Biosurfactants in plant protection against diseases: rhamnolipids and lipopeptides case study

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Abstract

Biosurfactants are amphiphilic surface-active molecules that are produced by a variety of microorganisms including fungi and bacteria. *Pseudomonas*, *Burkholderia* and *Bacillus* species are known to secrete rhamnolipids and lipopeptides that are used in a wide range of industrial applications. Recently, these compounds have been studied in a context of plant-microbe interactions. This mini-review describes the direct antimicrobial activities of these compounds against plant pathogens. We also provide the current knowledge on how rhamnolipids and lipopeptides stimulate the plant immune system leading to plant resistance to phytopathogens. Given their low toxicity, high biodegradability and ecological acceptance, we discuss the possible role of these biosurfactants as alternative strategies to reduce or even replace pesticide use in agriculture.

Introduction

Plant pathogens cause significant agricultural damages and up to 30% of crops are lost before or after harvest to plant diseases leading to huge economic losses (Jones et al., 2016). Diseases and pests are therefore major problems for sustainable agriculture in the world. Chemical pesticides are largely used to control plant disease worldwide (Popp et al., 2013). However, chemical pesticides can be detrimental to human and environmental health and therefore, development and optimization of alternative strategies to reduce their utilization for crop protection is becoming a necessity. Biocontrol is a promising strategy based on the use of organisms that decrease disease pressure by competing with the pathogen for space and nutrients, by inducing the plant's natural defense system, and/or by the production of antimicrobial substances (Berg et al., 2017; Bonanomi et al., 2018; Syed Ab Rahman et al., 2018). In addition, natural, ecofriendly and biodegradable compounds isolated from (micro)organisms can also be part of a biocontrol strategy. These compounds can act directly onto the pathogen *via* antimicrobial properties or by stimulating the plant immune system to prime plant protection against disease (Delaunois et al., 2014; Bardin et al., 2015; Keswani et al., 2019). Molecules from microbial origin stimulating the plant immune system are called invasion patterns or elicitors and are highly diverse both in nature and origins (Vatsa et al., 2010; Delaunois et al., 2014; Burketova et al., 2015; Schellenberger et al., 2019).

Application of biosurfactants like mannosylerythritol lipids (MEL), trehalose dimycolate (TDM), trehalolipids, sophorolipids, rhamnolipids or lipopeptides have been studied since decades in biomedical sciences especially for their antimicrobial properties and as modulators of human innate immunity (for extensive reviews see (Singh and Cameotra, 2004; Banat et al., 2010; Vatsa et al., 2010; Naughton et al., 2019; Coelho et al., 2020; Jahan et al., 2020; Sajid et al., 2020). Some of these microbial biosurfactants exhibit antimicrobial properties that are effective against a large panel of plant pathogens (Mnif and Ghribi, 2016; Penha et al., 2020). In addition, rhamnolipids and lipopeptides have recently been shown to stimulate the plant immune system conferring a better resistance to fungal and bacterial pathogens (Raaijmakers et al., 2010; Vatsa et al., 2010; Schellenberger et al., 2019). In this review, we will provide current knowledge and recent advances on the role of biosurfactants in plant protection. We will focus on rhamnolipids and lipopeptides that have been the most extensively studied in this context.

Biosurfactants as biopesticides

Rhamnolipids are efficient bio-fungicides

Among classical glycolipidic biosurfactants, sophorolipids, MEL and cellobiose lipids have marginally been studied for their antimicrobial properties towards plant pathogens (Yoshida et al., 2015; Mnif and Ghribi, 2016; Sen et al., 2017; Chen et al., 2020; Penha et al., 2020). Rhamnolipids are glycolipids produced by various bacterial species including some *Pseudomonas* sp. and *Burkholderia* sp. (Abdel-Mawgoud et al., 2010). Whereas no direct or robust evidences have been reported for rhamnolipid antibacterial or antiviral activities against plant pathogens, a large number of studies described their antifungal activities on pathogens affecting crops. These activities were mainly targeted to fungi and oomycetes including *Botrytis* sp., *Rhizoctonia* sp., *Fusarium* sp., *Alternaria* sp., *Pythium* sp., *Phytophthora* sp. or *Plasmopara* sp. species (Table 1). Because of their amphiphilic nature, glycolipids should be able to interact directly with plasma membranes (Otzen, 2017). It was thus proposed that the mode of action of rhamnolipids against zoospore-producing plant pathogens could be a direct lysis of zoospores *via* the intercalation of the glycolipids within plasma membranes which are not protected by a cell wall (Stanghellini and Miller, 1997). Rhamnolipids could also affect mycelial cells resulting in their destabilization or lysis. Rhamnolipid partition into membranes strongly depends on lipid composition (Aranda et al., 2007). It was shown that purified mono and di-rhamnolipids are able to intercalate into phosphatidylcholine and phosphatidylethanolamine bilayers, notably altering their packing (Ortiz et al., 2006; Sánchez et al., 2006; Sánchez et al., 2009; Abbasi et al., 2012; Abbasi et al., 2013). These insertions thus produce structural perturbations, which might affect the function of the membranes. These compounds also alter the physicochemical properties of the bilayer and disturb the hydration status of the water/lipid interface. Depending on the lipid composition of the membrane and on their concentration, rhamnolipids are also able to permeabilize membranes (Sánchez et al., 2010) that could result in their lysis.

Lipopeptides as antimicrobial agents

Cyclic lipopeptides (CLPs) represent a class of biosurfactant widely produced by various bacterial species referred as plant-beneficial bacteria. Among them, *Bacillus* and *Pseudomonas* are exploited as biocontrol agents and are also the best known for the production of a range of structurally distinct and multifunctional CLPs with strong biological activities related to plant protection (Raaijmakers et al., 2010). Bacterial CLPs are powerful biosurfactants retaining strong destabilizing activities on biological membranes. Their antimicrobial activity is well documented in a context of biocontrol *via* direct inhibition of phytopathogens. In vitro-based assays using purified CLPs combined or not with loss of function mutants of natural producers have highlighted the extremely wide range of fungal and oomycete plant pathogens that are affected by bacterial CLPs such as fengycins and iturins (see recent reviews (Caulier et al., 2019; Rabbee et al., 2019) for *Bacillus* and (Geudens and Martins, 2018; Götze and Stallforth, 2020) for *Pseudomonas* CLPs, respectively). Many studies indicate that CLP activity is linked to their capacity to compromise the fungal cell membrane stability, resulting in cytoplasm leakage and hyphae death or inhibition of spore germination (Chitarra et al., 2003; Romero et al., 2007; Etchegaray et al., 2008; Pérez-García et al., 2011; Gong et al., 2015; Qian et al., 2016). However, the mechanistic basis of antifungal activity may be more complex and, as for rhamnolipids, the lipid composition of the targeted cell membrane could play an essential role in the microbicidal activity (Grau et al., 1999; Tao et al., 2011; Wise et al., 2014). Like other antimicrobial peptides, CLPs are not only membrane disruptive but can also directly or indirectly act on intracellular targets and alter fungal cell functions (Latoud et al., 1987; Qi et al., 2010).

Antibacterial activity has also been occasionally reported for *Bacillus* CLPs such as iturin A, bacillomycin and locillomycins towards several plant pathogens of agronomic importance (Zeriouh et al., 2011; Luo et al., 2015; Cao et al., 2018). However, there are globally few convincing evidences for a direct bactericidal effect of *Bacillus* CLPs and surfactin in particular on phytopathogens or soil-borne bacterial attackers. The precise antibiotic mechanistic of *Bacillus* CLPs against bacterial phytopathogens remains unclear even if a direct interaction with the cellular membrane of the target is also obvious (Zeriouh et al., 2011; Gao et al., 2017). However, in some instances, the inhibitory effect of some *Bacillus* CLPs such as surfactin (or related lichenysin and pumilacidin) is not related to a direct effect on target cell viability but rather due to some interference with key developmental processes of the pathogen such as efficient biofilm formation by *Pseudomonas syringae* and *Ralstonia solanacearum* (Bais et al., 2004; Chen et al., 2013; Xiu et al., 2017) or inhibition of aerial hyphal development of *Streptomyces coelicolor* (Straight et al., 2006; Hoefler et al., 2012).

Stimulation of plant immunity by biosurfactants

Plants have developed complex defense mechanisms leading to enhance resistance to phytopathogens. After microbial perception, early signaling events are set up including ion fluxes, reactive oxygen species (ROS) accumulation and phosphorylation cascades (Garcia-Brugger et al., 2006; Bigeard et al., 2015). These early signaling and the activation of an intricate network of phytohormones, such as salicylic acid or jasmonic acid, regulate late defense-related responses

(Pieterse et al., 2012) including synthesis of antimicrobial metabolites and cell wall reinforcement. These defense responses collectively allow local plant immunity (Boller and Felix, 2009). In addition, microbial perception triggers systemic responses that are effective against a large panel of microorganisms in the whole plant (Fu and Dong, 2013; Pieterse et al., 2014). Activation of the plant immune system involves invasion patterns (IPs) molecules also known as elicitors which can originate from or be produced by the microbe (Schellenberger et al., 2019).

Rhamnolipids trigger local resistance against plant pathogens

Whereas most studies on glycolipid biosurfactants were focused on their antimicrobial and antifouling activities, it was recently discovered that rhamnolipids may also stimulate plant innate immunity (Vatsa et al., 2010) (Figure 1). Interestingly, despite their antimicrobial and mammalian immunomodulatory properties, no study on sophorolipids, trehalolipids, MELs or cellobiose lipids, to our knowledge, described their potential role in the activation of plant defense responses so far. Following plant sensing, rhamnolipids trigger early signaling events like accumulation of ROS in grapevine and *Brassica napus* (Varnier et al., 2009; Monnier et al., 2018) as well as a calcium influx and a phosphorylation cascade in grapevine (Varnier et al., 2009). Callose deposition, hormone production, defense gene activation and a hypersensitive reaction-like response are also hallmarks of rhamnolipid-triggered immunity in *Brassicaceae* and grapevine (Varnier et al., 2009; Sanchez et al., 2012; Monnier et al., 2018; Monnier et al., 2020). It was demonstrated in Arabidopsis that rhamnolipid-mediated local resistance to *Botrytis cinerea, Hyaloperonospora arabidopsidis* or *Pseudomonas syringae* pv. *tomato* (*Pst*) involves different signaling pathways that depend on the type of pathogen (Sanchez et al., 2012). In addition, rhamnolipid potentiate defense responses induced by other elicitors like chitosan. The immune response triggered by rhamnolipids also participates in local resistance against *B. cinerea* and the hemibiotrophic fungus *Leptosphaeria maculans* in *B. napus* (Monnier et al., 2018; Monnier et al., 2020). A large range of rhamnolipid concentrations from 0.005 to 1 mg/mL have been used to induce immunity on these various plant species (Varnier et al., 2009; Sanchez et al., 2012; Monnier et al., 2018; Monnier et al., 2020). Synthetic biosurfactants derived from rhamnolipid structure are also elicitors. For instance, synthetic rhamnolipid bolaforms, composed of two rhamnoses separated by a fatty acid chain, trigger an immune response in Arabidopsis that varies according to fatty acid chain length (Luzuriaga-Loaiza et al., 2018). In addition, RL harboring carboxylic acid (Ac-RL) and methyl (Alk-RL) induce ROS production in this plant (Nasir et al., 2017).

The way by which rhamnolipids are perceived by plant cells still remains unknown. Given their amphiphilic nature, it is postulated that they could interact with plant membrane lipids (Sanchez et al., 2012; Schellenberger et al., 2019). Recently it has been demonstrated that natural rhamnolipids fit into plant lipid-based membrane models and are located near the lipid phosphate group of the phospholipid bilayers, nearby phospholipid glycerol backbones (Monnier et al., 2019). Rhamnolipid insertion inside the lipid bilayer does not strongly affect lipid dynamics but the nature of the phytosterols could influence the effect of the glycolipids on plant plasma membrane destabilization. These subtle changes in lipid dynamics could be linked with plant defense induction (Monnier et al., 2019). Interestingly, whereas no receptor for rhamnolipid perception has been identified so far, the mc-3-OH-acyl building block of rhamnolipids is sensed by the lectin S-domain-1 receptor–like kinase LORE (Kutschera et al., 2019) (Figure 1).

For some studies, it is not demonstrated whether rhamnolipid-triggered protection is driven by activation of plant defense responses and/or antimicrobial properties. For instance, treatments of pepper plants with rhamnolipids result in an enhanced protection to *Phytophthora* blight disease and also prevent the development of *Colletotrichum orbiculare* infection on leaves of cucumber plants (Kim et al., 2000). Rhamnolipids significantly decrease the incidence of water-borne damping-off disease by *Phytophthora* sp. and *Pythium* sp. (Yoo et al., 2005). Similar results were obtained in field trials on chili pepper and tomato (Sharma et al., 2007a; Sharma et al., 2007b). Using bacterial mutants, it was demonstrated that phenazine and rhamnolipids interact in the biological control of soil-borne diseases caused by *Pythium* sp. (Perneel et al., 2008). Syringomycin E and rhamnolipids can also act synergistically to control pathogenic and opportunistic fungi recovered from diseased grape (Takemoto et al., 2010). The control of postharvest phytopathogens on seeds or fruits for a better conservation is often related to antimicrobial activities. But we cannot exclude that protection could also be due to plant defense responses (Borah et al., 2016). When applied alone, rhamnolipids induce antioxidative reactions in cherry tomato fruit, leading to a significant reduction of fungal disease (Yan et al., 2015). When applied in combination with the biocontrol yeast agent *Rhodotorula glutinis,* a synergistic inhibitory effect on *Alternaria alternata* infection could be observed in cherry tomato fruit, leading to an efficient protection (Yan et al., 2014). This protection is associated with a higher induction of defense-related enzymes and the accumulation of antimicrobial metabolites.

Lipopeptides as powerful inducers of plant systemic resistance

Several studies have reported the involvement of *Bacillus* CLPs in plant immunity induction on various pathosystems. The potential of fengycin and surfactin CLPs to trigger plant systemic resistance was first shown on bean and tomato plants. When applied as pure compounds at micromolar concentrations, surfactin and to a lower extend fengycin induced significant disease reduction in bean and tomato infected with *B. cinerea* (Ongena et al., 2007). More recently, a study performed with a large range of natural *Bacillus* isolates strengthened the role of surfactin as ISR (induced systemic resistance) inducer since strong correlation was observed between defense-inducing activity and the amount of surfactin produced by the different strains (Cawoy et al., 2014). In the same way, *B. velezensis* FZB42 mutant strains unable to synthesize surfactin are impaired in their ISR to *Rhizoctonia solani* in lettuce (Chowdhury et al., 2015). Further studies allowed enlarging the ISR elicitor role of surfactin to other plants. For example, purified surfactin was shown to increase resistance against the cucurbit powdery mildew in melon plants (García-Gutiérrez et al., 2013). In the pathosystem citrus fruit/*Penicillium digitatum*, surfactin stimulates defense responses involved in generating signal molecules for ISR activation (Waewthongrak et al., 2014). This lipopeptide activates a plant innate response effective against *Magnaporthe oryzae* in perennial ryegrass (Rahman et al., 2015) or *Plasmopara viticola* in grapevine (Li et al., 2019). It also reduces infection by the rhizomania disease vector *Polymyxa betae* in sugar beet (Desoignies et al., 2013) or by *Colletotrichum gloeosporioides* in strawberry leaves (Yamamoto et al., 2015). Finally, a recent study showed that *Sclerotium rolfsii* disease incidence was strongly reduced in *Arachis hypogaea* when pretreated with surfactin (Rodríguez et al., 2018). Interestingly, CLPs like surfactin do not globally provoke a strong plant defensive response associated with major genetic reprogramming and fitness cost but rather act by priming host defenses to trigger systemic resistance (Ongena et al., 2007; Jourdan et al., 2009; Debois et al., 2015). Induction of plant defenses by CLPs of the iturin group has also been occasionally reported. Iturin A was shown to have a similar role as surfactin in strawberry leaves (Yamamoto et al., 2015) and also acted as an inducer of plant defense gene expression in cotton plants upon *Verticillium dahliae* attack (Han et al., 2015). Mycosubtilin is the most efficient lipopeptide inducing an immune response in grapevine (Farace et al., 2015). Compared to surfactin, bacillomycin D produced by *B. velezensis* SQR9 has a comparable efficacy in *Arabidopsis* ISR elicitation to prevent infection by *P. syringae* or *B. cinerea* (Wu et al., 2018). In wheat plants, resistance towards *Zymoseptoria tritici*, was activated by pure surfactin used at concentrations ranging from 1 to 100 µM upon foliar application (Le Mire et al., 2018). Some CLPs synthesized by *Pseudomonas* sp. also display consistent ISR-triggering activity. It was first demonstrated that massetolide A produced by *Pseudomonas fluorescens* strain SS101 retains ISR-eliciting activity in tomato plants for the control of *Phytophthora infestans* (Tran et al., 2007). *Pseudomonas* sp. strain CMR12a is a soil isolate retaining high biocontrol potential against *R. solani* relying mainly on the interplay between two different lipopeptides (sessilin and orfamide) and phenazine for inducing plant immunity (D'Aes et al., 2011; D'aes et al., 2014). These CLPs were also active at protecting *Brassica chinensis* against *R. solani* (Olorunleke et al., 2015). In monocots, such as rice, orfamide and other *Pseudomonas* CLPs such as WLIP, lokisin and entolysin, successfully induced resistance towards *C. miyabeanus* or *M. oryzae* (Ma et al., 2016; Ma et al., 2017; Omoboye et al., 2019).

Up to now, how lipopeptides act and are recognized by plants cells to activate ISR remains unclear. CLPs are in most instances only active in micromolar concentrations, and defenses are more intensively induced at the highest surfactin doses. This suggests that the recognition mechanism at the plant cell surface should be of quite low affinity in contrast to other elicitors (Jourdan et al., 2009). Such a low specificity may be explained by the fact that CLPs like surfactin are not perceived by a protein receptor, but rather involve a process driven by an uncommon pathway based on interaction with the lipid bilayer fraction of plant plasma membranes (Henry et al., 2011). This is supported by some studies revealing that CLP structure plays an important role for the ISR eliciting activity. Both the fatty acid chain length, the cyclic conformation of the molecule and amino acid positions in the peptide chain impact the eliciting potential of surfactin in tobacco cells (Jourdan et al., 2009; Henry et al., 2011). The activation of defense genes in Arabidopsis upon iturin A foliar treatment was also dependent on the structure of the molecule, *i.e.* cyclization and/or nature of the fatty acid chain (Kawagoe et al., 2015). As for rhamnolipids, the hypothesis is that lipopeptides have the ability to create some disturbance in the plant plasma membrane and could consequently activate a cascade of molecular events leading to the activation of defense mechanisms (Schellenberger et al., 2019) (figure 1).

Conclusion

Biosurfactants, produced by bacteria, yeast, and fungi, are promising molecules for a wide variety of applications due to their potential to be commercially produced at large scales, their low toxicity and high biodegradability. In this mini-review, we provided evidences about the potential of rhamnolipids and lipopeptides for plant protection in a context of sustained agriculture. These molecules have similar dual effects by protecting plants through antimicrobial properties and stimulation of local and/or systemic plant immunity. These singular properties are essential for the efficiency of these biopesticides. Although numerous elicitors are perceived by plasma membrane receptors, recent studies on amphiphilic biosurfactants such as rhamnolipids or lipopeptides suggest that are sensed by an uncommon way involving lipids in the bilayer of the plant plasma membrane that could explain their singular elicitor activity. To better understand the mechanisms of action of biosurfactants, experiments or trials need to be realized not only on mixture but also on highly purified molecules in the future. Nevertheless, several obstacles to the development of rhamnolipid and lipopeptide applications still remain. Biosurfactant costs, their efficacies in the field and purity of compounds have to be improved to allow their use at a higher degree in crop protection. In this respect, combination of biosurfactants should be considered to increase efficacy in field conditions. Finally, given their interesting properties it is now time to really consider ecofriendly biosurfactants as biocontrol solutions in integrated pest management.

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References

Abbasi, H., Aranda, F.J., Noghabi, K.A., and Ortiz, A. (2013). A bacterial monorhamnolipid alters the biophysical properties of phosphatidylethanolamine model membranes. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1828(9)**,** 2083-2090. doi: https://doi.org/10.1016/j.bbamem.2013.04.024.

Abbasi, H., Noghabi, K.A., and Ortiz, A. (2012). Interaction of a bacterial monorhamnolipid secreted by *Pseudomonas aeruginosa* MA01 with phosphatidylcholine model membranes. *Chemistry and Physics of Lipids* 165(7)**,** 745-752. doi: https://doi.org/10.1016/j.chemphyslip.2012.09.001.

Abdel-Mawgoud, A.M., Lepine, F., and Deziel, E. (2010). Rhamnolipids: diversity of structures, microbial origins and roles. *Applied Microbiology and Biotechnology* 86(5)**,** 1323-1336. doi: 10.1007/s00253-010-2498-2.

Aranda, F.J., Espuny, M.J., Marqués, A., Teruel, J.A., Manresa, Á., and Ortiz, A. (2007). Thermodynamics of the interaction of a dirhamnolipid biosurfactant secreted by *Pseudomonas aeruginosa* with phospholipid membranes. *Langmuir* 23(5)**,** 2700-2705. doi: 10.1021/la061464z.

Bais, H.P., Fall, R., and Vivanco, J.M. (2004). Biocontrol of *Bacillus subtilis* against infection of Arabidopsis roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. *Plant physiology* 134(1)**,** 307-319. doi: 10.1104/pp.103.028712.

Banat, I.M., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M.G., Fracchia, L., et al. (2010). Microbial biosurfactants production, applications and future potential. *Applied Microbiology and Biotechnology* 87(2)**,** 427-444. doi: 10.1007/s00253-010-2589-0.

Bardin, M., Ajouz, S., Comby, M., Lopez-Ferber, M., Graillot, B., Siegwart, M., et al. (2015). Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides? *Frontiers in Plant Science* 6**,** 566. doi: 10.3389/fpls.2015.00566.

Benincasa, M., Abalos, A., Oliveira, I., and Manresa, A. (2004). Chemical structure, surface properties and biological activities of the biosurfactant produced by *Pseudomonas aeruginosa* LBI from soapstock. *Antonie Van Leeuwenhoek* 85(1)**,** 1-8. doi: 10.1023/B:ANTO.0000020148.45523.41

5105181 [pii].

Berg, G., Koberl, M., Rybakova, D., Muller, H., Grosch, R., and Smalla, K. (2017). Plant microbial diversity is suggested as the key to future biocontrol and health trends. *FEMS Microbiology Ecology* 93(5). doi: 10.1093/femsec/fix050.

Bigeard, J., Colcombet, J., and Hirt, H. (2015). Signaling mechanisms in pattern-triggered immunity (PTI). *Molecular Plant* 8(4)**,** 521-539. doi: 10.1016/j.molp.2014.12.022.

Boller, T., and Felix, G. (2009). A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annual Review of Plant Biology* 60**,** 379-406. doi: 10.1146/annurev.arplant.57.032905.105346.

Bonanomi, G., Lorito, M., Vinale, F., and Woo, S.L. (2018). Organic amendments, beneficial microbes, and soil microbiota: toward a unified framework for disease suppression. *Annual Review of Phytopathology* 56(1)**,** 1-20. doi: 10.1146/annurev-phyto-080615-100046.

Borah, S.N., Goswami, D., Lahkar, J., Sarma, H.K., Khan, M.R., and Deka, S. (2015). Rhamnolipid produced by *Pseudomonas aeruginosa* SS14 causes complete suppression of wilt by *Fusarium oxysporum* f. sp. *pisi* in *Pisum sativum*. *BioControl* 60(3)**,** 375-385. doi: 10.1007/s10526-014-9645-0.

Borah, S.N., Goswami, D., Sarma, H.K., Cameotra, S.S., and Deka, S. (2016). Rhamnolipid biosurfactant against *Fusarium verticillioides* to control stalk and ear rot disease of maize. *Frontiers in Microbiology* 7**,** 1505. doi: 10.3389/fmicb.2016.01505.

Burketova, L., Trda, L., Ott, P.G., and Valentova, O. (2015). Bio-based resistance inducers for sustainable plant protection against pathogens. *Biotechnology Advances* 33(6 Pt 2)**,** 994-1004. doi: 10.1016/j.biotechadv.2015.01.004.

Cao, Y., Pi, H., Chandrangsu, P., Li, Y., Wang, Y., Zhou, H., et al. (2018). Antagonism of Two Plant-Growth Promoting Bacillus velezensis Isolates Against Ralstonia solanacearum and Fusarium oxysporum. *Scientific Reports* 8(1)**,** 4360. doi: 10.1038/s41598-018-22782-z.

Caulier, S., Nannan, C., Gillis, A., Licciardi, F., Bragard, C., and Mahillon, J. (2019). Overview of the antimicrobial compounds produced by members of the *Bacillus subtilis* group. *Frontiers in Microbiology* 10(302). doi: 10.3389/fmicb.2019.00302.

Cawoy, H., Mariutto, M., Henry, G., Fisher, C., Vasilyeva, N., Thonart, P., et al. (2014). Plant Defense Stimulation by Natural Isolates of Bacillus Depends on Efficient Surfactin Production. *Molecular Plant-Microbe Interactions* 27(2)**,** 87-100. doi: 10.1094/mpmi-09-13-0262-r.

Chen, J., Liu, X., Fu, S., An, Z., Feng, Y., Wang, R., et al. (2020). Effects of sophorolipids on fungal and oomycete pathogens in relation to pH solubility. *Journal of Applied Microbiology* n/a(n/a). doi: 10.1111/jam.14594.

Chen, Y., Yan, F., Chai, Y., Liu, H., Kolter, R., Losick, R., et al. (2013). Biocontrol of tomato wilt disease by Bacillus subtilis isolates from natural environments depends on conserved genes mediating biofilm formation. *Environmental Microbiology* 15(3)**,** 848-864. doi: 10.1111/j.1462-2920.2012.02860.x.

Chitarra, G.S., Breeuwer, P., Nout, M.J.R., Van Aelst, A.C., Rombouts, F.M., and Abee, T. (2003). An antifungal compound produced by *Bacillus subtilis* YM 10–20 inhibits germination of Penicillium roqueforti conidiospores. *Journal of Applied Microbiology* 94(2)**,** 159-166. doi: 10.1046/j.1365-2672.2003.01819.x.

Chowdhury, S.P., Hartmann, A., Gao, X., and Borriss, R. (2015). Biocontrol mechanism by root-associated Bacillus amyloliquefaciens FZB42 – a review. *Frontiers in Microbiology* 6(780). doi: 10.3389/fmicb.2015.00780.

Coelho, A.L.S., Feuser, P.E., Carciofi, B.A.M., de Andrade, C.J., and de Oliveira, D. (2020). Mannosylerythritol lipids: antimicrobial and biomedical properties. *Applied Microbiology and Biotechnology* 104(6)**,** 2297-2318. doi: 10.1007/s00253-020-10354-z.

D'Aes, J., Hua, G.K., De Maeyer, K., Pannecoucque, J., Forrez, I., Ongena, M., et al. (2011). Biological control of *Rhizoctonia* root rot on bean by phenazine- and cyclic lipopeptide-producing *Pseudomonas* CMR12a. *Phytopathology* 101(8)**,** 996-1004. doi: 10.1094/phyto-11-10-0315.

D'Aes, J., Kieu, N.P., Léclère, V., Tokarski, C., Olorunleke, F.E., De Maeyer, K., et al. (2014). To settle or to move? The interplay between two classes of cyclic lipopeptides in the biocontrol strain Pseudomonas CMR12a. *Environmental Microbiology* 16(7)**,** 2282-2300. doi: 10.1111/1462-2920.12462.

De Jonghe, K., De Dobbelaere, I., Sarrazyn, R., and Höfte, M. (2005). Control of *Phytophthora cryptogea* in the hydroponic forcing of witloof chicory with the rhamnolipid-based biosurfactant formulation PRO1. *Plant pathology* 54**,** 219-226.

Debois, D., Fernandez, O., Franzil, L., Jourdan, E., de Brogniez, A., Willems, L., et al. (2015). Plant polysaccharides initiate underground crosstalk with bacilli by inducing synthesis of the immunogenic lipopeptide surfactin. *Environmental microbiology reports* 7(3)**,** 570-582. doi: 10.1111/1758-2229.12286.

Deepika, K.V., Ramu Sridhar, P., and Bramhachari, P.V. (2015). Characterization and antifungal properties of rhamnolipids produced by mangrove sediment bacterium *Pseudomonas aeruginosa* strain KVD-HM52. *Biocatalysis and Agricultural Biotechnology* 4(4)**,** 608-615. doi: https://doi.org/10.1016/j.bcab.2015.09.009.

Delaunois, B., Farace, G., Jeandet, P., Clement, C., Baillieul, F., Dorey, S., et al. (2014). Elicitors as alternative strategy to pesticides in grapevine? Current knowledge on their mode of action from controlled conditions to vineyard. *Environmental science and pollution research international* 21(7)**,** 4837-4846. doi: 10.1007/s11356-013-1841-4.

Desoignies, N., Schramme, F., Ongena, M., and Legrève, A. (2013). Systemic resistance induced by Bacillus lipopeptides in Beta vulgaris reduces infection by the rhizomania disease vector Polymyxa betae. *Molecular Plant Pathology* 14(4)**,** 416-421. doi: 10.1111/mpp.12008.

Etchegaray, A., de Castro Bueno, C., de Melo, I.S., Tsai, S.M., de Fátima Fiore, M., Silva-Stenico, M.E., et al. (2008). Effect of a highly concentrated lipopeptide extract of *Bacillus subtilis* on fungal and bacterial cells. *Archives of Microbiology* 190(6)**,** 611-622. doi: 10.1007/s00203-008-0409-z.

Farace, G., Fernandez, O., Jacquens, L., Coutte, F., Krier, F., Jacques, P., et al. (2015). Cyclic lipopeptides from Bacillus subtilis activate distinct patterns of defence responses in grapevine. *Molecular Plant Pathology* 16(2)**,** 177-187. doi: 10.1111/mpp.12170.

Fu, Z.Q., and Dong, X. (2013). Systemic acquired resistance: turning local infection into global defense. *Annual Review of Plant Biology* 64(1)**,** 839-863. doi: 10.1146/annurev-arplant-042811-105606.

Gao, L., Han, J., Liu, H., Qu, X., Lu, Z., and Bie, X. (2017). Plipastatin and surfactin coproduction by Bacillus subtilis pB2-L and their effects on microorganisms. *Antonie van Leeuwenhoek* 110(8)**,** 1007-1018. doi: 10.1007/s10482-017-0874-y.

Garcia-Brugger, A., Lamotte, O., Vandelle, E., Bourque, S., Lecourieux, D., Poinssot, B., et al. (2006). Early signaling events induced by elicitors of plant defenses. *Molecular Plant-Microbe Interactions* 19(7)**,** 711-724.

García-Gutiérrez, L., Zeriouh, H., Romero, D., Cubero, J., de Vicente, A., and Pérez-García, A. (2013). The antagonistic strain Bacillus subtilis UMAF6639 also confers protection to melon plants against cucurbit powdery mildew by activation of jasmonate- and salicylic acid-dependent defence responses. *Microbial Biotechnology* 6(3)**,** 264-274. doi: 10.1111/1751-7915.12028.

Geudens, N., and Martins, J.C. (2018). Cyclic lipodepsipeptides from *Pseudomonas* spp. – biological swiss-army knives. *Frontiers in Microbiology* 9(1867). doi: 10.3389/fmicb.2018.01867.

Gong, A.-D., Li, H.-P., Yuan, Q.-S., Song, X.-S., Yao, W., He, W.-J., et al. (2015). Antagonistic mechanism of Iturin A and Plipastatin A from *Bacillus amyloliquefaciens* S76-3 from wheat spikes against *Fusarium graminearum*. *PLOS one* 10(2)**,** e0116871. doi: 10.1371/journal.pone.0116871.

Goswami, D., Borah, S.N., Lahkar, J., Handique, P.J., and Deka, S. (2015). Antifungal properties of rhamnolipid produced by *Pseudomonas aeruginosa* DS9 against *Colletotrichum falcatum*. *Journal of Basic Microbiology* 55(11)**,** 1265-1274. doi: 10.1002/jobm.201500220.

Goswami, D., Handique, P.J., and Deka, S. (2014). Rhamnolipid biosurfactant against *Fusarium sacchari*--the causal organism of pokkah boeng disease of sugarcane. *Journal of Basic Microbiology* 54(6)**,** 548-557. doi: 10.1002/jobm.201200801.

Götze, S., and Stallforth, P. (2020). Structure, properties, and biological functions of nonribosomal lipopeptides from pseudomonads. *Natural product reports* 37(1)**,** 29-54. doi: 10.1039/c9np00022d.

Grau, A., Gómez Fernández, J.C., Peypoux, F., and Ortiz, A. (1999). A study on the interactions of surfactin with phospholipid vesicles. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1418(2)**,** 307-319. doi: https://doi.org/10.1016/S0005-2736(99)00039-5.

Haba, E., Pinazo, A., Jauregui, O., Espuny, M.J., Infante, M.R., and Manresa, A. (2003). Physiochemical characterization and antimicrobial properties of rhamnolipids produced by *Pseudomonas aeruginosa* 47T2 NCBIM 40044. *Biotechnology and Bioengineering* 81(3)**,** 316-322.

Han, Q., Wu, F., Wang, X., Qi, H., Shi, L., Ren, A., et al. (2015). The bacterial lipopeptide iturins induce Verticillium dahliae cell death by affecting fungal signalling pathways and mediate plant defence responses involved in pathogen-associated molecular pattern-triggered immunity. *Environmental Microbiology* 17(4)**,** 1166-1188. doi: 10.1111/1462-2920.12538.

Henry, G., Deleu, M., Jourdan, E., Thonart, P., and Ongena, M. (2011). The bacterial lipopeptide surfactin targets the lipid fraction of the plant plasma membrane to trigger immune-related defence responses. *Cellular Microbiology* 13(11)**,** 1824-1837. doi: 10.1111/j.1462-5822.2011.01664.x.

Hoefler, B.C., Gorzelnik, K.V., Yang, J.Y., Hendricks, N., Dorrestein, P.C., and Straight, P.D. (2012). Enzymatic resistance to the lipopeptide surfactin as identified through imaging mass spectrometry of bacterial competition. *Proceedings of the National Academy of Sciences* 109(32)**,** 13082-13087. doi: 10.1073/pnas.1205586109.

Jahan, R., Bodratti, A.M., Tsianou, M., and Alexandridis, P. (2020). Biosurfactants, natural alternatives to synthetic surfactants: Physicochemical properties and applications. *Advances in Colloid and Interface Science* 275**,** 102061. doi: https://doi.org/10.1016/j.cis.2019.102061.

Jones, J.D., Vance, R.E., and Dangl, J.L. (2016). Intracellular innate immune surveillance devices in plants and animals. *Science* 354(6316). doi: 10.1126/science.aaf6395.

Jourdan, E., Henry, G., Duby, F., Dommes, J., Barthelemy, J.P., Thonart, P., et al. (2009). Insights into the defense-related events occurring in plant cells following perception of surfactin-type lipopeptide from *Bacillus subtilis*. *Molecular Plant-Microbe Interactions* 22(4)**,** 456-468. doi: 10.1094/MPMI-22-4-0456.

Kawagoe, Y., Shiraishi, S., Kondo, H., Yamamoto, S., Aoki, Y., and Suzuki, S. (2015). Cyclic lipopeptide iturin A structure-dependently induces defense response in Arabidopsis plants by activating SA and JA signaling pathways. *Biochemical and Biophysical Research Communications* 460(4)**,** 1015-1020. doi: https://doi.org/10.1016/j.bbrc.2015.03.143.

Keswani, C., Singh, H.B., Hermosa, R., Garcia-Estrada, C., Caradus, J., He, Y.W., et al. (2019). Antimicrobial secondary metabolites from agriculturally important fungi as next biocontrol agents. *Applied Microbiology and Biotechnology* 103(23-24)**,** 9287-9303. doi: 10.1007/s00253-019-10209-2.

Kim, B.S., Lee, J.Y., and Hwang, B.K. (2000). *In vivo* control and *in vitro* antifungal activity of rhamnolipid B, a glycolipid antibiotic, against *Phytophthora capsici* and *Colletotrichum orbiculare*. *Pest Management Science* 56(12)**,** 1029-1035.

Kutschera, A., Dawid, C., Gisch, N., Schmid, C., Raasch, L., Gerster, T., et al. (2019). Bacterial medium-chain 3-hydroxy fatty acid metabolites trigger immunity in Arabidopsis plants. *Science* 364(6436)**,** 178-181. doi: 10.1126/science.aau1279.

Latoud, C., Peypoux, F., and Michel, G. (1987). Action of iturin A, an antifungal antibiotic from Bacillus subtilis, on the yeast Saccharomyces cerevisiae: modifications of membrane permeability and lipid composition. *J Antibiot (Tokyo)* 40(11)**,** 1588-1595. doi: 10.7164/antibiotics.40.1588.

Le Mire, G., Siah, A., Brisset, M.-N., Gaucher, M., Deleu, M., and Jijakli, M.H. (2018). Surfactin Protects Wheat against Zymoseptoria tritici and Activates Both Salicylic Acid- and Jasmonic Acid-Dependent Defense Responses. *Agriculture* 8(1)**,** 11.

Li, Y., Héloir, M.-C., Zhang, X., Geissler, M., Trouvelot, S., Jacquens, L., et al. (2019). Surfactin and fengycin contribute to the protection of a Bacillus subtilis strain against grape downy mildew by both direct effect and defence stimulation. *Molecular Plant Pathology* 20(8)**,** 1037-1050. doi: 10.1111/mpp.12809.

Luo, C., Zhou, H., Zou, J., Wang, X., Zhang, R., Xiang, Y., et al. (2015). Bacillomycin L and surfactin contribute synergistically to the phenotypic features of Bacillus subtilis 916 and the biocontrol of rice sheath blight induced by Rhizoctonia solani. *Applied Microbiology and Biotechnology* 99(4)**,** 1897-1910. doi: 10.1007/s00253-014-6195-4.

Luzuriaga-Loaiza, P., Schellenberger, R., De Gaetano, Y., Obounou Akong, F., Villaume, S., Crouzet, J., et al. (2018). Synthetic rhamnolipid bolaforms trigger an innate immune response in *Arabidopsis thaliana*. *Scientific Reports*.

Ma, Z., Hua, G.K.H., Ongena, M., and Hofte, M. (2016). Role of phenazines and cyclic lipopeptides produced by pseudomonas sp. CMR12a in induced systemic resistance on rice and bean. *Environ Microbiol Rep* 8(5)**,** 896-904. doi: 10.1111/1758-2229.12454.

Ma, Z., Ongena, M., and Hofte, M. (2017). The cyclic lipopeptide orfamide induces systemic resistance in rice to Cochliobolus miyabeanus but not to Magnaporthe oryzae. *Plant Cell Rep* 36(11)**,** 1731-1746. doi: 10.1007/s00299-017-2187-z.

Mnif, I., and Ghribi, D. (2016). Glycolipid biosurfactants: main properties and potential applications in agriculture and food industry. *Journal of the Science of Food and Agriculture* 96(13)**,** 4310-4320. doi: 10.1002/jsfa.7759.

Monnier, N., Cordier, M., Dahi, A., Santoni, V., Guenin, S., Clement, C., et al. (2020). Semi-purified rhamnolipid mixes protect *Brassica napus* against *Leptosphaeria maculans* early infections. *Phytopathology* 110(4)**,** 834-842. doi: 10.1094/phyto-07-19-0275-r.

Monnier, N., Furlan, A., Botcazon, C., Dahi, A., Mongelard, G., Cordelier, S., et al. (2018). Rhamnolipids from *Pseudomonas aeruginosa* are elicitors triggering *Brassica napus* protection against *Botrytis cinerea* without physiological disorders. *Frontiers in Plant Sciences* 9**,** 1170. doi: 10.3389/fpls.2018.01170.

Monnier, N., Furlan, A.L., Buchoux, S., Deleu, M., Dauchez, M., Rippa, S., et al. (2019). Exploring the dual interaction of natural rhamnolipids with plant and fungal biomimetic plasma membranes through biophysical studies. *International Journal of Molecular Sciences* 20(5). doi: 10.3390/ijms20051009.

Nalini, S., and Parthasarathi, R. (2014). Production and characterization of rhamnolipids produced by *Serratia rubidaea* SNAU02 under solid-state fermentation and its application as biocontrol agent. *Bioresource Technology* 173**,** 231-238. doi: https://doi.org/10.1016/j.biortech.2014.09.051.

Nasir, M.N., Lins, L., Crowet, J.M., Ongena, M., Dorey, S., Dhondt-Cordelier, S., et al. (2017). Differential interaction of synthetic glycolipids with biomimetic plasma membrane lipids correlates with the plant biological response. *Langmuir* 33(38)**,** 9979-9987. doi: 10.1021/acs.langmuir.7b01264.

Naughton, P.J., Marchant, R., Naughton, V., and Banat, I.M. (2019). Microbial biosurfactants: current trends and applications in agricultural and biomedical industries. *Journal of Applied Microbiology* 127(1)**,** 12-28. doi: 10.1111/jam.14243.

Olorunleke, F.E., Hua, G.K.H., Kieu, N.P., Ma, Z., and Höfte, M. (2015). Interplay between orfamides, sessilins and phenazines in the control of Rhizoctonia diseases by Pseudomonas sp. CMR12a. *Environmental Microbiology Reports* 7(5)**,** 774-781. doi: 10.1111/1758-2229.12310.

Omoboye, O.O., Oni, F.E., Batool, H., Yimer, H.Z., De Mot, R., and Höfte, M. (2019). Pseudomonas Cyclic Lipopeptides Suppress the Rice Blast Fungus Magnaporthe oryzae by Induced

Resistance and Direct Antagonism. *Frontiers in Plant Science* 10(901). doi: 10.3389/fpls.2019.00901.

Ongena, M., Jourdan, E., Adam, A., Paquot, M., Brans, A., Joris, B., et al. (2007). Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. *Environmental Microbiology* 9(4)**,** 1084-1090. doi: EMI1202 [pii]

10.1111/j.1462-2920.2006.01202.x.

Ortiz, A., Teruel, J.A., Espuny, M.J., Marqués, A., Manresa, Á., and Aranda, F.J. (2006). Effects of dirhamnolipid on the structural properties of phosphatidylcholine membranes. *International Journal of Pharmaceutics* 325(1)**,** 99-107. doi: https://doi.org/10.1016/j.ijpharm.2006.06.028.

Otzen, D.E. (2017). Biosurfactants and surfactants interacting with membranes and proteins: Same but different? *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1859(4)**,** 639-649. doi: https://doi.org/10.1016/j.bbamem.2016.09.024.

Penha, R.O., Vandenberghe, L.P.S., Faulds, C., Soccol, V.T., and Soccol, C.R. (2020). Bacillus lipopeptides as powerful pest control agents for a more sustainable and healthy agriculture: recent studies and innovations. *Planta* 251(3)**,** 70. doi: 10.1007/s00425-020-03357-7.

Pérez-García, A., Romero, D., and de Vicente, A. (2011). Plant protection and growth stimulation by microorganisms: biotechnological applications of Bacilli in agriculture. *Current Opinion in Biotechnology* 22(2)**,** 187-193. doi: https://doi.org/10.1016/j.copbio.2010.12.003.

Perneel, M., D'Hondt, L., De Maeyer, K., Adiobo, A., Rabaey, K., and Hofte, M. (2008). Phenazines and biosurfactants interact in the biological control of soil-borne diseases caused by *Pythium* spp. *Environmental Microbiology* 10(3)**,** 778-788.

Pieterse, C.M., Van der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S.C. (2012). Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology* 28**,** 489-521. doi: 10.1146/annurev-cellbio-092910-154055.

Pieterse, C.M.J., Zamioudis, C., Berendsen, R.L., Weller, D.M., Wees, S.C.M.V., and Bakker, P.A.H.M. (2014). Induced Systemic Resistance by Beneficial Microbes. *Annual Review of Phytopathology* 52(1)**,** 347-375. doi: 10.1146/annurev-phyto-082712-102340.

Popp, J., Pető, K., and Nagy, J. (2013). Pesticide productivity and food security. A review. *Agronomy for Sustainable Development* 33(1)**,** 243-255. doi: 10.1007/s13593-012-0105-x.

Qi, G., Zhu, F., Du, P., Yang, X., Qiu, D., Yu, Z., et al. (2010). Lipopeptide induces apoptosis in fungal cells by a mitochondria-dependent pathway. *Peptides* 31(11)**,** 1978-1986. doi: https://doi.org/10.1016/j.peptides.2010.08.003.

Qian, S., Lu, H., Sun, J., Zhang, C., Zhao, H., Lu, F., et al. (2016). Antifungal activity mode of *Aspergillus ochraceus* by bacillomycin D and its inhibition of ochratoxin A (OTA) production in food samples. *Food Control* 60**,** 281-288. doi: https://doi.org/10.1016/j.foodcont.2015.08.006.

Raaijmakers, J.M., De Bruijn, I., Nybroe, O., and Ongena, M. (2010). Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. *FEMS Microbiology Reviews* 34(6)**,** 1037-1062. doi: FMR221 [pii]

10.1111/j.1574-6976.2010.00221.x.

Rabbee, M.F., Ali, M.S., Choi, J., Hwang, B.S., Jeong, S.C., and Baek, K.H. (2019). *Bacillus velezensis:* A valuable member of bioactive molecules within plant microbiomes. *Molecules* 24(6). doi: 10.3390/molecules24061046.

Rahman, A., Uddin, W., and Wenner, N.G. (2015). Induced systemic resistance responses in perennial ryegrass against Magnaporthe oryzae elicited by semi-purified surfactin lipopeptides and live cells of Bacillus amyloliquefaciens. *Molecular Plant Pathology* 16(6)**,** 546-558. doi: 10.1111/mpp.12209.

Rodrigues, A.I., Gudina, E.J., Teixeira, J.A., and Rodrigues, L.R. (2017). Sodium chloride effect on the aggregation behaviour of rhamnolipids and their antifungal activity. *Scientific Reports* 7(1)**,** 12907. doi: 10.1038/s41598-017-13424-x.

Rodríguez, J., Tonelli, M.L., Figueredo, M.S., Ibáñez, F., and Fabra, A. (2018). The lipopeptide surfactin triggers induced systemic resistance and priming state responses in Arachis hypogaea L. *European Journal of Plant Pathology* 152(3)**,** 845-851. doi: 10.1007/s10658-018-1524-6.

Romero, D., de Vicente, A., Rakotoaly, R.H., Dufour, S.E., Veening, J.-W., Arrebola, E., et al. (2007). The Iturin and Fengycin families of lipopeptides are key factors in antagonism of *Bacillus subtilis* toward *Podosphaera fusca*. *Molecular Plant-Microbe Interactions* 20(4)**,** 430-440. doi: 10.1094/mpmi-20-4-0430.

Sajid, M., Ahmad Khan, M.S., Singh Cameotra, S., and Safar Al-Thubiani, A. (2020). Biosurfactants: Potential applications as immunomodulator drugs. *Immunology Letters* 223**,** 71-77. doi: 10.1016/j.imlet.2020.04.003.

Sanchez, L., Courteaux, B., Hubert, J., Kauffmann, S., Renault, J.H., Clément, C., et al. (2012). Rhamnolipids elicit defense responses and induce disease resistance against biotrophic, hemibiotrophic, and necrotrophic pathogens that require different signaling pathways in *Arabidopsis* and highlight a central role for salicylic acid. *Plant Physiology* 160(3)**,** 1630-1641. doi: 10.1104/pp.112.201913.

Sánchez, M., Aranda, F.J., Teruel, J.A., Espuny, M.J., Marqués, A., Manresa, Á., et al. (2010). Permeabilization of biological and artificial membranes by a bacterial dirhamnolipid produced by *Pseudomonas aeruginosa*. *Journal of Colloid and Interface Science* 341(2)**,** 240-247. doi: https://doi.org/10.1016/j.jcis.2009.09.042.

Sánchez, M., Aranda, F.J., Teruel, J.A., and Ortiz, A. (2009). Interaction of a bacterial dirhamnolipid with phosphatidylcholine membranes: a biophysical study. *Chemistry and Physics of Lipids* 161(1)**,** 51-55. doi: https://doi.org/10.1016/j.chemphyslip.2009.06.145.

Sánchez, M., Teruel, J.A., Espuny, M.J., Marqués, A., Aranda, F.J., Manresa, Á., et al. (2006). Modulation of the physical properties of dielaidoylphosphatidylethanolamine membranes by a dirhamnolipid biosurfactant produced by *Pseudomonas aeruginosa*. *Chemistry and Physics of Lipids* 142(1)**,** 118-127. doi: https://doi.org/10.1016/j.chemphyslip.2006.04.001.

Sathi Reddy, K., Yahya Khan, M., Archana, K., Gopal Reddy, M., and Hameeda, B. (2016). Utilization of mango kernel oil for the rhamnolipid production by *Pseudomonas aeruginosa* DR1 towards its application as biocontrol agent. *Bioresource Technology* 221**,** 291-299. doi: 10.1016/j.biortech.2016.09.041.

Schellenberger, R., Touchard, M., Clement, C., Baillieul, F., Cordelier, S., Crouzet, J., et al. (2019). Apoplastic invasion patterns triggering plant immunity: plasma membrane sensing at the frontline. *Molecular Plant Pathology* 20(11)**,** 1602-1616. doi: 10.1111/mpp.12857.

Sen, S., Borah, S.N., Bora, A., and Deka, S. (2017). Production, characterization, and antifungal activity of a biosurfactant produced by *Rhodotorula babjevae* YS3. *Microbial Cell Factories* 16(1)**,** 95. doi: 10.1186/s12934-017-0711-z.

Sha, R., Jiang, L., Meng, Q., Zhang, G., and Song, Z. (2012). Producing cell-free culture broth of rhamnolipids as a cost-effective fungicide against plant pathogens. *Journal of Basic Microbiology* 52(4)**,** 458-466. doi: 10.1002/jobm.201100295.

Sha, R., and Meng, Q. (2016). Antifungal activity of rhamnolipids against dimorphic fungi. *The Journal of General and Applied Microbiology* 62(5)**,** 233-239. doi: 10.2323/jgam.2016.04.004.

Sharma, A., Jansen, R., Nimtz, M., Johri, B.N., and Wray, V. (2007a). Rhamnolipids from the rhizosphere bacterium *Pseudomonas* sp. GRP(3) that reduces damping-off disease in Chilli and tomato nurseries. *Journal of Natural Products* 70(6)**,** 941-947. doi: 10.1021/np0700016.

Sharma, A., Wray, V., and Johri, B.N. (2007b). Rhizosphere *Pseudomonas* sp. strains reduce occurrence of pre- and post-emergence damping-off in chile and tomato in Central Himalayan region. *Archives of Microbiology* 187(4)**,** 321-335. doi: 10.1007/s00203-006-0197-2.

Singh, P., and Cameotra, S.S. (2004). Potential applications of microbial surfactants in biomedical sciences. *Trends in Biotechnology* 22(3)**,** 142-146. doi: 10.1016/j.tibtech.2004.01.010.

Stanghellini, M.E., and Miller, R.M. (1997). Biosurfactants : their identity and potential efficacy in the biological control of zoosporic plant pathogen. *Plant disease* 81(1)**,** 4-12.

Straight, P.D., Willey, J.M., and Kolter, R. (2006). Interactions between Streptomyces coelicolor and Bacillus subtilis: Role of Surfactants in Raising Aerial Structures. *Journal of Bacteriology* 188(13)**,** 4918-4925. doi: 10.1128/jb.00162-06.

Syed Ab Rahman, S.F., Singh, E., Pieterse, C.M.J., and Schenk, P.M. (2018). Emerging microbial biocontrol strategies for plant pathogens. *Plant Science* 267**,** 102-111. doi: 10.1016/j.plantsci.2017.11.012.

Takemoto, J., Bensaci, M., De Lucca, A., Cleveland, T., Gandhi, N., and V, P.S. (2010). Inhibition of fungi from diseased grapeby syringomycin E-rhamnolipid mixture. *American Journal of Enology and Viticulture* 61**,** 120-124.

Tao, Y., Bie, X.-m., Lv, F.-x., Zhao, H.-z., and Lu, Z.-x. (2011). Antifungal activity and mechanism of fengycin in the presence and absence of commercial surfactin against Rhizopus stolonifer. *The Journal of Microbiology* 49(1)**,** 146-150. doi: 10.1007/s12275-011-0171-9.

Tran, H., Ficke, A., Asiimwe, T., Hofte, M., and Raaijmakers, J.M. (2007). Role of the cyclic lipopeptide massetolide A in biological control of *Phytophthora infestans* and in colonization of tomato plants by *Pseudomonas fluorescens*. *New Phytologist* 175(4)**,** 731-742.

Varnier, A.L., Sanchez, L., Vatsa, P., Boudesocque, L., Garcia-Brugger, A., Rabenoelina, F., et al. (2009). Bacterial rhamnolipids are novel MAMPs conferring resistance to *Botrytis cinerea* in grapevine. *Plant, Cell & Environment* 32(2)**,** 178-193. doi: PCE1911 [pii]

10.1111/j.1365-3040.2008.01911.x.

Vatsa, P., Sanchez, L., Clément, C., Baillieul, F., and Dorey, S. (2010). Rhamnolipid biosurfactants as new players in animal and plant defense against microbes. *International Journal of Molecular Sciences* 11**,** 5095-5108.

Waewthongrak, W., Leelasuphakul, W., and McCollum, G. (2014). Cyclic LIPopeptides from Bacillus subtilis ABS-S14 elicit defense-related gene expression in citrus fruit. *PloS one* 9(10)**,** e109386-e109386. doi: 10.1371/journal.pone.0109386.

Wise, C., Falardeau, J., Hagberg, I., and Avis, T.J. (2014). Cellular Lipid Composition Affects Sensitivity of Plant Pathogens to Fengycin, an Antifungal Compound Produced by Bacillus subtilis Strain CU12. *Phytopathology* 104(10)**,** 1036-1041. doi: 10.1094/phyto-12-13-0336-r.

Wu, L., Huang, Z., Li, X., Ma, L., Gu, Q., Wu, H., et al. (2018). Stomatal Closure and SA-, JA/ET-Signaling Pathways Are Essential for Bacillus amyloliquefaciens FZB42 to Restrict Leaf Disease Caused by Phytophthora nicotianae in Nicotiana benthamiana. *Frontiers in Microbiology* 9(847). doi: 10.3389/fmicb.2018.00847.

Xiu, P., Liu, R., Zhang, D., and Sun, C. (2017). Pumilacidin-Like Lipopeptides Derived from Marine Bacterium *Bacillus* sp. Strain 176 Suppress the Motility of *Vibrio alginolyticus*. *Applied and Environmental Microbiology* 83(12)**,** e00450-00417. doi: 10.1128/aem.00450-17.

Yamamoto, S., Shiraishi, S., and Suzuki, S. (2015). Are cyclic lipopeptides produced by Bacillus amyloliquefaciens S13-3 responsible for the plant defence response in strawberry against Colletotrichum gloeosporioides? *Letters in Applied Microbiology* 60(4)**,** 379-386. doi: 10.1111/lam.12382.

Yan, F., Xu, S., Chen, Y., and Zheng, X. (2014). Effect of rhamnolipids on *Rhodotorula glutinis* biocontrol of *Alternaria alternata* infection in cherry tomato fruit. *Postharvest Biology and Technology* 97**,** 32-35. doi: https://doi.org/10.1016/j.postharvbio.2014.05.017.

Yan, F., Xu, S., Guo, J., Chen, Q., Meng, Q., and Zheng, X. (2015). Biocontrol of post-harvest *Alternaria alternata* decay of cherry tomatoes with rhamnolipids and possible mechanisms of action. *Journal of the Science of Food Agriculture* 95(7)**,** 1469-1474. doi: 10.1002/jsfa.6845.

Yoo, D.S., Lee, B.S., and Kim, E.K. (2005). Characteristics of microbial biosurfactant as an antifungal agent against plant pathogenic fungus. *Journal of Microbiology and Biotechnology* 15(6)**,** 1164-1169.

Yoshida, S., Koitabashi, M., Nakamura, J., Fukuoka, T., Sakai, H., Abe, M., et al. (2015). Effects of biosurfactants, mannosylerythritol lipids, on the hydrophobicity of solid surfaces and infection behaviours of plant pathogenic fungi. *Journal of Applied Microbiology* 119(1)**,** 215-224. doi: 10.1111/jam.12832.

Zeriouh, H., Romero, D., García-Gutiérrez, L., Cazorla, F.M., de Vicente, A., and Pérez-García, A. (2011). The Iturin-like Lipopeptides Are Essential Components in the Biological Control Arsenal of Bacillus subtilis Against Bacterial Diseases of Cucurbits. *Molecular Plant-Microbe Interactions®* 24(12)**,** 1540-1552. doi: 10.1094/mpmi-06-11-0162.

Figure and Table Legends

Figure 1. Schematic representation of dual effects of rhamnolipids and lipopeptides: antimicrobial activities and plant defense induction. mc-3-OH-acyl building block of rhamnolipids is perceived by plant through the LORE receptor ①; Rhamnolipid could be sensed through their direct insertion in plasma membrane ②. Recognition of rhamnolipids leads to early signalling events like ion fluxes (Ca^{2+}), reactive oxygen species production (H_2O_2) and MAPK phosphorylation cascade ③. These early responses trigger defense gene expression, probably through activation of transcription factors (TF) and hormonal signaling pathways ④. This leads to defense mechanisms like cell wall reinforcement and PR protein accumulation ⑤ triggering the resistance to the microbes ⑥. Plant immunity due to lipopeptides does not involve a protein receptor and rely on interaction between lipopeptides and the plant membrane ⑦. Both rhamnolipids and lipopeptides can also have direct antimicrobial effects through direct insertion into the microbial plasma membrane ⑧. These insertions trigger loss of cell morphology leading to pore formation ⑨. The pore formation causes cellular component leakage triggering microbial cell death ⑩. Cell death due to lipopeptides can also be indirectly due to the inhibition or activation of microbial cell functions ⑪.

Table 1: Anti-phytopathogenic properties of rhamnolipids

Composition	**Source organism**	**Organism affected**	**Activity/Effect**	**References**
Rha-Rha-C_{10}-C_{10}, Rha-C_{10}-C_{10}	*Pseudomonas aeruginosa*	*Phytophthora capsici, Pythium aphanidermatum, Plasmopara lactucae-radicis*	Zoospore lysis	(Stanghellini and Miller, 1997)
Rha-Rha-C_{10}-C_{10}	*Pseudomonas aeruginosa* B5	*Cercospora kikuchii, Cladosporium cucumerinum, Colletotrichum orbiculare, Cylindrocarpon destructans, Magnaporthe grisea, Phytophthora capsici*	Zoospore lysis, spore germination and hyphal growth inhibition	(Kim et al., 2000)
Rha-Rha-C_8-C_{10}, Rha-C_{10}-C_8/Rha-C_8-C_{10}, Rha-Rha-C_8-$C_{12:1}$, Rha-Rha-C_{10}-C_{10}, Rha-Rha-C_{10}-$C_{12:1}$, Rha-C_{10}-C_{10}, Rha-Rha-C_{10}-C_{12}/Rha-Rha-C_{12}-C_{10}, Rha-C_{10}-$C_{12:1}$/Rha-$C_{12:1}$-C_{10}, Rha-Rha-$C_{12:1}$-C_{12}, Rha-Rha-C_{10}-$C_{14:1}$, Rha-C_{10}-C_{12}/Rha-C_{12}-C_{10}	*Pseudomonas aeruginosa* 47T2	*Penicillium funiculosum*, *Fusarium solani*, *Botrytis cinerea*, *Rhizoctonia solani*	Growth inhibition (MIC)	(Haba et al., 2003)
Rha-Rha-C_{10}-C_{10}, Rha-C_{10}-C_{10}, Rha-Rha-C_{10}-$C_{12:1}$, Rha-C_{10}-$C_{12:1}$, Rha-Rha-C_{10}-C_{12}, Rha-C_{10}-C_{12}	*Pseudomonas aeruginosa* LBI	*Penicilium funiculosum, Alternaria alternata*	Growth inhibition (MIC)	(Benincasa et al., 2004)
Biosurfactant PRO1 (formulation of 25% RLs) Plant support (the Netherlands)	*Pseudomonas aeruginosa*	*Phytophthora cryptogea*	Zoospore lysis, reduction of sporangia formation	(De Jonghe et al., 2005)
Mono- and di-rhamnolipids	*Pseudomonas aeruginosa* IGB 83	*Phytophthora capsici*, *Phytophthora nicotianae*, *Phytophthora cactorum*, *Phytophthora infestans*, *Pythium aphanidermatum*, *Pythium ultimum*	Motility inhibition, zoospore lysis, mycelial growth inhibition	(Yoo et al., 2005)
Rha-Rha-C_{10}-C_{10}, Rha-C_{10}-C_{10} (Jeneil Biosurfactant Company JBR599)	*Pseudomonas aeruginosa*	*Pythium myriotylum*	Mycelial growth inhibition	(Perneel et al., 2008)

Biosurfactant PRO1 (formulation of 25% RLs) Plant support (the Netherlands)				
Rha-Rha-C$_{10}$-C$_{10}$, Rha-C$_{10}$-C$_{10}$ (Jeneil Biosurfactant Company JBR599)	*Pseudomonas aeruginosa*	*Botrytis cinerea*	Spore germination and mycelial growth inhibition	(Varnier et al., 2009; Monnier et al., 2018)
Rha-Rha-C$_{10}$-C$_{10}$, Rha-C$_{10}$-C$_{10}$	*Pseudomonas aeruginosa* ZJU211	*Phytophthora infestans, Phytophthora capsici, Botrytis cinerea, Fusarium graminearum*, *Fusarium oxysporum*	Mycelial growth Inhibition	(Sha et al., 2012)
Rha-C$_{8:1}$, Rha-C$_{10}$-C$_{10:1}$, Rha-C$_{10:1}$-C$_{10}$, Rha-Rha-C$_{10}$-C$_{12:1}$, Rha-Rha-C$_{12:1}$-C$_{10}$	*Pseudomonas aeruginosa* DS9	*Fusarium sacchari*	Mycelial growth Inhibition	(Goswami et al., 2014)
Mono- and di-rhamnolipids	*Pseudomonas aeruginosa* ZJU-211	*Alternaria alternata*	Spore germination and mycelial growth inhibition	(Yan et al., 2014; Yan et al., 2015)
Rha-C$_{10}$-C$_{10}$, Rha-Rha-C$_{10}$-C$_{8}$ Other Rha or Rha-Rha : -C$_{10}$-C$_{10}$, -C$_{8}$-C$_{10}$, -C$_{10}$-C$_{12}$, -C$_{12}$-C$_{12}$, -C$_{14}$-C$_{10}$, -C$_{10}$-C$_{16}$	*Serratia rubidaea* SNAU02	*Fusarium oxysporum, Colletotrichum gloeosporioides*	Mycelial growth Inhibition	(Nalini and Parthasarathi, 2014)
Rha-C$_{9:2}$, Rha-C$_{10}$, Rha-C$_{12:3}$, Rha-C$_{8}$-C$_{8}$, Rha-C$_{10}$-C$_{10:1}$, Rha-C$_{10:1}$-C$_{10}$, Rha-C$_{10}$-C$_{8}$, Rha-C$_{8}$-C$_{10}$, Rha-Rha-C$_{10}$-C$_{12}$, Rha-Rha-C$_{12}$-C$_{10}$	*Pseudomonas aeruginosa* SS14	*Fusarium oxysporum* f. sp. *pisi*	Fungal growth inhibition	(Borah et al., 2015)
Rha-C$_{10}$-C$_{10}$, Rha-Rha-C$_{10}$-C$_{10}$	*Pseudomonas aeruginosa* KVD-HM52	*Fusarium oxysporum*	Mycelial growth and fungal biomass accumulation inhibition	(Deepika et al., 2015)
Rha-C$_{8:2}$, Rha-C$_{8:1}$, Rha-C$_{10}$, Rha-C$_{12:1}$, Rha-Rha-C$_{10:1}$, Rha-C$_{10}$-C$_{10:1}$/Rha-C$_{10:1}$-C$_{10}$	*Pseudomonas aeruginosa* DS9	*Colletotrichum falcatum*	Spore germination and mycelial growth inhibition	(Goswami et al., 2015)
Rha-C$_{8}$, Rha-C$_{10}$-C$_{10}$	*Pseudomonas aeruginosa* SS14	*Fusarium verticillioides*	Spore germination and mycelial growth inhibition	(Borah et al., 2016)
Rha-Rha-C$_{10}$, Rha-Rha-C$_{8}$-	*Pseudomonas*	*Sclerotium rolfsii*, *Fusarium oxysporium*,	Mycelial growth	(Sathi Reddy et

C_{10}, Rha-Rha-C_{10}-C_{10}	*aeruginosa* DR1	*Phytophthora* *nicotianae*, *Macrophomina phaseolina*	inhibition	al., 2016)
Rha-Rha-C_{10}-C_{10}, Rha-C_{10}-C_{10}	*Pseudomonas aeruginosa* ZJU211	*Verticillium dahliae*	Spore germination and mycelial growth inhibition	(Sha and Meng, 2016)
Rha-C_{10}-C_8, Rha-C_{10}-C_{10}, Rha-C_{10}-$C_{12:1}$, Rha-C_{10}-C_{12}, Rha-Rha-C_8-C_{10}, Rha-Rha-C_{10}-C_{10}, Rha-Rha-C_{10}-$C_{12:1}$, Rha-Rha-C_{10}-C_{12}	Pseudomonas *aeruginosa* #112	*Aspergillus carbonarius*	Mycelial growth inhibition	(Rodrigues et al., 2017)
Semipurified rhamnolipid mixture (RL90-A, AGAE Technologies, Corvalis, USA) and RL90-N, NatSurFact, Fairfax, USA)	*Pseudomonas aeruginosa*	*Leptosphaeria maculans*	Mycelial growth inhibition	(Monnier et al., 2020)