BIOSURFACTANT-BASED BIOCONTROL OF AGRICULTURALLY IMPORTANT PLANT PATHOGENS

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Abstract

Most chemical fertilizers and pesticides used in contemporary agriculture are frequently linked to several environmental issues. The use of biosurfactants as a biocontrol agent against agriculturally important plant pathogens is a promising approach, biosurfactants have shown promise as a sustainable alternative to chemical pesticides. Biological agents, which include naturally chosen microorganisms like bacteria, fungi, and viruses, provide several advantages for agriculture. Further research is needed to optimize their production and application in agriculture, but the potential benefits are significant, including reduced use of chemical pesticides, improved crop yields, and a more sustainable agricultural system. Overall, it can be concluded that the use of biosurfactant-based biocontrol is an effective practice against microbial plant pathogens.

Keywords: Biosurfactants, Biocontrol agents, Agriculture, Microbes, Plant pathogens.

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Introduction

Biosurfactants are surface-active compounds produced by microorganisms, which have applications in various fields, including agriculture. Biosurfactants have been shown to have the potential as biocontrol agents against agriculturally important plant pathogens [1]. Biosurfactants can inhibit the growth of plant pathogens by disrupting the cell membrane, altering the pH of the environment, and interfering with the attachment of the pathogen to the plant surface [2]. Additionally, they can make other biocontrol agents more effective, such as antibiotics and enzymes, by increasing their solubility and stability [3].

1.1 Types of Biosurfactant

Several types of biosurfactants are effective in controlling plant pathogens [56]. Some examples of biosurfactants that are effective in controlling plant pathogens include rhamnolipids, surfactin, sophorolipids, and lipopeptides [57]. These biosurfactants are produced by a variety of microorganisms such as *Pseudomonas*, *Bacillus*, *Candida*, and *Rhodococcus* [58]. Rhamnolipids, produced by *Pseudomonas aeruginosa* have been shown to control the growth of various plant pathogens including *Fusarium oxysporum*, *Pythium ultimum*, and *Rhizoctonia solani*. Surfactin, produced by *Bacillus subtilis* is effective against *Fusarium oxysporum* and *Rhizoctonia solani*. Sophorolipids produced by *Candida bombicola* is effective against *Botrytis cinerea* and *Fusarium oxysporum* [59].

Lipopeptides produced by various bacteria including *Pseudomonas*, *Bacillus* and *Rhodococcus* have broad-spectrum activity against many plant pathogens [60].

1.2 Methods to screen biosurfactant

Numerous techniques have been used to date to verify the capability of biosurfactants. However, all biosurfactants have comparable characteristics, which is a crucial factor in the use of various screening techniques. However, the majority of screening techniques are based on the surface tension or interfacial tension phenomenon. Each technique has a benefit and a drawback for locating the producer of the biosurfactant. An earlier study Poonguzhali *et al.* (2017) has also exclusively adapted various screening methods for biosurfactant production. Every method has its advantage and limitation in identifying the biosurfactant producer. The methods available to affirm biosurfactant production are summarized and tabulated below [7] (Table 1).

1.3 Production of biosurfactants

Biosurfactants are typically produced by microorganisms, such as bacteria, yeast, and fungi, through fermentation processes. Some of the most common biosurfactant-producing bacteria include *Pseudomonas*, *Bacillus* and *Rhodococcus* [52]. The production process involves growing the microorganisms in a nutrient-rich medium under controlled conditions, such as temperature and pH, to promote biosurfactant production [53]. Once the biosurfactants are produced, they can be extracted and purified for use in biocontrol applications.

1.3.1 Advances in biosurfactant production

Recent advances in the production of biosurfactants, including the use of microbial consortia to produce biosurfactants with enhanced properties. For example, a recent study demonstrated that a consortium of *Pseudomonas aeruginosa* and *Candida albicans* produced a biosurfactant with better wetting properties and antifungal activity compared to biosurfactants produced by individual strains.

1.4 Formulation of Biosurfactant

Eventually, many processes in the industrial sector are in need of potential surfactants/biosurfactants. Formulation of a low-cost product with high durability retains the original characteristics for a longer period by extending its prolonged shelf life is an essential criterion in the current scenario not only to reduce the product cost, but also for the maintainability of the product quality. It can, therefore, be used as a substitute for replacing the toxic chemicals prevailing in the applications of various fields including medical, pharmaceutical, biotechnology, food and agriculture. The formulations for agrochemicals currently use the special qualities of biosurfactants, such as emulsification, foaming, dispersion, wetting, penetrating, and thickening.

The biosurfactant produced from the yeast, *Candida tropicalis* UCP0996 proved to exhibit its ideal characteristics in the formulations prepared using the potassium sorbate (0.2% w/v) in the cell-free supernatant [30]. The study also revealed the dispersion index of the formulated biosurfactant around 60 % between 45 and 120 days of evaluation and suggested it as an effective bio-dispersant in the dispersion of motor oils for applications in petrochemical industries. However, the study of Bafghi & Fazaelipoor (2012) recognized the application of rhamnolipid in the formulation of detergent and documented the effectiveness of biosurfactants in the oil removal of samples during analysis. It also acknowledged that the formulation prepared was comparably competent to the commercial powders employed regarding stain removal [31].

Furthermore, biosurfactants are also recommended for the use of cosmetic products. The current trend of concentrating based on natural cosmetic products by the consumer attracted the cosmetic industries to focus on the usage of biosurfactants. Vecino *et al.* (2017) suggested that in this sense, biosurfactants with their prebiotic property can efficiently be employed in the preparation of cosmetic products [32]. The glycolipopeptide obtained from the *Lactobacillus pentosus* was reported by Vecino *et al.* (2015) as the better stabilizing agent of oil-in-water (O/W) emulsions formulated with rosemary oil [33]. In a similar study by Bai and McClements (2016), employing rhamnolipid for formulating oil-in-water (O/W) nano-emulsion was found to be satisfactory with relatively small droplet diameters lesser than150 nm at low surfactant/oil ratios lesser than 1:10 respectively [34]. Yet another study by Freitas *et al.* (2016), reported that the formulations of biosurfactant extracted from the microbial source of the yeast *Candida bombicola* URM 3718 [35]. The usage of the potassium sorbate in preparing the formulation of biosurfactant has been highlighted revealing a high stability at a wide range of pH, temperatures and in the presence of sodium chloride and emphasizing the compatibility of the cost-wise product. Certain biosurfactants are utilized in the formulations of agro-based products. The biosurfactant has been promisingly used in the formulations of pesticides, insecticides, and herbicides for the reason that it could act as the penetrants and carriers in those preparations as stated by Awada *et al.* (2011) [36]. The study also revealed that the rhamnolipid has efficiently been employed in the formulation of the insecticide Imidacloprid improving both the solubility of the insecticide as well as the translocating ability of the insecticide into the plant system considerably. There was also a report on the usage of sophorolipids as the adjuvants in the fungicide, Opus and the herbicide, Cato [37].

Biosurfactants also occupied the position to be implemented in the formulation of detergent making. The report of Elazzazy *et al*. (2015) insisted on the production of the detergent using biological surfactants/biosurfactants [38]. Production of bio-detergents employing biosurfactants from *P. aeruginosa* and *Yarrowia lipolytica* emulsified and produced a better result in foaming power too with corresponding CMC of 30 mg/l. Likewise, the formulation of the hair dye has the influence of the biosurfactant composition has been reported by the study of Rincon-Fontan *et al.* (2017) [39]. The study clearly demonstrated the influence of the micelle formation on the adsorption capacity of the Fengycin, a biosurfactant extracted from the agro industrial waste, corn steep liquor of corn wet-milling industry on dyed hair.

One of the challenges of using biosurfactants in agriculture is their formulation for effective delivery and application [74]. Biosurfactants can be formulated in different ways, such as microemulsions, nanoparticles, and liposomes, to improve their stability and efficacy. Formulating biosurfactants into nanoparticles can increase their stability and make them easier to apply while encapsulating them in liposomes can help to target specific plant tissues or pathogens. Biosurfactants can be formulated in various ways, such as emulsions, suspensions, or powders, depending on the target pathogen and the intended application method [68]. Biosurfactants can be applied to plant surfaces as a foliar spray or as a seed treatment. The effectiveness of biosurfactants can be enhanced by combining them with other biocontrol agents, such as beneficial microorganisms or plant extracts [75].

1.4.1 Bioformulations of biosurfactants for plant disease management

Bioformulations of biosurfactants have been proposed as a potential alternative to chemical pesticides for managing plant diseases [78]. Bioformulations can enhance the stability, efficacy, and delivery of biosurfactants, leading to improved biocontrol efficacy. Wong *et al*. (2019) described an overview of the bioformulations of biosurfactants for plant disease management [79]. There are various types of bioformulations of biosurfactants that can be used for plant disease management, including:

i. Microbial consortia

Microbial groups made up of bacteria and fungus can create biosurfactants that can enhance the biocontrol efficacy of biosurfactants. Microbial consortia can produce a range of biosurfactants with different modes of action, leading to improved biocontrol efficacy [79, 80].

ii. Encapsulation

Biosurfactants can be encapsulated in various materials, including liposomes, polymers, and nanoparticles, that can improve their stability, delivery, and biocontrol efficacy [81]. Encapsulation can protect biosurfactants from degradation, increase their solubility, and improve their adhesion and penetration to the target organism [82].

iii. Co-formulation

Biosurfactants can be co-formulated with other biocontrol agents, including bacteria, fungi, and viruses, that can enhance their biocontrol efficacy [83]. Co-formulation can improve the stability and delivery of biosurfactants and can result in a synergistic effect, leading to enhanced biocontrol efficacy [84].

1.5 Application of biosurfactants

Biosurfactants can be applied to plants in a variety of ways, including foliar spray, seed treatment, or soil amendment [66]. The effectiveness of biosurfactants can be enhanced by combining them with other biocontrol agents, such as beneficial microorganisms or plant extracts [67]. The timing and frequency of biosurfactant application can also affect their effectiveness and may need to be tailored to specific crops and pathogens [68].

1.6 Challenges in formulating and applying biosurfactants

Despite the potential benefits of biosurfactants, there are still challenges that need to be addressed before they can be widely used in agriculture [63]. One challenge is the high cost of production, which can limit their availability and affordability. Another challenge is the variability in biosurfactant production, which can affect their efficacy and consistency. Environmental elements like soil pH and temperature can also have an impact on how effective biosurfactants are.

1.7 Development of novel biosurfactants

Researchers are also developing novel biosurfactants with unique properties and modes of action. For example, a recent study reported the isolation and characterization of a new biosurfactant produced by a *Bacillus* strain that showed strong antifungal activity against *Fusarium oxysporum*. Most research has shown that biosurfactants can effectively control various plant pathogens, including *Fusarium oxysporum*, *Botrytis cinerea*, and *Phytophthora infestans*. In addition to their efficacy in controlling plant pathogens, biosurfactants are also environmentally friendly and biodegradable, making them a sustainable alternative to chemical pesticides. Several studies have demonstrated the effectiveness of biosurfactants in controlling plant pathogens under field conditions. For example, a study conducted in Brazil found that a biosurfactant produced by *Bacillus subtilis* was effective in reducing the incidence of Fusarium wilt in tomato plants. Another study conducted in India found that a biosurfactant produced by *Pseudomonas aeruginosa* was effective in controlling root rot caused by *Rhizoctonia solani* in mustard plants.

1.8 Field trials

Field trials have been conducted to evaluate the efficacy of biosurfactants for the biocontrol of plant pathogens in real-world agricultural settings. For example, a recent study conducted field trials in tomato plants to evaluate the efficacy of a biosurfactant produced by a *Pseudomonas* strain against the plant pathogen *Rhizoctonia solani*. The biosurfactant was found to be effective in reducing disease incidence and severity in the field.

1.9 Efficacy and specificity of Biosurfactants over targeted pathogens

Several studies have shown that biosurfactants can be effective in controlling a wide range of plant pathogens, including fungi, bacteria, and nematodes [69]. It has been demonstrated that some biosurfactants have broad-spectrum action, while others are more effective against specific pathogens [70]. The efficacy of biosurfactants can be influenced by various factors, including the type and concentration of biosurfactant used the type of pathogen, and the environmental conditions [71]. Biosurfactants are effective against a wide range of agriculturally important targeted plant pathogens, including fungi, bacteria, and oomycetes [54]. Some of the most common pathogens that can be controlled with biosurfactants include *Fusarium* spp. *Pythium* spp., *Rhizoctonia* spp., and *Botrytis cinerea* [55].

2. Biosurfactant influencing plant microbiome and soil ecology

The soil ecology is greatly concerned with the organic content available to the plants for enhanced growth. Further, the flora and fauna of the soil have been directly influenced by the soil quality. The soil environment though rich in nutrients (micro & macronutrients), the major portion of them exists in the unavailable form and it can be made available only after the mineralization/ solubilization. Nevertheless, pollutants and other adverse conditions also affect the bioavailability of the soil nutrition to plant system making it unfit for the plant/ agricultural crop growth. The addition of biosurfactant or the biosurfactant producers to the soil facilitates the physiological/chemical changes over the unavailable nutrients via., sorption, desorption, and other chemical changes by way of sequestration with the formation of metal biosurfactant complex [18]. Thereby, it aids the availability of even the micronutrients for the uptake of plant roots by decreasing its interfacial tension at the soil-root interface, i.e., in the rhizosphere region. Perhaps, the biosurfactant form micelle formation in the soil environment itself. Critical micelle concentration (CMC) is more significant for expressing solubilization and is achieved at the specific concentration below which there will not be any more drop-off in surface tension [19] Certain study Stacey *et al*. (2008) has established the availability and absorption of trace elements like Cu, Mn, and Zn in the plant system such as *Brassica napus* and *Triticum durum* by the rhamnolipid type of biosurfactant. Furthermore, biosurfactant-producing bacteria at the rhizosphere serve as the plant growth-promoting rhizobacteria (PGPR) and influences the balance of other beneficial microbes in that community of ecological niche [20]. The earlier reports [21, 22] have suggested the recurrent microbiome colonization of plant rhizosphere in the category of biosurfactant producers stood up for *Pseudomonas* and *Bacillus* in certain plants such as tomato and wheat.

2.1 Role of Biosurfactant in plant-microbe interactions

Usually, plant-microbe interaction is considered the major concern for the induction of either beneficial or harmful effects owing to its association. Such association is boosted by the availability of certain exudates or signalling molecules from the rhizosphere. Indeed, biosurfactant favours the beneficial plant-microbe interactions [4]. However, acyl homoserine lactone (AHL) is one such quorum sensing molecule attributing to the beneficial microbial colonization overcoming the competent microbes by exerting certain antimicrobial properties and provides an impact over the microbial mobilization in addition, AHL triggers the synthesis and production of rhamnolipid [5]. Moreover, a certain study has evidenced that the biosurfactant of rhamnolipid type from *Pseudomonas* species have established the quorum sensing signalling/ molecules among them to communicate within cells of their colonization [6]. Such plant-microbe interaction may be due to the reason that the biosurfactant producers as the rhizobacteria would have elevated the bioavailability and reduced the nutrient deprivation.

Table 1. Screening methods to identify biosurfactant producer

Screening Method/ Assay	Nature of the method	Reference	Principle/Advantage of the method
Haemolytic activity	Rapid method	Mulligan *et al.* (1984) [8]; Satpute *et al.* (2010); [9] Joice and Parthasarathi, (2014)[10]	✓ The principle of biosurfactant lysing erythrocytes is used for the hemolytic assay. ✓ Much recommended method at the initial level
Oil spreading technique	Reliable method	Youssef *et al.* (2004) [11]; Satpute *et al.* (2010) [12]; Joice and Parthasarathi (2014) [10]	✓ Oil spreading method as a sign of wetness and surface activity. ✓ Accession of special instruments is not needed and a low amount of samples is used
Drop collapse technique	Rapid method	Jain *et al.* 1991 [13]; Satpute *et al.* 2010 [12]; Joice and Parthasarathi (2014) [10]	✓ Accession of special instruments is not needed and a low amount of sample is used. ✓ Indication of biosurfactant activity
Tilted glass slide method	Alternative or modified method of drop collapse technique	Persson and Molin, (1987) [14]; Satpute *et al.* (2010) [12]	✓ When the oil drop starts to descend quickly, biosurfactant activity is detected. ✓ Instant method.
CTAB agar assay or Blue agar method	A rapid semi-quantitative method for glycolipids	Siegmund and wagner (1991) [15]; Satpute *et al.* (2008) [9]	✓ The principle of anionic surfactant forms an insoluble complex with the cationic bromide salt and the complexes are revealed using methylene blue present in the medium. ✓ A frequent method for the

			indication of anionic biosurfactant
Emulsification index (E24%)	Accurate method	Willumsen and Karlson (1997) [16]; Nalini and Parthasarathi (2016) [17]	✓ A more accurate technique for measuring the amount of soluble biosurfactant in the medium. ✓ Reveals the potentiality of biosurfactant activity
Pendant drop method	An alternative method for surface tension measurement	Agarry *et al.* 2015	✓ Accurate but rarely preferred method
Du-Nouy-Ring method	Most accurate method for surface tension measurement	Satpute *et al.* (2010) [12]	✓ Measures surface tension accurately and directly determines the potentiality of biosurfactant activity

2.2 Biosurfactant in bioremediation and soil fertility

Numerous factors contribute to soil pollution, including hydrocarbons, chemical pesticides, and the build-up of heavy metals in soil, which pose a risk to the survival of both terrestrial and aquatic creatures. Factors like pollutants and microbial flora influence soil fertility to a great extent. Agricultural lands are known for their prone use of pesticides, including fungicides, insecticides, etc., which are the major sources of heavy metal and hydrocarbon pollution. The change in the biotic community depends on the stress of the soil environment. Biosurfactants also employ better wettability of soil. Biosurfactants increase the water solubility of the bound component present in soil aiding the substrate availability for bioaugmentation. It is due to the phenomenon that biosurfactant increases the state of pollutant being accelerated from the soil particles. The major drawback in the bioavailability of certain molecules is not only their molecular weight but also their nature of not being available for the microbes to involve in a series of degradative mechanisms [23, 24]. The literature of Pacwa-Plociniczak *et al.* (2011) suggests that biosurfactants in technologies such as soil washing, and combined clean-up are efficient for hydrocarbon and heavy metal removal. Biosurfactants by inducing bioremediation and mineralization of components increase soil fertility, thereby indirectly enhancing crop development too [25]. During the remediation of heavy metal, biosurfactant forms a much stronger ionic bond with metal ions than the bond with soil. The metal ion biosurfactant complexes are thus desorbing from the soil matrix and mobilizing to the water phase due to low interfacial tension [26]. Biosurfactants are also familiar for their even distribution, anti-caking of fertilizers and replacing the toxicants of pesticides [27].

In consideration of these hostile takeovers of surfactants and pesticides, it is essential to implement the biosurfactant as an environmentally safe approach in the million-dollar pesticide industries to prevent pollution [28]. Industrial sectors involving pesticide synthesis are keen on these properties and thus replace the hazardous surfactant with biosurfactants in pesticide formulations. There is a huge need for such agricultural products in the future to retain the soil quality, fertility and to minimize the hazardous organic pollutant accumulation in the soil environment as well as their residues in food (in the edible part of the vegetables and fruits) to a maximum extent. The nitrogen-fixing *Azotobacter chrococcum* strain in the study of Thavasi *et al.* (2009) possesses biosurfactant and crude oil degrading activity. These types of soil strains break down hydrocarbons and also fix nitrogen, hence enhancing soil fertility [29].

3. Biosurfactant in plant disease resistance and biocontrol over phytopathogens

Biosurfactant mediates induced systemic resistance (ISR) in different plants and thereby acts as plant immune enhancers. Moreover, the report of Vatsa *et al.* (2010) insists the stimulation of plant immunity by rhamnolipid and decreases the infection of plant pathogens [40]. Ongena & Jacques (2007) demonstrates an ISR-mediated mechanism of defense against the grey mould fungi, *Botrytis cinerea* in the tomato plants by fengycin and surfactin from *B. subtilis* S499. The application of the crude biosurfactant extract and biosurfactant-producing cell controls many plants disease causing phytopathogens. Biosurfactants are well known to exhibit antagonist properties from the environmental source of rhizosphere region [41].

Investigation on the Surfactin from *Brevibacillus brevis* strain HOB1 exhibits a great extent of antibacterial and antifungal property in controlling phytopathogens [42]. The study of Kubra *et al*. (2013) reveals sorpholipid derivatives exhibit significant, *Alternaria solani*, *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Phytophthora infestans*, *Ustilago maydis*, and 18 plant fungal pathogens as well as 8 plant bacterial pathogens, such as *Erwinia amylovora*, *Pseudomonas cichorii*, *P. syringae*, *Ralstonia solanace*) [43]. Gamalero and Glick (2011) insist on the fact that rhizobacteria aids in the promotion of plant growth [44]. However, *Pseudomonas* and *Bacillus* from the rhizosphere region produce biosurfactant showing a potent biocontrol activity against the soft rot by *Pectobacterium* and *Dickeya* species from the study of Krzyzanowska *et al.* (2012) most publications on rhamnolipid focus on its antifungal efficacy against plant infections such Zygomycetes, Ascomycota, and Oomycetes fungi [45]. Further, the biocontrol activity of biosurfactant against Fusarium wilt disease among Bhendi *Abelmoschus esculentus* L. has been exclusively evaluated by Poonguzhali *et al*. (2022) insisting on the rhamnolipid type of biosurfactant in effectively controlling the phytopathogen *Fusarium oxysporum f. sp. vasinfectum* and promotes the plant growth [46]. Yet another study of Yoshida *et al*. (2015) has suggested the biocontrol activity of Mannosylerythritol lipids (biosurfactant) in suppressing the powdery mildew disease among wheat [47].

3.1 Biosurfactant for controlling mycotoxigenic fungi in stored agricultural products

The report of Newton *et al*. (2004) states the significant release of quorum sensing (cell to cell communication) molecules like acyl homoserine lactone in establishing its association or interaction with the plant system [48]. The Biosurfactant producing pseudomonads from Vietnam reveals the *Pseudomonas putida* strains of the black pepper's rhizosphere region provides a significant control over *Phytophthora capsici* root rot. It also promotes the shoot and root development of black pepper, stem cuttings enhancing both plant protection and plant growth promotion.

The biosurfactant enhances bioavailability of the nutritive molecules from the soil to the plant system. Similar investigation of Kurijt *et al.* (2009), characterize the biosurfactant producing PGPR as *Pseudomonas putida* 267 by proving the role of putisolvin-like biosurfactants in controlling *Phytophthora* damping-off (of cucumber) thereby concludes plant protection through the interaction towards plant system [49].

Mycotoxigenic fungi are a significant problem in the agricultural industry as they can cause spoilage of stored agricultural products and contaminate them with toxic secondary metabolites called mycotoxins [118]. Mycotoxins can pose a significant threat to human and animal health and result in economic losses due to reduced quality and marketability of the agricultural products [119]. Conventional methods for controlling mycotoxigenic fungi involve the use of synthetic fungicides, but their use has been associated with adverse environmental effects and health hazards [120]. As a result, there has been growing interest in the use of biosurfactants as a potential alternative to synthetic fungicides for controlling mycotoxigenic fungi in stored agricultural products [95].

Biosurfactants have shown potential in controlling mycotoxigenic fungi in stored agricultural products. They could damage fungi's cell membrane, causing cell death and leaking [121]. Biosurfactants can also alter the surface properties of fungi, making them more susceptible to other antifungal agents [122]. In addition, biosurfactants can act as signal molecules that trigger defense responses in plants, leading to enhanced resistance against mycotoxigenic fungi [123]. Biosurfactants produced by microorganisms have been shown to have antifungal activity against mycotoxigenic fungi [124]. For example, rhamnolipids produced by *Pseudomonas aeruginosa* have been shown to inhibit the growth of *Aspergillus flavus* and *Aspergillus parasiticus*, which are common mycotoxigenic fungi in stored agricultural products [125]. Similarly, sophorolipids produced by *Candida bombicola* have been shown to inhibit the growth of *Fusarium graminearum*, another mycotoxigenic fungus that causes *Fusarium* head blight in cereal crops [126]. It has also been demonstrated that biosurfactants produced by plants themselves have antifungal effect against mycotoxigenic fungus [127]. For example, saponins, a group of plant-derived biosurfactants, have been shown to inhibit the growth of *Aspergillus flavus* and *Aspergillus parasiticus*. Saponins are found in various plant species, including soybean, alfalfa, and quinoa [128], [129]. Biosurfactants have the potential to control mycotoxigenic fungi in stored agricultural products. They are environmentally friendly, biodegradable, and non-toxic, making them a promising alternative to synthetic fungicides [130].

Role of Biosurfactants in enhancing plant defence mechanisms

Biosurfactants are amphipathic compounds that can decrease the surface tension between two immiscible phases [131]. They are naturally occurring compounds produced by microorganisms, plants, and animals. Biosurfactants are environmentally friendly and can be used in various applications, including bioremediation, food, and pharmaceutical industries [132]. In recent years, their potential in enhancing plant defense mechanisms has gained attention.

Plants have developed various mechanisms to defend against biotic and abiotic stress [133, 134]. Biotic stress includes insect herbivores, pathogens, and competing plants, while abiotic stress includes drought, high salinity, and extreme temperatures. Plants have evolved several structural and biochemical defenses to combat these stresses [135]. Thorns, spines, and trichomes are examples of structural defenses, whereas alkaloids, terpenoids, and flavonoids are examples of biochemical defenses [136].

Biosurfactants have shown promising results in enhancing plant defense mechanisms against biotic and abiotic stress [137]. The amphipathic nature of biosurfactants allows them to interact with plant membranes and alter their properties, leading to enhanced stress tolerance [138]. Biosurfactants produced by microorganisms have been shown to enhance plant growth and stress tolerance. For example, rhamnolipids produced by *Pseudomonas aeruginosa* have been shown to enhance drought tolerance in maize by increasing root length and biomass [139]. Similarly, sophorolipids produced by *Candida bombicola* have been shown to enhance salinity tolerance in *Arabidopsis thaliana* by increasing root length, biomass, and chlorophyll content [140]. Biosurfactants produced by plants themselves have also been shown to enhance plant defense mechanisms. Saponins, a group of plant-derived biosurfactants, have been shown to have antifungal and antibacterial properties [141].

3.2 Advantages of biosurfactant-based biocontrol

Biosurfactants have several advantages over chemical pesticides, including biodegradability, low toxicity to humans and animals, and environmental friendliness [50]. They also have the potential to improve crop yields and reduce the use of chemical pesticides, which can have negative impacts on the environment and human health [51].

4. Mechanisms of Biosurfactant-based Biocontrol

Biosurfactants work by reducing surface tension at the interface of water and other substances, such as soil or plant surfaces. They can inhibit the growth of plant pathogens by disrupting their cell membranes and altering the pH of the environment, which can make it difficult for the pathogens to thrive [61]. Biosurfactants can also prevent pathogen attachment to the plant surface by interfering with the adhesion process [62].

4.1 Resistance to biosurfactant-based biocontrol

Like chemical pesticides, some plant pathogens may develop resistance to biosurfactants over time [64]. Understanding the mechanisms of resistance is important for developing effective strategies to prevent or manage resistance. Some studies have shown that pathogens can produce extracellular polymeric substances (EPS) that can reduce the efficacy of biosurfactants [65]. Other pathogens may develop efflux pumps that can pump out biosurfactants from their cells.

4.2 Molecular mechanisms of biosurfactant-based biocontrol

Biosurfactants have been proposed as a potential alternative to chemical pesticides for controlling pests, diseases, and weeds in agriculture [78, 95]. They possess various modes of action, including membrane disruption, signalling, and competition, that can contribute to their biocontrol efficacy. Naughton *et al*. (2019) provided an overview of the molecular mechanisms underlying biosurfactant-based biocontrol [96].

i. Membrane disruption

Biosurfactants can disrupt the cell membrane of pests and pathogens, leading to cell leakage and death. They can destabilize the lipid bilayer of the cell membrane by inserting themselves between the lipid molecules [2]. This can lead to an increase in membrane permeability, which can cause ions and molecules to leak out of the cell, leading to cell death. The membrane disruption activity of biosurfactants has been demonstrated against various pests and pathogens, including fungi, bacteria, and insects [96].

ii. Signalling

Biosurfactants can act as signal molecules that trigger defense responses in plants, leading to enhanced resistance against pests and diseases [97]. They can activate plant defense pathways, including the Jasmonic acid and Salicylic acid pathways, leading to the production of defenserelated compounds. This can result in enhanced resistance against pests and diseases [98].

iii. Competition

Biosurfactants can compete with pests and pathogens for nutrients and space. They can outcompete pathogens for nutrients, leading to a reduction in their growth and virulence. This can result in a reduction in disease incidence and severity [99]. Similarly, biosurfactants can compete with pests for space, leading to a reduction in their population size. Biosurfactants possess various modes of action that contribute to their biocontrol efficacy [78]. Their membrane disruption activity can lead to cell death in pests and pathogens. They can act as signal molecules that trigger plant defense responses, leading to enhanced resistance against pests and diseases [100]. Biosurfactants can also compete with pests and pathogens for nutrients and space, leading to a reduction in their population size [101].

5. Synergistic effects of biosurfactants with other biocontrol agents

Biocontrol agents, including bacteria, fungi, and viruses, have been used for controlling pests and diseases in agriculture [85]. Biosurfactants have also been proposed as a potential biocontrol agent due to their various modes of action [86]. Chandra *et al*. (2012) provided an overview of the synergistic effects of biosurfactants with other biocontrol agents [87].

The combination of biosurfactants with other biocontrol agents can result in a synergistic effect, leading to enhanced biocontrol efficacy [88, 89]. Biosurfactants can improve the activity and stability of other biocontrol agents, leading to increased efficacy [90]. For example, biosurfactants can enhance the activity of *Bacillus thuringiensis*, a commonly used biocontrol agent against insects, by improving its adhesion and penetration to the insect cuticle. Similarly, biosurfactants can improve the activity of fungal biocontrol agents by increasing their spore germination and penetration into the host plant [91]. Biosurfactants can also enhance the effectiveness of other biocontrol agents by stimulating plant defense responses. They may function as signal molecules that initiate plant defense mechanisms and cause the synthesis of chemicals involved in defense [89]. This can result in enhanced resistance against pests and diseases and can improve the efficacy of other biocontrol agents.

The synergistic effects of biosurfactants with other biocontrol agents can depend on the application method [92]. For example, the combination of biosurfactants with fungal biocontrol agents can result in a synergistic effect when applied as a co-formulation [93]. However, when applied separately, the biosurfactant may inhibit the germination of fungal spores, leading to a reduction in biocontrol efficacy [94]. Biosurfactants can improve the activity and stability of other biocontrol agents, stimulate plant defense responses, and increase their adhesion and penetration to the target organism [90]. The application method can affect the synergistic effects of biosurfactants with other biocontrol agents [95].

Biosurfactants can be combined with other biocontrol agents, such as plant extracts and beneficial microorganisms, to enhance their efficacy. Recent studies have shown that combining biosurfactants with plant extracts, such as neem oil and ginger extract, can improve their antifungal and antibacterial activity against plant pathogens.

6. Comparative analysis of biosurfactants with chemical pesticides

In agriculture, chemical pesticides are frequently employed to eradicate weeds, illnesses, and pests [109]. However, their use has been associated with adverse environmental effects, including soil and water contamination, resistance development, and health hazards. As a result, there has been growing interest in the use of biosurfactants as a potential alternative to chemical pesticides. Inamuddin & Adetunji (2022) evaluated the comparative analysis the effectiveness of biosurfactants compared to chemical pesticides [95].

Biosurfactants are naturally occurring compounds that possess the ability to lower surface tension between two immiscible phases [110]. They are produced by microorganisms, plants, and animals and have various functions, including emulsification, solubilization, and dispersal of hydrophobic compounds. Biosurfactants are environmentally friendly, biodegradable, and non-toxic, making them a promising alternative to chemical pesticides.

Chemical pesticides are synthetic compounds that are designed to control pests, diseases, and weeds [111]. They are effective in controlling pests, but their use has been associated with various environmental and health hazards [112]. They may linger in the environment and build up in soil and water, causing pollution and having negative impacts on creatures that aren't the target [113].

In agricultural settings, biosurfactants have proven to be successful in reducing weeds, illnesses, and pests. They can disrupt the cell membrane of pests and pathogens, leading to cell leakage and death. Biosurfactants can also alter the surface properties of plants, making them more resistant to pests and diseases [115]. In addition, biosurfactants can act as signal molecules that trigger defense responses in plants, leading to enhanced resistance against pests and diseases [97]. Chemical pesticides, on the other hand, are effective in controlling pests, diseases, and weeds, but their use has been associated with various environmental and health hazards [116]. They can lead to soil and water contamination, resistance development, and adverse effects on non-target organisms. Furthermore, chemical pesticides can persist in the environment for a long time, leading to long-term adverse effects on ecosystems [117]. Biosurfactants have the potential to be an effective alternative to chemical pesticides in agriculture. They are environmentally friendly, biodegradable, and non-toxic, making them a promising alternative to chemical pesticides.

7. Impact of biosurfactant over beneficial microorganisms and environment

Biosurfactants have several environmental benefits over chemical pesticides, including biodegradability and low toxicity to non-target organisms [72]. Biosurfactants can also help to reduce the environmental impact of agriculture by reducing the use of chemical pesticides, which can contaminate soil and water resources [73].

Biosurfactants can have both positive and negative impacts on beneficial microorganisms in soil and plant surfaces [76]. While biosurfactants can enhance the growth of some beneficial microorganisms, they can also inhibit the growth of others. It is important to consider the potential impact of biosurfactants on beneficial microorganisms when developing biocontrol strategies [77].

8. Safety and regulatory aspects of biosurfactant-based biocontrol

The use of biosurfactants in agriculture is subject to regulatory oversight in many countries [102]. Before biosurfactants can be commercialized for agricultural use, they must undergo rigorous safety and efficacy testing to ensure their safety for humans, animals, and the environment [103]. Regulatory agencies may also require data on the environmental fate and impact of biosurfactants.

The broad consensus is that biosurfactants are safe for the environment and human health. [104]. They are biodegradable and non-toxic, and their use is associated with minimal risks compared to chemical pesticides. However, the safety of biosurfactants can vary depending on their source, production process, and application method. Therefore, it is essential to assess the safety of biosurfactants on a case-by-case basis before their use in agriculture [105].

The use of biosurfactants in agriculture is subject to various regulatory frameworks, depending on the country or region [102]. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) governs the use of biosurfactants in the United States, and the Environmental Protection Agency (EPA) oversees this regulation [106]. Biosurfactants that are intended for use in agriculture must undergo a rigorous evaluation process to determine their safety and effectiveness. The evaluation process includes various studies, including toxicity, environmental fate, and efficacy studies [95]. Similarly, the European Union (EU) regulates the use of biosurfactants under the Biocidal Products Regulation (BPR) and the Plant Protection Products Regulation (PPPR) [107]. Biosurfactants that are intended for use as biocontrol agents must undergo a similar evaluation process to determine their safety and efficacy [108].

9. Market Potential

In the upcoming years, it is anticipated that the global market for biopesticides, including biosurfactants, would expand significantly. The market for biopesticides is expanding because of rising consumer demand for ecologically friendly and sustainable farming methods as well as innovative biosurfactant products. However, the high cost of production and lack of regulatory guidance is currently limiting the commercialization of biosurfactants for agricultural use.

10. Commercialization of biosurfactants and availability

Although they have showed potential as a biocontrol agent in agriculture, biosurfactants are still in the early phases of commercialization [145]. The high cost of manufacture is one of the key obstacles to the commercialization of biosurfactants, which can limit their availability and affordability [146]. For example, a recent startup company in the United States is developing a biosurfactant-based product for the biocontrol of fungal plant pathogens. Another challenge is the lack of regulatory guidelines and standards for biosurfactant use in agriculture [126]. Although biosurfactants are still in the research and development phase, some companies have begun to commercialize biosurfactant-based products for use in agriculture [78]. For example, the company Aqtiva Biotech has developed a biosurfactant-based product called AqtivEmbrace, which is designed to enhance the efficacy of other biocontrol agents and reduce the use of chemical pesticides [147].

11. Future directions and opportunities

Further research is needed to optimize the production and application of biosurfactants in agriculture [142]. This includes identifying new microorganisms that produce effective biosurfactants, improving production processes to reduce costs and increase yields, and developing effective application methods that can be easily integrated into existing agricultural practices [143]. Additionally, more field trials are needed to evaluate the long-term effectiveness of biosurfactants in controlling plant pathogens under different environmental conditions and in different cropping systems [60]. More research is needed to optimize the use of biosurfactants in combination with other biocontrol agents for effective pest and disease management in agriculture [95], to elucidate the molecular mechanisms underlying biosurfactant-based biocontrol and to optimize their use in agriculture [144], to explore the potential of biosurfactants in agriculture and food technology, including the development of biosurfactant-based formulations for practical applications in controlling mycotoxigenic fungi in stored agricultural products, pests, diseases, and weeds [95], to investigate the possibilities of biosurfactants in plant biotechnology and agriculture [144].

Declarations

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