets and modify fungal cell activities.

Antibacterial action has been sporadically documented for *Bacillus* cyclic lipopeptides, including iturin A, bacillomycin, and locillomycins, against many agronomically significant plant diseases. Nonetheless, there is a paucity of compelling evidence worldwide about the direct bactericidal impact of *Bacillus* cyclic lipopeptides, particularly surfactin, on phytopathogens or soil-borne bacterial pathogens. The specific antibiotic mechanism of *Bacillus* CLPs against bacterial phytopathogens is not fully understood, despite evident direct interaction with the target's cellular membrane. In certain cases, the inhibitory action of specific *Bacillus* cyclic lipopeptides (CLPs), such as surfactin, lichenysin, and pumilacidin, is not directly associated with the viability of target cells. Instead, it pertains to interference with critical developmental processes of pathogens, including the disruption of efficient biofilm formation in *Pseudomonas syringae* and *Ralstonia solanacearum*, as well as the inhibition of aerial hyphal development in *Streptomyces coelicolor*. Plants have evolved intricate defense mechanisms that strengthen their resistance to phytopathogens. Upon the detection of microbial pathogens, plants initiate a series of rapid and intricate signalling events that serve as the foundation for their immune response. These early signalling processes include dynamic changes such as ion fluxes, particularly the influx of calcium ions (Ca^{2+}) and efflux of potassium ions (K^{+}), which play crucial roles in transmitting defense signals. Another hallmark of this phase is the rapid production of reactive oxygen species (ROS), which act as both antimicrobial agents and secondary messengers in immune signalling pathways.

Concurrently, phosphorylation cascades mediated by mitogen-activated protein kinases (MAPKs) and other protein kinases amplify and relay the immune signal, coordinating downstream responses. These initial signalling events lead to the activation of a sophisticated network of phytohormones, including salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). These hormones act synergistically or antagonistically, depending on

the type of pathogen encountered, to fine-tune the plant's defense mechanisms. SA is typically associated with systemic acquired resistance (SAR) and defenses against biotrophic pathogens, while JA and ET are more closely linked to induced systemic resistance (ISR) and defenses against necrotrophic pathogens and herbivores. This hormonal crosstalk orchestrates late-stage defense responses, which include the synthesis of antimicrobial compounds such as phytoalexins and pathogenesis-related (PR) proteins. Additionally, plants reinforce their physical barriers by fortifying cell walls through the deposition of callose, lignin, and other structural polymers, creating an inhospitable environment for pathogen invasion. Together, these responses establish a robust defense framework, enabling plants to resist a wide range of microbial threats while maintaining energy efficiency and growth. These defensive mechanisms jointly facilitate local plant immunity.

Furthermore, microbial awareness initiates systemic responses that are effective against a broad spectrum of microbes throughout the entire plant. The activation of the plant immune system entails invasion pattern (IP) molecules, often referred to as elicitors, which may derive from or be synthesized by the pathogen. While the majority of research on glycolipid biosurfactants has been on their antibacterial and antifouling properties, recent findings indicate that rhamnolipids may also enhance plant innate immunity. Notably, despite their antibacterial and immunomodulatory capabilities in mammals, there has been little research to date on sophorolipids, trehalolipids, MELs, or cellobiose lipids on their possible significance in activating plant defensive responses. Subsequent to plant sensing, rhamnolipids initiate preliminary signalling processes, including the formation of reactive oxygen species (ROS) in grapevine and *Brassica napus*, along with a calcium influx and a phosphorylation cascade in grapevine.

Rhamnolipids play a pivotal role in plant immunity, inducing a variety of defense responses in species such as Brassicaceae and grapevine. Characteristic features of rhamnolipid-triggered immunity include the deposition of callose, synthesis of defense-related hormones, activation of specific defense genes, and the initiation of a hypersensitive reaction-like response. These responses have been well-documented in studies by Varnier et al. (2009), Sanchez et al. (2012), and Monnier et al. (2018, 2020).

In *Arabidopsis thaliana*, rhamnolipid-induced resistance to various pathogens, including the necrotrophic fungus *Botrytis cinerea*, the oomycete *Hyaloperonospora arabidopsidis*, and the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (Pst), was found to involve distinct signalling pathways tailored to the specific pathogen. This indicates that rhamnolipids can differentially modulate immune signalling networks based on the type of microbial threat. Additionally, rhamnolipids have been shown to synergize with other elicitors, such as chitosan, enhancing the overall defense response. In Brassicaceae, particularly in *Brassica napus*, rhamnolipids contribute to local resistance not only against *B. cinerea* but also against the hemibiotrophic fungus *Leptosphaeria maculans*. This dual functionality underscores their potential as versatile tools for bolstering plant immunity in both dicotyledonous and monocotyledonous crops (Monnier et al., 2018, 2020). A broad spectrum of rhamnolipid concentrations, ranging from 0.005 to 1 mg/mL, has been employed to stimulate immunity in several plant species (Varnier et al., 2009; Sanchez et al., 2012; Monnier et al., 2018, 2020). Synthetic biosurfactants derived from rhamnolipid structures have shown potential as elicitors of plant immune responses. Among these, synthetic rhamnolipid bolaforms—comprising two rhamnose sugar molecules linked by a single fatty acid chain—are particularly noteworthy. These molecules are capable of activating an immunological response in *Arabidopsis thaliana*, a widely studied model organism in plant biology. Importantly, the efficiency and nature of this elicitation depend on the structural properties of the bolaform, especially the length of the fatty acid chain connecting the rhamnose units. Variations in chain length influence the molecule's ability to interact with plant receptors or other cellular components, thus modulating the plant's immune signaling pathways.

The ability of rhamnolipids to activate multifaceted immune responses, tailored to pathogen type and enhanced in combination with other elicitors, highlights their promise for sustainable agricultural practices aimed at reducing chemical pesticide use while improving crop resilience. This highlights the potential of structural modifications in synthetic biosurfactants for tailoring their function as elicitors, providing valuable insights for agricultural and biotechnological. Furthermore, RL containing

carboxylic acid (Ac-RL) and methyl (Alk-RL) stimulates ROS generation in this plant. Some investigations do not establish whether the protective effects induced by rhamnolipid were a result of activated plant defense responses and/or antibacterial characteristics. The application of rhamnolipids has demonstrated significant potential in enhancing plant resistance to various diseases caused by fungal and oomycete pathogens. In pepper plants (*Capsicum annuum*), treatment with rhamnolipids has been shown to provide substantial protection against *Phytophthora* blight, a destructive disease caused by *Phytophthora capsici*. This protective effect extends to other crops as well; for example, rhamnolipids effectively inhibit the growth of *Colletotrichum orbiculare*, the pathogen responsible for anthracnose disease, on cucumber (*Cucumis sativus*) leaves. These findings highlight the versatility of rhamnolipids as a broad-spectrum defense agent. Rhamnolipids also play a critical role in controlling water-borne soil pathogens. They significantly reduce the incidence of damping-off disease, a common and devastating condition in seedlings caused by pathogens such as *Phytophthora* spp. and *Pythium* spp.

The disease, often prevalent in wet conditions, leads to seedling death and poor crop establishment. By suppressing the growth and spread of these pathogens, rhamnolipids enhance plant survival and vigour during early growth stages. Field trials have further validated the efficacy of rhamnolipids under natural conditions. In studies conducted on chili pepper (*Capsicum frutescens*) and tomato (*Solanum lycopersicum*), rhamnolipid treatments significantly reduced disease incidence and improved crop health. These trials underscore the practical applicability of rhamnolipids in agricultural settings, offering an environmentally friendly alternative to chemical fungicides. The success of these treatments in diverse crops and conditions highlights the potential of rhamnolipids as a sustainable tool for integrated disease management. Collectively, these studies illustrate that rhamnolipids not only enhance plant immunity but also provide direct antimicrobial effects against a variety of pathogens. Their ability to mitigate disease outbreaks across multiple crops and conditions makes them a promising candidate for advancing sustainable agriculture and reducing dependence on chemical pesticides.

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