Trends in Entomology
Vol. 14, 2018

Review

Biosurfactants: Production and potential application in insect pest management

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ABSTRACT

Biosurfactants are amphipathic surface-active molecules produced by bacteria, fungi, and yeast. Owing to their low toxicity, high degree of biodegradability, optimal activity at extreme environmental conditions, and environmental friendly nature, biosurfactants have received attention in pest management. Most recently, the insecticidal activities of the biosurfactants obtained from different bacterial species have been reported. Therefore, considering the role of biosurfactants in novel insecticide production and environmentally safe insect pest management, we reviewed the isolation and identification of the biosurfactant-producing bacteria, the production methods of biosurfactants, and their potential applications in insect pest management, as well as delineated the possible future research areas. Finally, we concluded that, in the near future, biosurfactants will be the green pesticides that will replace the synthetic pesticides, and thus, for the discovery of novel insecticides, biochemical and molecular approaches, such as genomic and transcriptomic studies, are appreciable.

KEYWORDS: *Bacillus*, biosurfactant, biocides, biocontrol, bioprocessing, peptides.

1. Introduction

Biosurfactants are the surface-active biomolecules produced by bacteria, fungi, and yeast [1]. Basically, these microorganisms produce different biosurfactants for various purposes; for instance, rhamnolipids increase the solubility of hydrophobic hydrocarbons [2], cause changes in microbial surface properties [3], and enhance the bioavailability of potential hydrophobic carbon sources [4]. Unlike the chemically synthesized surfactants, biosurfactants are generally categorized based on their microbial origin and chemical composition [5]. Chemically, biosurfactants are categorized into glycolipids (rhamnolipids) [6], trehalolipids [7], sophorolipids [8], lipopeptides and lipoproteins (surfactin, lichenysin), fatty acids, phospholipids and neutral lipids [8], polymeric, and particulate biosurfactants [9]. Owing to their safe properties such as, low toxicity [10], high degree of biodegradability [11], high foaming capacity [12], and optimal activity at extreme environmental conditions [13], biosurfactants have recently received attention for their different applications in various fields (food industry, removal of oil and petroleum contamination, bioremediation of toxic pollutants and biopesticides). Currently, the interest in the use of biosurfactants as biopesticides has been growing fast [14-17] because of their environment-friendly characteristics and high degree of degradability. Thus, to reduce the adverse effects of synthetic pesticides on the environment and human health, biosurfactants could be one of the promising alternative options in the management of agricultural pests. Hence, this review mainly emphasizes on the contribution of biosurfactants in the management of agricultural insect pests, production and application of biosurfactants, and the way forward.

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2. History of biosurfactants as biopesticides

Insecticides have a long history in insect pest control. Synthetic surfactants are popularly used as adjuvant, emulsifying, dispersing, spreading and wetting agents and enhance the efficiency of pesticides. As a result, they are highly used in several pesticide-manufacturing industries [18]. However, these synthetic surfactants are non-degradable and accumulate in soil and ground water, as well as on agricultural products [19]. As a result, they cause adverse effects on the environment and humans. Alternatively, different bacterial and fungal species have recently been reported to produce biosurfactants [16, 17, 20, 21]. These biosurfactants have well known applications in the food industry and bioremediation of toxic pollutants, and therefore, have recently received attention in the pesticide industry and pest control [18, 22-24].

3. Types of biosurfactants

Biosurfactants are classified based on their microbial origin and chemical composition [5]. Originally, biosurfactants are naturally produced by bacteria, fungi, and/or yeast. However, bacterial species are well known for producing different biosurfactants, with *Pseudomonas* being the dominant genus in biosurfactant production [1]. The *Bacillus* species, which is the most commonly known bacteria used in insect pest management, predominantly produces lipopeptides, lichenysin, surfactin, lipid protein complexes, and subtilisin [25-29]. Chemically, they are categorized into glycolipids, lipopeptides and lipoproteins, fatty acids, phospholipids and neutral lipids, polymeric biosurfactants, and particulate biosurfactants. For easy understanding, [30], these biosurfactants are grouped into two types, based on their molecular weights: low- and high-molecular weight biosurfactants.

3.1. High-molecular weight biosurfactants

High-molecular weight biosurfactants are produced by a number of diverse bacterial species and are generally termed as polymeric biosurfactants, which consist of lipoproteins, proteins, polysaccharides, lipopolysaccharides or complexes [31, 32]. The *Acinetobacter* species has been identified as the bacterial species producing most high-molecular weight biosurfactants (Table 1).

3.2. Low-molecular weight biosurfactants

Low-molecular weight biosurfactants include glycolipids, lipopeptides and lipoproteins, fatty acids, phospholipids, and neutral lipids. The major role of these surfactants is to increase the surface

Table 1. High-molecular weight biosurfactants, their chemical compositions and origins.

Biosurfactant name	Components	Origin	References
Alasan	Complex of an anionic polysaccharide and a protein	*Acinetobacter radioresistens*	[33]
Complex biopolymer	Proteins, carbohydrates, and lipids	*Candida lipolytica IA 1055*	[34]
Non-dialyzable bioemulsifier	Complex of a protein, polysaccharide, and lipid	*Acinetobacter junii SC14*	[35]
Alasan	Complex of an anionic polysaccharide and a protein	*Acinetobacter radioresistens KA53*	[36, 37]
Biodispensan	Polysaccharide	*Acinetobacter calcoaceticus*	[38]
Mannoprotein	Carbohydrate (mannose) and protein	*Saccharomyces cerevisiae*	[39]
Capsule polysaccharide	Polysaccharide	*Acinetobacter calcoaceticus BD4*	[40]
Liposan	Carbohydrate and protein	*Yeast (Yarrowia lipolytica)*	[41]
Vesicles of *Acinetobacter*	Protein, phospholipid, and lipopolysaccharide	*Acinetobacter* sp. strain *HO1-N*	[42]
Emulsan	Lipopolysaccharide	*Acinetobacter calcoaceticus RAG-1 ATCC 31012*	[43]

area of hydrophobic substrates, thereby enhancing the bioavailability of hydrophobic substrates through solubilization/desorption and regulating the attachment and removal of microorganisms from the surface [5]. The *Pseudomonas* and *Bacillus* species are widely known for producing low-molecular weight biosurfactants. Some of these biosurfactants, their chemical components, and microbial origins are summarized in Table 2.

4. Production strategies of biosurfactants

Most of the biosurfactant-producing bacterial species are soil-inhabiting bacteria. Admittedly, the foremost step for biosurfactant production is the isolation and characterization of the target bacterial species (Fig. 1A). The chemical nature of a biosurfactant depends on the microbial species producing it. Thus, identifying the effective bacterial species has paramount importance in the production of biosurfactants for the purpose of insect pest control.

4.1. Isolation and identification of biosurfactant-producing bacteria

The source of a sample for bacterial isolation varies with the purpose of isolation. For instance, it could be from a traditional fermented food or from crude petroleum-oil-contaminated soil [60]. The isolation, taxonomic identification, and characterization of the biosurfactant-producing microorganism strains could be done by following the standard biochemical and morphological tests [61, 62]. Briefly, the sample is diluted and spread on the specific agar nutrient, and then incubated overnight at appropriate incubation temperature, which differs based on the targeted bacterial species. For example, if thermophilic bacterial species are targeted, an incubation temperature of >50 °C is recommendable. Subsequently, the morphological tests, such as, colony size, shape and color; biochemical tests such as, Gram positive and negative; and molecular

Table 2. Low molecular weight biosurfactants, their chemical composition and origin.

Biosurfactant name	Components	Origin	Reference
Rhamnolipids	Glycolipids	*Pseudomonas aeruginosa* *Pseudomonas chlororaphis*, *Serratia rubidaea*	[44-46]
Trehalolipids	Glycolipids	*Arthrobacter paraffineus* *Rhodococcus erythropolis*	[47, 48]
Sophorolipids	Glycolipids	*Torulopsis bombicola*, *T. apicola*, *T. petrophilum*, *Candida bombicola*, *C. antarctica*, *C. batistae*, *C. apicola*, *C. riodocensis*, *C. stellata*, *C. bogoriensis*	[49-53]
Glucose lipids	Glycolipids	*Alcanivorax borkumensis*	[54]
Surfactin	Lipopeptides	*Bacillus subtilis* *B. amyloliquefaciens*	[25, 31, 55]
Lipopeptides	Lipopeptides	*Bacillus licheniformis 86*	[28]
Peptide lipids	Peptide lipids	*Bacillus licheniformis*	[27]
Streptofactin	Hydrophobic peptide	*Streptomyces tendae*	[56]
Phosphatidylethanolamine	Fatty Acids, Phospholipids	*Rhodococcus erythropolis*	[57]
Glycolipid bioemulsifier	Disaccharides Fatty acids	*Rhodococcus erythropolis*	[58]
Corynomycolic acid	Fatty Acids	*Corynebacterium diphtheriae*	
Mannosylerythritol lipids	Glycolipids	*Pseudozyma antarctica*, *Pseudozyma siamensis*	[59]

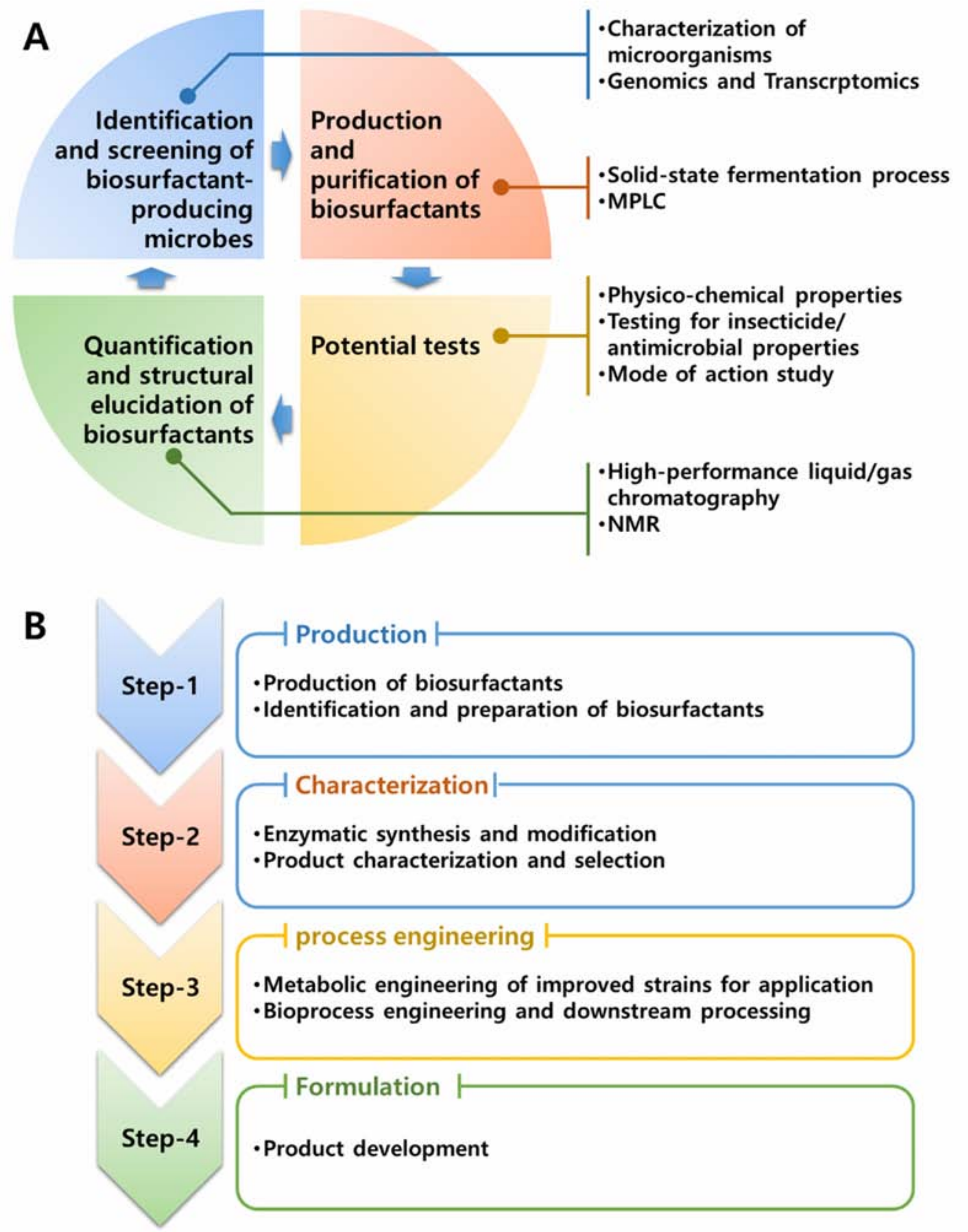

Fig. 1. Biosurfactant and novel bio-pesticide production strategies. **A**) Biosurfactant production and application strategies: The foremost step in the production of biosurfactants is the isolation, screening, and characterization of the biosurfactant-producing microbes using molecular (genomics and transcriptomic) and biochemical tests. Subsequently, biosurfactants are produced by solid-state fermentation and purified by middle pressure liquid chromatography (MPLC). The third step involves conducting insecticidal potential tests against target insects; this step also includes the mode of action study. Finally, the quantification and structural elucidation is carried out by high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) methods, respectively, for the production of novel bio-pesticide. **B**) Novel bio-pesticide production Strategies: Step 1 (production) involves the identification of appropriate production methods, which are cost effective and produce quality biosurfactants. Step 2 (characterization) includes enzymatic synthesis and product characterization. Step 3 (process engineering) includes recovery, concentration, and purification of the samples. Step 4 (formulation) is the last step in which the product is developed and disseminated.

identification tests like, polymerase chain reaction (PCR) amplification of the 16s rRNA gene and DNA sequencing could be employed as the identification and characterization tools. In general, the *Bacillus* species are well known for producing various types of biosurfactants [63-65].

4.2. Production and identification of biosurfactants as biopesticides

For experimental purposes, biosurfactants have been successfully produced in small quantities [15, 16, 66]. However, the successful production of a novel bio-pesticide from biosurfactants and its application relies mostly on the production processes and its quality [67] (Fig. 1B). These days, researchers have made an effort to improve the yield and quality of biosurfactants by using agricultural byproducts [15, 68, 69]. Besides cost effectiveness, the yield optimization process conditions have been extensively studied [60, 70, 71].

Solid-state and submerged fermentation are the common methods used in the production of biosurfactants for use as biopesticides [15, 60, 71]. However, the solid-state fermentation has several advantages over the submerged fermentation [72]. Consequently, this process has recently received considerable attention of researchers [71] and has potential applications in the production of microbial enzymes, bioinsecticides, secondary metabolites, and pharmaceuticals [73, 74].

The solid-state fermentation procedure was well developed for the extraction of biosurfactants [14, 15]. The industrial and agricultural byproducts could be used as carbon-source substrates. For example, banana peels, potato peels, chick-pea flour, corn bran, corn starch, millet, soya meal, barley flour, barley bran, rice flour, cornstarch, and orange peels [14] are carbon-rich substrates. These substrates need to be autoclaved at 120 °C for 30 min. To these substrates, Na_2HPO_4 (6.0 g/l), KH_2PO_4 (3.0 g/l), NH_4NO_3 (1.0 g/l), NaCl (1.0 g/l), $CaCl_2$ (0.014 g/l), $MgSO_4.7H_2O$ (0.245 g/l), thiamine-HCl solution (1.0 ml), and 1 ml of a solution of micronutrients are added, mixed thoroughly, and autoclaved at 121 °C and 15 lbs pressure for 15 min. Finally, 2 ml of 24-h grown bacterial culture is inoculated into the prepared substrate under sterile conditions and incubated at appropriate temperature for 2 to 5 days [60]. The fermented bacterial culture is mixed with distilled water (1:5, w/v) and stirred on a magnetic stirrer for 30 min at room temperature, then centrifuged at 10,000 × g for 10 min at 4 °C to remove the insoluble material. The identification of biosurfactants from the crude mixture is carried out by performing the high-performance liquid chromatography (HPLC) and mass spectrometric analyses [62].

5. Application of biosurfactants in insect pest control

Biosurfactants are known to have low toxicity [10], high degree of biodegradability [11], and optimal activity at extreme environmental conditions [13]. These properties enable them to have wide applications in different fields.

For decades, microbial bioinsecticides have displayed the potential to control agricultural pests [66, 70] (Table 3). The *Bacillus* species are the most famous biosurfactant-producing bacteria. They produce broad-spectrum lipopeptides, including surfactin, iturin, bacillomycin, fengycin, and lichenysin [21, 62]. These are bioactive metabolites that cause hemolysis with potent larvicidal activity [75, 76]. Mnif and Ghribi (2015) [65] have intensively reviewed the role of *Bacillus* and *Pseudomonas* bacterial species-derived biopesticides in pest management.

Surfactin produced by *Bacillus amyloliquefaciens G1* showed insecticidal potency against the green peach aphid, *Myzus persicae*, by affecting the aphid cuticles and inducing significant dehydration of the cuticle membrane to cause death [77]. Moreover, Khedher *et al*. (2015) [16] have reported the potential of *B. amyloliquefaciens* AG1 biosurfactant to control the *Tuta absoluta* larvae. The biosurfactant from this bacterium consists of lipopeptides and polyketides. This biosurfactant acts by binding to the receptors located in the brush-border membrane vesicles of the larvae. Similarly, *Bacillus thuringiensis Vip3Aa16 and B. amyloliquefaciens AG1* biosurfactants showed insecticidal potency against *Spodoptera littoralis*. The histopathological examination of the treated larval midgut revealed the vacuolization, necrosis, and disintegration of the basement membrane [17].

Since 1900s, *Bacillus subtilis*, which is commonly found in the upper layers of the soil, has been used

Table 3. Some of the biosurfactants used as biopesticides to control insects.

Bacterial species	Biosurfactants	Composition	Activity	Insect	References
Bacillus amyloliquefaciens AG1	Lipopeptides	Surfactin, fengycin, iturin, and bacillomycin	Larvicidal	*Tuta absoluta*	[16]
	Polyketides	Bacillaene, macrolactin, and difficidin			
Bacillus amyloliquefaciens AG1	Lipopeptides	Surfactin, fengycin, iturin, and bacillomycin	Larvicidal	*Spodoptera littoralis*	[17]
B. subtilis	Cyclic lipopeptides	Surfactin	Larvicidal	Mosquito	[76, 79, 80]
	Lipopeptide	Not given	Larvicidal	*Spodoptera littoralis*	[14]
B. amyloliquefaciens	Cyclic lipopeptides	Not given	Pupicidal		[76]
B. amyloliquefaciens G1	Lipopeptides	Surfactin	Insecticidal	*Myzus persicae*	[77]
Bacillus thuringiensis	Not given	*Cry3Aa* toxin	Larvicidal	Colorado potato beetle	[81]
Bacillus thuringiensis	Not given	Cry1Da toxin	Larvicidal	*Spodoptera littoralis*	[82]
Bacillus subtilis	Cyclic lipopeptides	Surfactin	Larvicidal	*Aedes aegypti* L.	[83]

in human disease treatment. Presently, the biosurfactants of this species have been reported to have applications in insect pest control. *B. subtilis* SPB1 has been shown to produce a lipopeptide biosurfactant and insecticidal activity against the storage insect pests, carob moth, *Ectomyelois ceratoniae* [15]. The histopathological effects of *B. subtilis* SPB1 biosurfactant on *E. ceratoniae* midgut was also studied and showed vesicle formation in the apical region of cells and lysis and strong vacuolization of columnar cells. Similar histopathological effects were observed in *Ephestia kuehniella* [21], *Spodoptera littoralis* [70, 78], and *Prays oleae* [70].

From this, we can understand that the biosurfactants produced by *Bacillus* species target the midgut tissue to cause death of the treated insects; thus, the oral application method is the effective way of treatment. Furthermore, the molecular mechanisms of these biosurfactants need to be studied to fully understand their exact modes of action. Additionally, the large-scale production of novel insecticides from this bacterial species needs further investigation.

6. Conclusion and future direction

Currently, biosurfactants have become one of the promising biopesticides in the management of insect pests. Though dozens of bacterial and fungi species have been reported to produce biosurfactants, only a few biosurfactants produced by the *Bacillus* species against insect pests have been well studied. Most of the exploited biosurfactants have been produced in small-scale in laboratories. Appreciating the efforts of some researchers, the mass production of these biosurfactants needs detailed study to produce the quality product. The targets of most biosurfactants and their modes of action in insects remain elusive. Therefore, the advanced biochemical and molecular approaches, such as genomic and transcriptomic studies, are crucial in studying their modes of action. This study will lead to the discovery of novel biopesticides. Hence, it can be concluded that, in the near future, biosurfactants will be the green pesticides that will replace the synthetic pesticides, and thus, the efforts of researchers in the fields of molecular biology, biochemistry, microbiology, and environmental science are appreciable.

ACKNOWLEDGEMENTS

This research was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through the Advanced Production Technology Development Program (no. 116110-2) and Export Promotion Technology Development Program (No. 617077-5), funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA), Korea.

AUTHORS’ CONTRIBUTION

Yeon soo Han, Tariku Tesfaye Edosa and Young Hu Jo, generated the idea and content of the review. Tariku Tesfaye Edosa and Mariam Keshavarz conducted the literature search. Tariku Tesfaye Edosa explored the literature and wrote the paper.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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