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Biosurfactants as Green Biostimulants for Seed Germination and Growth

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Abstract: *Microorganisms, particularly fungi, are well-known to significantly affect crop production. In 2011, the United States produced over 35 million tons of grains. Grain rust diseases were estimated to lead a US$5 billion loss. Thus, to enhance the crop production, pesticides are extensively used in agriculture, most synthetic ones. Consequently, humans are often exposed to pesticides mainly by residues in food and water. In this sense, natural-based pesticides have been drawn attention from the world due to their sustainable approach. Biosurfactants is one of the most promising molecules as green pesticides. In addition, biosurfactants have biostimulants properties such as metabolism enhancers. This review paper highlights the key features in biosurfactants, in particular lipopeptides and glycolipids as green biostimulants for seed germination and growth. In conclusion, on the one hand, lipopeptides, rhamnolipids and sophorolipids have already been studied as biostimulants; on the other hand mannosylerythritol lipids have been poorly investigated - mostly due to their antimicrobial properties.*

Keywords: *Lipopeptides, Glycolipids, Biostimulants.*

1. Introduction

The Pre-Sowing Seed Stimulation Is A Technique That Aims To Accelerating The Germination. It Covers The Seed Surfaces With Remarkable Molecules That Protect Them Against Microorganisms And/Or Enhance Their Metabolism, For Example Higher Plant Resistance Against Bacteria, Fungi, Yeasts And Virus. The Seed Stimulation Can Decrease The Initial Dose Of Fertilizers (Krawczyńska Et Al., 2012). In This Sense, Natural-Based Stimulation Methods Have Been Drawn Attention From The World Due To The Their Sustainable Approach. Biosurfactants Is One Of The Most Promising Molecules As Biostimulants.

Biosurfactants Are Amphiphilic Compounds Which Structure Is Composed By Hydrophilic And Lipophilic Moieties. They Are Mostly Derived From Plants And Microorganisms. Nevertheless, The Microbial Production Has Advantages When Compared To Plant-Based Surfactants Due To Their Multifunctional Properties, Rapid Production, And Scale-Up Capacity (Xu Et Al., 2011). In Addition, Their High Biodegradability, High Temperature Stability And Insignificant Effect On Environmental Ph Are Exceptional Advantages For Agricultural Applications. Biosurfactants Can Be Classified Into Five Groups, According To Their Chemical Structure: (I) Lipopeptides And Lipoproteins, (II) Glycolipids, (III) Fatty Acids, Neutral Lipids And Phospholipids, (IV) Polymeric Surfactant And (V) Particulate (Andrade Et Al., 2016a). Lipopeptides And Glycolipids Are The Most Scientifically Well-Known Biosurfactants.

As Already Mentioned, Lipopeptides Have Been Exhaustively Investigated, In Which *Bacillus Subtilis* Lipopeptides Such As Surfactin, Iturin And Fengycin Families Have Risen To Prominence. In 2003, The Use Of Daptomycin, A Semi-Synthetic Cyclic Lipopeptide, As Antibiotic Was Approved In The USA. The Chemical Amphiphilic Structure Of Surfactin, Iturin And Fengycin Families Is Basically Composed Of A Cyclic Peptide Linked To Fatty Acid Chain, *Β*-OH (Lactone), *Β*-NH_2 (Lactam) And *Β*-OH (Lactone), Respectively. The Peptide Moiety Of The Surfactin And Iturin Families Contains A Heptapeptide Whereas The Fengycin Family, A Decapeptide. It Is Worth Noting That Subtle Chemical Structural Differences Are The Key For Biological Activities Of Lipopeptides

Such As Antimicrobial Agents. The Amphiphilic Structure Of *B. Subtilis* Lipopeptides Can Interfere With The Integrity Of Microbial Membrane, In Which The Presence Of Specific Sterols In The Microbial Membrane Seems To Lead To Lower Destabilizing Effects. Regarding *B. Subtilis* Lipopeptides As (Bio)Controllers Of Plant Diseases, They Were First Studied Due To Their Antagonistic Activity Against A Wide Range Of Phytopathogens Including Bacteria, Fungi And Oomycetes. However, They Also Have Significant Effects On Soil Microbiota (Rhizosphere).

Glycolipids Are Chemical Molecules Composed By Two Moieties: Hydrophilic Carbohydrate And Hydrophobic Lipid. Similar To Lipopeptides, Glycolipids Also Presents Amphiphilic And Interface-Active Characteristics. Thus, Glycolipids Also Have Biostimulants Properties.

Based On The Promising Applications Of These Molecules In Agriculture, This Review Paper Aims To Put A Light On The Chemical Structures Of Biosurfactants And Also Some Biological Properties, In Particular As Biostimulants.

2. Biosurfactants

Biosurfactants are amphiphilic compounds which structure is formed by hydrophilic and lipophilic groups. They are mostly derived from plants and microorganisms. Nevertheless, the microbial production have advantages when compared to plant-based surfactants because of the multifunctional properties, rapid production, and scale-up capacity (Xu et al., 2011).

Increasing the solubility and bioavailability of hydrophobic compounds, promoting the swarming motility of microorganism, signaling and differentiation cellular physiological processes (Kearns and Losick, 2003), are some of the biosurfactants capabilities, that may also contribute to agricultural sustainability by antimicrobial action for disease control (Ahmad et al., 2018).

These compounds can be classified into five groups, according to their chemical structure: (I) lipopeptides and lipoproteins, (II) glycolipids, (III) fatty acids, neutral lipids and phospholipids, (IV) polymeric surfactant and (V) particulate (Andrade et al., 2016a). Lipopeptides and glycolipids are being successfully applied in agricultural environment and are the central point of this discussion.

2.1. Lipopeptides

The lipopeptides consist of a lipophilic fatty acid(s) chemically linked to hydrophilic peptide ring moiety. *Bacillus* produces lipopeptides, which most common families are iturin, fengycin, and surfactin. Each family has a specific number of amino acids, but with different residues at specific positions. It also has different length and isomer of β-hydroxyl fatty acid, that is, lipopeptides have remarkable heterogeneity of molecular weight (Andrade et al., 2016b). Iturin are heptapeptides and C_{14}-C_{17} β-amino fatty acids. Fengycins are formed by β-hydroxy fatty acid chains and decapeptides, which originates cyclic lactone rings. Surfactins contain cyclic lactone rings involving C_{13}-C_{16} β-hydroxy fatty acids and heptapeptides (Yang et al., 2015).

2.1.1. Surfactin

Surfactin is an anionic structure composed by heptapeptide ring ($_{L}$-Glu-$_{L}$-Leu-$_{D}$-Leu-$_{L}$-Val-$_{L}$-Asp-$_{D}$-Leu-$_{L}$-Leu) linked to one β-OH fatty acid chain C_{12}-C_{16} (Figure 1). The amino acid sequence can suffer changes according to bioprocess conditions, culture medium, the surfactin producer, etc. Between their properties: can reduce the surface tension (ST) of water from 72 to 27 mN/m at concentration as low as 10 mg/L; bioactive properties such as antiviral, antitumor, and antibiotic (Andrade and Pastore, 2017).

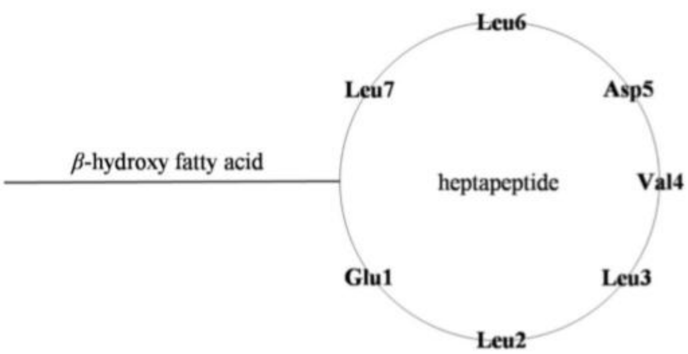


Figure1. *Structural formula of surfactin (Andrade and Pastore, 2017).*

2.2.Glycolipids

Glycolipids are a group of chemical compounds formed by moieties of hydrophilic carbohydrate and hydrophobic lipid. They also present amphiphilic and interface-active characteristics. They are commonly hydroxylated fatty acids connected to a sugar residue by a glycosidic bond. This class could be produced by microbial metabolism, for example from the *Pseudomonas aeruginosa* bacteria which originates rhamnolipids; the *Candida bombicola* yeast that produces sophorolipids, and the consortium of yeasts and higher fungi (e.g., *Candida antarctica* and *Pseudozyma aphidis*) which generates mannosylerythritol lipids (Giessier-Blank et al., 2016).

2.1.2. Rhamnolipids

Rhamnolipids are glycolipids which chemical structures consist of a β-hydroxy fatty acid-based fraction and a rhamnose-based moiety which have one or two rhamnose residues; originating two classes of rhamnolipids: monorhamnolipids or dirhamnolipids. Likewise, the lipid portion can be represented by one or two ester-bonded β-hydroxy fatty acids, with different chain length and degree of saturation (Figure 2). *Pseudomonas aeruginosa* are the best bacterium group for rhamnolipid production.

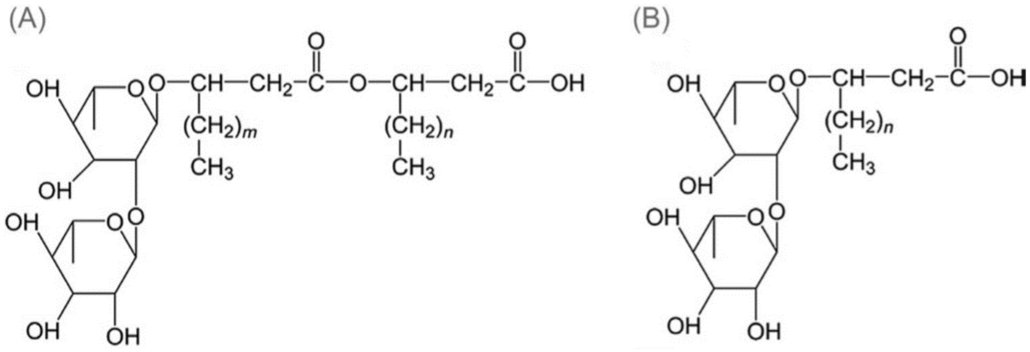


Figure2. *Chemical structure of rhamnolipid: a) mono-rhamnolipid and b) di-rhamnolipid (adapted from Suh, Invally, & Ju, 2019).*

2.1.3. Sophorolipids

Sophorolipids (SLs) are synthesized by specific yeast species (*Candida* sp.) from renewable resources. Characteristically, they are formed by a mixture of lactonic and acidic varieties in the acetylation standards of the constituting glucose moieties, in the type of the hydrophobic portion, and in its bond to the hydrophilic sugar (sophorose) (Figure 3).

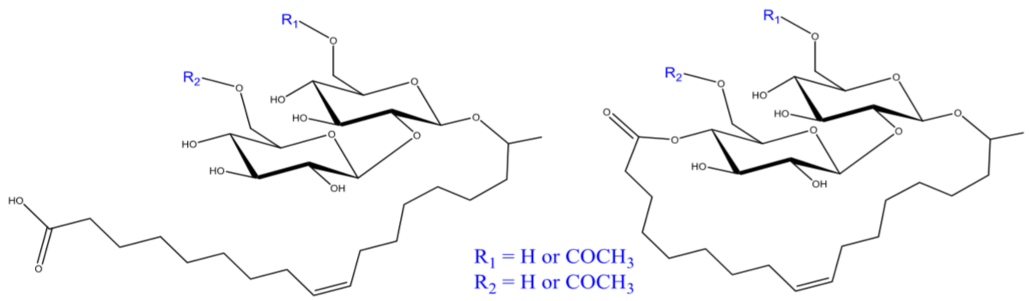
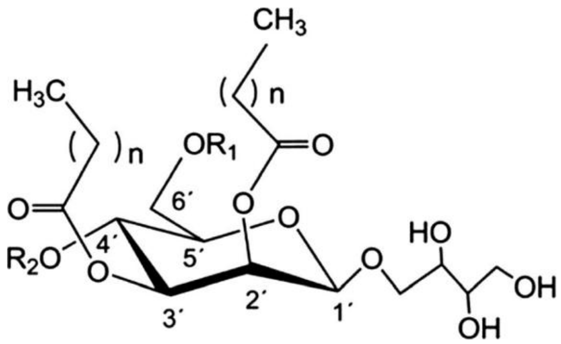


Figure3. *Chemical structure of wild-type SLs: acidic (left) and lactonic (right) (Roelants et al., 2019).*

2.1.4. Mannosylerythritol lipids

Mannosylerythritol lipids (MELs) are nonionic biosurfactants, originated by plant- associated fungi of the genera *Pseudozyma* and *Ustilago*, also recognized by their low toxicity, excellent dispersion, emulsification, foaming, functional properties, production yields, solubilization, and wetting (Arutchelvi et al., 2008; Yu et al., 2015). MELs are amphiphilic molecules with an acetyl group and/or a fatty acid as the hydrophobic portion and a 4-O-β-$_D$-mannopyranose-erythritol as a hydrophilic fraction (Arutchelvi and Doble, 2010). They can be classified according to the different location and number of the acetyl group at C-$_4$ and C-$_6$ as MEL-A, MEL-B, MEL-C, and MEL-D as represented at Figure 4 (Günther et al., 2015).



Chemical structure of MELs. MEL-A the radicals R_1 and R_2 are acetyl; MEL-B: R_1 is acetyl and R_2 is H; MEL-C: R_1 is H and R_2 is acetyl; MEL-D: R_1/R_2 are H (Niu et al., 2019).

3. Biosurfactants As Biostimulants For Seed Germination And Growth

The production biosurfactants and their application as antimicrobials have been extensively studied. However, biosurfactants interact with bacterial, animal and vegetable cells in a wide range of ways. These interactions can act as, for instance as agricultural enhancers of plant immunity. In this sense, the applications of biosurfactants (groups) as biostimulants (agricultural) are briefly described below.

3.1. Lipopeptides

Lipopeptides are the main class of antimicrobial peptides produced by *Bacillus* sp. with a wide range of biotechnological applications (Shafi et al., 2017; Xu et al., 2013; Ye et al., 2012). In agriculture, in addition to reducing surface tension, dispersion, pesticide and powdered fertilizer suspension; emulsifying pesticide solutions, facilitating the action of biocontrol mechanisms, lipopeptides are also used to eliminate plant pathogens and stimulate defense responses, acting as exogenous eliciting molecules (Dangl and Jones, 2001; Garcia-Brugger et al., 2006; Henry et al., 2012; Santos et al., 2016). Plants produce phytohormones which can regulate and stimulate defense responses or act as precursors to physical defense systems by strengthening the cell wall and accumulating phytoalexin (Dangl and Jones, 2001; Garcia-Brugger et al., 2006).

The use of lipopeptides for eliciting induced systemic resistance (ISR) in plants was reported by Ongena et al. (2007), where surfactin and pure fengycin were applied directly to the plant, providing a significant ISR response as a protective effect, similar to that induced by a strain of *Bacillus subtilis* cells inoculated at the root of bean and tomato plants. The results show a significant decrease of the disease (28%) using surfactin, but not with fengycin, which showed a disease reduction of 14%, which was equivalent to the control plants. Compared with the root treatment done with *B. subtilis* S499 cells (33%), the levels of disease control of pure surfactin was considered satisfactory (Ongena et al., 2007).

Waewthongrak et al. (2014) also applied the lipopeptides surfactin and fengycin, produced by *B. subtilis*, in *Citrus sinensis*, triggering a defense response against *Penicillium digitatum*, positively regulating the expression of the plants' defense genes. Another application of such lipopeptides was investigated by Yamamoto et al. (2015), relating surfactin and iturin as ISR molecules in plants; in this study, the expression of defense proteins was induced in strawberry plants against *Colletotrichum gloeosporioides*, indicating the important role of these lipopeptides. Le Mire et al. (2018) also reported the effectiveness of pure surfactin in protecting up to 70% wheat against *Zymoseptoria tritici*. Surfactin did not show antifungal activity but significantly induced ISR in plants by stimulating signaling pathways dependent on salicylic acid and jasmonic acid (Le Mire et al., 2018; Waewthongrak et al., 2014; Yamamoto et al., 2015).

Despite the induction of resistance to pathogens in plants by the use of surfactin being efficient, this lipopeptide exerts no direct antagonism to the growth of pathogens. Surfactin by itself is not fungitoxic, but when combined with iturin it exerts a synergistic antifungal effect (Maget-Dana et al., 1992). In contrast, fengycins and iturins exhibit high fungitoxicity (Vanittanakom et al., 1986). Few papers report the use of lipopeptides directly associated to plant growth. However, some studies indicate the passive use of lipopeptides as potential biostimulants, increasing the bioavailability of nutrients to plant-associated beneficial microorganisms (Santos et al., 2016).

To improve the immune system and serve as a support to plant metabolism, a biostimulator should be able to penetrate plant tissue. However, penetration time is limited since the biostimulator should remain in liquid form. This must be taken into account, since plants undergoing treatment are exposed to different climatic conditions and other extrinsic factors (Kolomazník et al., 2012). However, the permeability of the pores can be influenced by reducing the surface tension of the solution (Gaskin et al., 1996), by means of, for example, using surfactants or biosurfactants.

Another very important factor for plant growth is the microbial consortium in the soil. Some microbiological tests are useful in assessing the quality of the soil before planting. To determine the effects of contaminants or even of soil management and the dynamics of microbial communities, one of the tests most commonly used as an indicator is the release of CO_2 by microbial respiration (De Paula et al., 2010; Marchiori Júnior and Melo, 1999; Zhong et al., 2010). According to Machado (2018), who used respirometry techniques to test the influence of adding surfactin to the soil, surfactin acted as a biostimulant, increasing the release of CO_2 from 9.78 to 216.29 mg C-CO_2 / kg of soil with the addition of 4,000 mg / kg of surfactin (Machado, 2018).

Therefore, lipopeptides play a crucial role in agriculture, and could be better exploited for plant growth and disease management. The biodegradability of biosurfactants is a positive factor because they do not persist in the environment, are less toxic and more biocompatible (Ławniczak et al., 2013). Recent research investigates the applicability of lipopeptide biosurfactants as an alternative to pesticides and chemical fertilizers in order to improve the sustainability of current agricultural practices. The relevance of the topic and the few reports in the literature justify a more thorough research regarding the application of biosurfactants as biostimulants in plants.

3.2. Rhamnolipids

Rhamnolipids are well-known biosurfactants that can be used in a wide variety of applications including detergent formulation, food and pharmaceutical industry, cosmetics and bioremediation. More recently, some studies have shown that rhamnolipids can also be used as agriculture additives since they can act as direct antimicrobial agents as well as a stimulant to enhance plant's immunity (Vatsa et al., 2010).

Rhamnolipids can be applied as biocontrol agents in crops, controlling the growth and spread of several phytopathogenic fungi, such as *Fusarium oxysporum*, *Fusarium graminearum*, *Botrytis cinerea*, *Penicillium* sp., *Alternaria* sp., *Chaetomium globosum*, *Phytophthora infestans*, *Phytophthora capsica*, *Colletotrichum orbiculare*, and *Mucorcircinelloides* (Benincasa et al., 2004; Deepika et al., 2015; Kim et al., 2000; Sha et al., 2012). Kim et al. (2000) also reported that a rhamnolipid produced by *Pseudomonas* sp. was able to control and act as an insecticide against green peach aphid (*Myzus persicae*) (Kim et al., 2011).

In addition to the antimicrobial properties, rhamnolipids can also be used as a stimulant for plant immunity. Studies have shown that they can induce genes involved in the defense systems of plants as grapevine (*Vitis vinifera* L.), thale cress (*Arabidopsis thaliana*), cherry tomato (*Lycopersicon esculentum*), and rapeseed (*Brassica napus*) (Monnier et al., 2018; Sanchez et al., 2012; Varnier et al., 2009; Yan et al., 2014). Other researches showed that rhamnolipids can also induce the synthesis of hormones responsible for signaling important pathways for plant immunity (Chong and Li, 2017).

Varnier et al. (2009) studied the use of rhamnolipids from *Pseudomonas aeruginosa* to trigger grapevine defense responses against *B. cinerea*. The authors detected Ca^{2+} influx, reactive oxygen species production and mitogen-activated protein kinase activation in the first two minutes after the application. These signaling events can induce plant defense mechanisms, including the expression of a wide variety of genes and hypersensitive response. The cytosolic concentration of Ca^{2+}reached 6 µM with the addition of 0.05 mg/mL of rhamnolipids. The calcium influx generally leads to the activation of mitogen-activated protein kinase and the production of reactive oxygen species, which is frequently involved in plant cell signaling succeeding pathogen perception. The authors also demonstrated that 0.01 mg/mL of rhamnolipids was enough to induce early signaling events and expression of defense genes such as chitinase genes. The authors propose that rhamnolipids are acting as microbe-associated molecular patterns (MAMPs) in grapevine. MAMPs, also known as general elicitors, are involved in non-specific immunity in plants, being effective against a wide variety of pathogens (Bent and Mackey, 2007; Varnier et al., 2009).

Sanchez et al. (2012) reported that rhamnolipids were able to stimulate an innate immune response in thale cress including accumulation of signaling molecules and activation of defense genes. The use of rhamnolipids increased the plant resistance against the bacterium *Pseudomonassyringae* pv *tomato*, the oomycete *Hyaloperonospora arabidopsidis*, and the fungus *B. cinerea*. The authors demonstrated that the increased resistance induced by rhamnolipids can involve different signaling pathways depending on the nature of the pathogen. For example, ethylene is involved in the resistance to *H. arabidopsidis* and *P. syringae* pv *tomato*, jasmonic acid is crucial for the resistance to *B.cinerea*, while salicylic acid takes part of the defense against all pathogens tested. Sanchez et al. presented evidence that plant defense mechanisms involving salicylic acid are potentiated by rhamnolipids when the plant is exposed to *B. cinerea* or *P. syringae* pv *tomato*. As well as Varnier et al. (2009), the authors concluded that rhamnolipids can act as MAMPs to trigger non-specific immunity in plant cells.

According to Monnier et al. (2018), rhamnolipids from *P. aeruginosa* stimulate an effective defense of rapeseed foliar tissues toward *B. cinerea*. The defense triggered by rhamnolipids includes, besides chemical mechanisms as the production of reactive oxygen species and the expression of defense genes, physical protections as callose deposits and stomatal closure.

Table 1 summarizes the researchers found on literature about the use of rhamnolipids as plant immunity stimulants against phytopathogenic microorganisms.

Table1. *Literature reports about the use of rhamnolipids to stimulate plant immunity*.

Ref.	Plant	Pathogen	Defense mechanisms activated
(Varnier et al., 2009)	Grapevine	*B. cinerea*	- Ca^{2+} influx; - Mitogen-activated protein kinase; - Production of reactive oxygen species; - Expression of defense genes; - Hypersensitive response.
(Sanchez et al., 2012)	Thale cress	*P. syringae* pv *tomato* *H. arabidopsidis* *B. cinerea*	- Accumulation of signaling molecules (ethylene, jasmonic acid, and salicylic acid); - Expression of defense genes.
(Yan et al., 2014)	Cherry tomato	*A. alternata*	- Peroxidase, polyphenoloxidase and phenylalanine ammonialyase activities of cherry tomato fruit.
(Nasir et al., 2017)	Tobacco	-	- Early signaling activation; - Oxidative burst.
(Luzuriaga-Loaiza et al., 2018)	Thale cress	*P. syringae* pv *tomato*	- Interaction with plant plasma membrane; - Early signaling activation; - Expression of defense genes.
(Monnier et al., 2018)	Rapeseed	B. *cinerea*	- Production of reactive oxygen species; - Expression of defense genes; - Callose deposits; - Stomatal closure.

The analysis of Table 1 indicates that there are a reduced number of publications on the application of rhamnolipids as agricultural enhancers of plant immunity. Thus, it should be deeper investigates, since rhamnolipids can replace the current agrochemicals, leading to more sustainable agriculture productions.

3.3. Sophorolipids

The DE102014209346A1 German patent presents the use of sophorolipids to increase a crop yield, achieved even if the plant pathogen is not combated or the clinical picture is not changed (Sieverding, 2017). Experiments at field, greenhouse and laboratory were projected to treat each crop plants (barley, wheat, soybeans, and tomato) with distinguish sophorolipid samples in variated growth stages. The application was done to plant seeds or leaves considering sophorolipid samples alone or in mixture with commercial plant protection products (insecticides, fungicides, and nutrients). Such procedures can defend plants from insect infestation and illness at a primary stage of growth. At field tests, sophorolipids provided an increase in yield for agricultural crops. Sophorolipid seed treatment hurries and improves initial plant growth in sprouting and rooting (Sieverding, 2017). An additional report about the advantage of the sophorolipids use is an increase in yield, when used as adjuvants as shown at the US 2012/0220464 patent (Giessier-Blank et al., 2016).

A landfill with contaminated sludge derived originated in copper mineral production represent a threat to the nearby ecosystem due to the dust emission with heavy metals and the complete absence of vegetation. With the view to enrich the quality of the landfill and promote plants development for future cultivation, a researcher's group of Wroclaw University of Technology analyzed the performance, regarding germination and plant growth, of biopreparations with biosurfactants and microorganisms in these plant species: *Avena sativa*, *Lupinus luteus*, *Pisum sativum*, *Sinapsis alba*, and *Zea mays*. The results made it possible to conclude that biosurfactants application before germination promotes a stimulation of all studied seeds. Nevertheless, the direct efficiency of biosurfactants for plant growth development was not certified (Krawczyńska et al., 2012).

Numerous surfactants or wetting agents used in crop protection exhibit an elevated spreading behavior. One example is the trisiloxanes, that are harmful to health and could be either included directly in the crop protection product formulations or as an increment to the aqueous spray solutions, as a tank mix additive, at any moment before the application. In this context, Giessler-Blank et al. (2016) patented the application of sophorolipids and their derivates with pesticides as formulation additive and/or as tank mix additive for the industrial non-crop sector and for crop safety. Sophorolipids produce practically no spreading/wetting, besides they are capable to intensify or support a pesticide's efficiency. The adjuvants act to balance the weaknesses of the active component, such as the water inconstancy of sulfonylureas or the UV sensitivity of avermectins (Geoffrey et al., 2004). The adjuvant elevates the effectiveness and/or increases the pesticide activity, with a dose range of the adjuvant between 10-3000 mL/ha, preferably 50-700 mL org/ha which are the application rates of commercially offered adjuvants for agriculture. For a synergistically action of the adjuvant and pesticide mixture, it is proposed a ratio of pesticidal ingredient to adjuvant of 1:120 to 30:1 (Giessier-Blank et al., 2016).

Antibiotics, heavy metals, elevated abundance of genes with antibiotic resistance genes (ARGs), and their associations, present in soils prosperous to vegetable cultivation, can threaten human health over the food chain (Heuer et al., 2011; Ji et al., 2012; Peng et al., 2015; Zhu et al., 2013). So, new methods to soil remediation and assurance food security are targeted for new studies development. One of them, was proposed by Ye et al. (2016), based on the association of sophorolipid washings, which is an effective technique for removing organic and inorganic contaminates, and ultrasonication (Cao et al., 2015; Kulikowska et al., 2015). The best results were obtained by two successive washings with 20 g/L of sophorolipid solution plus ultrasonication (35 kHz) that effectively extracted 71.2% Cd, 100% roxithromycin, 96.6% sulfadiazine, and 88.2% tetracycline. Concomitantly, the rates of ARGs decreased to 10^{-7} and 10^{-8}. Supplementary, lettuce cultivation after the second washed soil exposed a reduction in ARG abundance in lettuce tissues and separate amounts of antibiotic-resistant bacterial endophytes, besides that a relevant enhancement in vegetable growth indices was confirmed by the measurement of chlorophyll content dry/fresh weight, root surface area and soluble protein content (Ye et al., 2016). These results evidenced that lettuce farming in the washed soil was an appropriate and effective tactic for vegetable safety control, and also provided a more appropriate environment for the establishment and subsistence of microorganisms to reinstate microbial metabolism in the washed soil.

Extras researches about the utilization of sophorolipids in agriculture are presented on Table 2. In front of the reduced number of published works, this class of biosurfactant is opened for more studies related to improve plant immunity in the agricultural environment.

Table2. *Extras reports about sophorolipid application in agricultural environment.*

References	Sophorolipid function
(Gross and Shofield, 2014; Yoo et al., 2005)	Biocide for phytopathogenic control, such as *Pythium* and *Phytophthora* sp.
(Baek et al., 2003; Sun et al., 2004)	Control of algal blooms
(Vaughn et al., 2014)	Adjuvants in postemergence herbicides, for greater adherence to plant surface and improved penetration of plant cuticle
(Sachdev and Cameotra, 2013)	Adjuvants in insecticides or fungicides
(Habibi and Babaei, 2017; He et al., 2017; Kang et al., 2010; Mulligan et al., 2001; Rufino et al., 2012; Schippers et al., 2000)	Improvement of soil quality by solubilizing and breaking down hydrocarbons (e.g., phenanthrene), crude oils and heavy metal removal (e.g., zinc/copper/arsenic).
(Sun et al., 2018)	Stimulation of dissipation of antibiotics and antibiotic resistance genes in soil

3.4. Mannosylerythritol Lipids

MELs have been used in several industrial processes due to their specific properties, such as low toxicity, biodegradability, and excellent surface activity (such as emulsification, dispersion, solubilization, foaming, and wetting) (Yu et al., 2015; Beck et al., 2019; Coelho et al., 2020). However, besides these characteristics, no practical use in agriculture has been developed. According to Yoshida et al. (2015), one potential agronomic application of MELs is as pesticides against phytopathogenic microorganisms. Some studies had shown that MELs, as well as the other biosurfactants, can act as antimicrobial agents (Yoshida et al., 2015). Kitamoto et al. (1993) demonstrated the antimicrobial property of MEL-A and MEL-B against bacteria (*Bacillus subtilis*, *Micrococcus luteus*, *Rhodococcus rhodochrous*, *Staphylococcus aureus* and *Pseudomonas riboflavina*) with a minimum inhibitory concentration of 25 $mg.L^{-1}$ or less. However, MELs inhibitory mechanisms have not yet been well elucidated (Kitamoto et al., 1993).

It is known that the hydrophobicity of plant surfaces plays an important role in its infection by pathogens. Fukuoka et al. (2015), evaluated the wetting ability of MELs solutions in hydrophobic surfaces through contact angle (θ) measurements for each second in a 100 s period, where θ varies inversely with the strength of its attraction to the solid surface. It was analyzed the performance of several surfactant solutions on abiotic and biotic surfaces using the Drop Master DM500 (Kyowa Interface Science Co.) with distilled water as a control. The contact angle of MELs solutions reduced to approximately 10° on diverse plant leaf surfaces (wheat, rice, strawberry and mulberry). They have efficiently reduced the contact angle even on surfaces of the Gramineae plant on which mostly nonionic surfactant solutions could not because of their little wettable property. Additionally, it was observed that the MELs solution used as surface pretreatment promoted a greater facility, comparing to numerous conventional surfactants, for microbial cells spreading and fixing on the plant leaf surface. Thus, it was demonstrated that MELs have the potential to be used as agrochemical spreaders, particularly for biological pesticides (Fukuoka et al., 2015).

Yoshida et al. (2015) demonstrated that the application of MEL-A in wheat leaves was able to suppress the development of powdery mildew fungus *Blumeria graminis* f. sp. *tritici* strain T-10, possibly due to the inhibition of conidial germination. On the other hand, when applied on rice leaves, no effect was noticed, and on strawberry leaves, the application of MEL enhanced significantly the disease symptoms. Thus, the ability of MELs to act as pesticides by decreasing the hydrophobicity of plant surfaces seems to depend on the plant and on the target pathogen (Yoshida et al., 2015).

Some species of *Pseudozyma* (*Ustilaginales*) genus are associated with beneficial function for plants, like bringing plant pathogens resistance (Buxdorf et al., 2013), and biological control (Avis and Belanger, 2002; Cheng et al., 2003; Jarvis et al., 1989). MELs have the ability, when in liquid culture, to persuade morphological variations in *P. antarctica* cells (Morita et al., 2013). In front of this, Yoshida et al. (2014) verified that MELs can also cause similar morphological changes in *P. antarctica* strain T-34 cells on solid surfaces, by comparing their phenotypic traits on fresh plant surfaces and modified solid surfaces. It was observed that the MELs, exogenously added to the mutant cells on plant surfaces, promotes enlargement of their colonized area (Yoshida et al., 2014).

Up to date, MELs have been poorly explored as agricultural additives and few studies can be found on literature. The mechanisms behind their action as antimicrobial agents and their interaction with plant surfaces must still be elucidated, so more research is needed in this area, using different plant species and pathogenic microorganisms. Others approaches of MELs utilization are exhibited on Table 3.

Table3. *Extras reports about mannosylerythritol application in agricultural environment*.

References	**MELs function**
(Ga'al et al., 2020)	Larvicidal and pupicidal toxicity of biosurfactant against *Ae. albopictus*, and the possibility of synthesizing silver nanoparticles using (MELs) as reducing and stabilizing agent
(Sha et al., 2012)	They exhibited a wide spectrum of antifungal activities against phytopathogenic fungi permitting their use in agriculture for plant protection.
(Kim et al., 2002)	They have antimicrobial action against Gram-positive bacteria when produced from *C. antarctica cephae*.
(Isoda et al., 1997)	Regular ability as tyrosine kinase activities in K562 cells to inhibit the cell proliferation and induction of differentiation, stimulation or stimulation of esterase activity.

4. Conclusion

The main applications of biosurfactants are related to their antimicrobial properties. In this sense, biosurfactants are the most promising sustainable alternative to pesticides (synthetic molecules). Nevertheless, biosurfactants also have biostimulant properties such as:

- Lipopeptides can induce systemic resistance in plants, in particular bean, tomato, wheat and strawberry;
- Rhamnolipids can induce systemic resistance in plants, in particular as grapevine (*Vitis vinifera* L.), thale cress (*Arabidopsis thaliana*), cherry tomato (*Lycopersicon esculentum*), and rapeseed (*Brassica napus*);
- Sophorolipids can enhance the initial plant growth in sprouting and rooting, in particular barley, wheat, soybean, and tomato.

In conclusion, on the one hand, lipopeptides, rhamnolipids and sophorolipids have already been studied as biostimulants, on the other hand mannosylerythritol lipids have been poorly investigated - mostly due to their antimicrobial properties. Therefore, the application of biosurfactants in agriculture can lead to more sustainable agriculture productions, in which simultaneous studies on their antimicrobial and biostimulant properties should be investigated.

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