ISBN: 978-93-90847-17-4

6. Biosurfactants Based Biocontrol in Agriculture

Parthasarathi Rengasamy, Elango Rasavel, Harini Sundaresan,

Akash Krishnamoorthy, Kavinilavu Narayana samy

Department of Agricultural Microbiology,
Faculty of Agriculture, Annamalai University,
Annamalai Nagar, Tamilnadu, India.

6.1 Introduction:

Biosurfactants, widely known as surface-active agents of biological origin. First biosurfactant "surfactin" was purified and characterized by Arima *et al*. (1968). Data reveals there are more than 250 patents obtained on these wonder biodegradable molecules so far (Shete *et al.,* 2006; Rahman and Gakpe, 2008). There are five major categories of biosurfactants viz. glycolipids, phospholipids and fatty acids, lipopeptides and lipoproteins, polymeric biosurfactants and particulate biosurfactants that have found applications in agricultural, pharmaceutical, food, cosmetics, and detergent industries.

Among the various categories of biosurfactants the glycolipid biosurfactants "rhamnolipids" stand apart. Rhamnolipid, primarily a crystalline acid, is composed of β-hydroxy fatty acid connected by the carboxyl end to a rhamnose sugar molecule.

Rhamnolipids are predominantly produced by *Pseudomonas aeruginosa* and classified as: mono and di-rhamnolipids. *Pseudomonas* species that have been reported to produce rhamnolipids are *P. chlororaphis, P. plantarii, P. putida, and P. fluorescens.*

In 1984, the first patent for the production of rhamnolipids was filed by Kaeppeli and Guerra-Santos (US 4628030) and obtained in 1986 for their work on Pseudomonas aeruginosa DSM 2659, Kaeppeli and Guerra-Santos, 1986.

Subsequently, Wagner et al. filed a patent (US 4814272) in 1985 for the biotechnical production of rhamnolipids from Pseudomonas sp. DSM 2874 and obtained the same in 1989 (Wagner et al., 1989). The reason behind the current global interest in rhamnolipid production in various industries was, "eco-friendly" properties.

6.1.1 Inimitable Applications of Rhamnolipids:

Over the years rhamnolipids are becoming broadly pertinent in various industries and are posing a serious threat to the synthetic surfactants. Increasing the demand for the biosurfactant.

Five major applications of rhamnolipids that cater to the wide range of industrial demands includes:

a. Bioremediation and enhanced oil recovery (EOR): Rhamnolipids show excellent emulsification properties, efficiently remove crude oil from contaminated soil and facilitate bioremediation of oil spills (Rahman et al., 2003; Costa et al., 2010).

b. Pharmaceuticals and therapeutics: Rhamnolipids show low toxicity, surface active properties and antimicrobial activities against several microbes (*Bacillus cereus, Micrococcus luteus, Staphylococcus aureus, Listeria monocytogenes*) thereby showing promising applications in pharmaceuticals and therapeutics (Magalhaes and Nitschke, 2013).

c. Cosmetics: Rhamnolipid as an active ingredient is found to be effective for several skin treatments i.e., wound healing with reduced fibrosis, cure of burn shock, treatment of wrinkles hence are in demand in the health and beauty industry (Piljac and Piljac, 2007).

d. Detergents and cleaners: Rhamnolipids are natural emulsifiers and surface active agents leading to their wide spread usage in detergent compositions, laundry products, shampoos and soaps (Parry *et al*., 2013).

e. Agriculture: Rhamnolipids are already used for soil remediation for improving soil quality and are now further getting explored for plant pathogen elimination, for aiding the absorption of fertilizers and nutrients through roots and as biopesticides (Sachdev and Cameotra, 2013).

6.1.2 Leading Producers of Biosurfactants:

Rhamnolipids are highly applicable in various activities with some researchers advancing the technology from laboratory to higher scale. However, there still are very limited companies in the field which are producing biosurfactants at a marketable scale. Leading producers of biosurfactant in India includes Evonik India Pvt. Ltd., Mitsubishi India, Vetline (Unit of Simfa Labs Pvt. Ltd.,) They focus on producing Cleaners, Food processing, Agricultural chemicals, Detergents, Oil field chemicals and the products used are; Glycolipids, Alkyl Polyglucosides, Methyl Ethyl sulfonates, Sucrose Esters, Sorbitan Ester, etc.,

Table 6.a: Biosurfactant Producing Companies Globally.

Company	Location	Products	Focus On
TeeGene Biotech	UK	Rhamnolipids and Lipopeptides	Pharmaceuticals, cosmetics, antimicrobials and anti-cancer ingredients
AGAE Technologies LLC	USA	Rhamnolipids (R95, an HPLC/MS grade rhamnolipid)	Pharmaceutical, cosmeceutical, cosmetics, personal care, bioremediation (*in*

Company	Location	Products	Focus On
			situ & *ex situ*), Enhanced oil recovery (EOR)
Jeneil Biosurfactant Co. LLC	USA	Rhamnolipids (ZONIX, a bio-fungicide and RECO, a rhamnolipid used in cleaning and recovering oil from storage tanks)	Cleaning products, EOR
Ecover Belgium	Belgium	Sophorolipids	Cleaning products, cosmetics, bioremediation, pest control, pharmaceuticals
Rhamnolipid Companies, Inc.	USA	Rhamnolipids	Agriculture, cosmetics, EOR, bioremediation, food products, pharmaceutical
Synthezyme LLC	USA	Sophorolipids	Cleaning products, cosmetics, food products, fungicides, crude oil emulsification
Allied Carbon Solutions (ACS) Ltd	Japan	Sophorolipids (ACS-Sophor—first bio-based surfactant from Indian mahua oil)	Agricultural products, ecological research
Lion Corporation	Japan	Methyl ester sulfonates (MES)	Detergent's formulations, cleaning products

6.2 Biosurfactant:

Surfactants are amphiphilic molecules with both hydrophilic and hydrophobic regions attributing towards decrease in surface tension by the formation of aggregates at interfaces between fluids of different polarities. Biosurfactants are the surface-active biomolecules produced by microorganism. Basically, these microorganisms produce different biosurfactants for various purposes like detergency, emulsification, adhesion, coatings, wetting, foaming, soil and water remediation, paints, chromatographic separation, medicine, agriculture, cosmetics etc,

For instance, Rhamnolipids increases the solubility of hydrophobic hydrocarbon, cause changes in microbial surface properties, and enhance the bioavailability of potential hydrophobic carbon sources.

6.3 Biosurfactants Supposedly Act Through Three Distinct Activities:

- Modification of surface properties,
- Alteration of compound bioavailability,
- Interaction with membranes.

6.4 Properties:

- Surface and interface activity,
- Low toxicity,
- Emulsion forming and emulsion breaking,
- Antiadhesive agents,
- Temperature and pH tolerance,
- High degree of biodegradability,
- High foaming capacity.
- Optimal activity at extreme environmental conditions.

6.5 Classification of Biosurfactants:

6.5.1 Glycolipid:

They are carbohydrates linked to long-chain aliphatic acids or hydroxyaliphatic acids by an ester group. Biosurfactants are majorly glycolipids. Among the glycolipids, the best known are.

- Rhamnolipids
- Trehalolipids
- Sophorolipids

6.5.2 Rhamnolipids:

One or two molecules of rhamnose are linked to one or two molecules of hydroxydecanoic acid. The principal glycolipids produced by *P. aeruginosa*.

6.5.3 Trehalolipids:

Trehaloselipids from *Rhodococcus erythropolis* and *Arthrobacter* spp. Lowered the surface and interfacial tension in culture broth from 25-40 and 1-5 mNm, respectively. Enhance the bioavailability of hydrocarbons.

6.5.4 Sophorolipids:

Produced by yeast like *Torulopsis bombicola, T. apicola* and *Wickerhamiella domericqiae,* consist of a dimeric carbohydrate sophorose linked to a long-chain hydroxyl fatty acid by glycosidic linkage. Sophorolipids, generally a mixture of at least six to nine different

hydrophobic sophorolipids and lactone form of the sophorolipid is preferable for many applications. Sophorolipids have the capacity to lower the surface tension of water, Recovery of hydrocarbons from dregs and muds and Removal of heavy metals from sediments, enhancement of oil recovery.

6.5.5 Lipopeptides and Lipoproteins:

These consist of a lipid attached to a polypeptide chain. Several biosurfactants have shown antimicrobial action against various bacteria, algae, fungi and viruses. The antifungal property and the antibacterial property of the lipopeptide, iturin which was produced by ***Bacillus subtilis.*** Iturin from *B. subtilis* was found to be active even after autoclaving, pH 5-11 and with a shelf life of 6 months at -18°C.

6.5.6 Surfactin:

A cyclic lipopeptide which contain a -hydroxy fatty acid in its side chain., one of the most effective biosurfactants known so far, which was first reported in ***B. subtilisATCC-21332***. Recent studies indicate that surfactin shows potent antiviral, antimycoplasma, antitumoral, anticoagulant activities as well as inhibitors of enzymes. Potential applications in medicine or biotechnology but have not been exploited extensively till date.

6.5.7 Lichenysin:

Bacillus licheniformis produces several biosurfactants which exhibit excellent stability under extreme temperature, pH and salt conditions which are similar to surfactin. Lichenysin from *B. licheniformis* are able to reduce the surface tension and interfacial tension of water to 27 and 0.36 mN m^{-1}, respectively. Lichenysin is a more efficient cation chelator compared with surfactin.

6.5.8 Iturin:

The iturin group of compounds are cyclic lipoheptapeptides which contain α - amino fatty acid in its side chain. Iturin-A was isolated from a *Bacillus subtilis* strain taken from the soil in Iturin (Zaire). They are potent antifungal agents which can also be used as biopesticides for plant protection.

6.5.9 Polymeric Biosurfactants:

a. Emulsan: Effective emulsifying agent for hydrocarbons in water, even at a concentration as low as 0.001-0.01%

b. Liposan: Extracellular water-soluble emulsifier synthesized by *Candida lipolytica* and is composed of 83% carbohydrate and17% protein and used as emulsifier in food and cosmetic industries.

c. **Alasan**: Produced by *A. radioresistensKA-53* was reported to solubilise and degrade polyaromatic hydrocarbons.

d. **Biodispersan:** Produced by *Acinetobacter calcoaceticu* A2 used in dispersion of limestone in water.

e. **Mannoprotein**: Produced by *Saccharomyces cerevisiae used in* Stabilization of hydrocarbon-in-water emulsions.

6.5.10 Biosurfactant as an Antiadhesive Agent:

Implementing the biosurfactant to the solid surface may prevent the adsorption of pathogenic or spoilage-causing microorganisms in food surface, equipment, pipeline and other food processing materials, thereby preventing the contamination in food industries to a great extent. In reference to the report of (McL and sborough *et al*. (2006), a surface-active agent interferes with the biofilm matrix and reduces the interfacial tension among solid surface and biofilm, thereby eliminating the biofilm.

6.6 Application in Agriculture:

6.6.1 Improvement of Soil Quality:

The productivity of agriculture land is affected by presence of organic and inorganic pollutants that impart abiotic stress on the cultivated crop plant. To increase the quality of such soil contaminated by hydrocarbon and heavy metals, process of bioremediation is required. Biosurfactant can be effectively used for removal of hydrocarbons as well as heavy metals and known to enhance bioavailability and carry out biodegradation of hydrophobic compounds, different technologies such as soil washing technology and clean up combined technology employ biosurfactant for effective removal of hydrocarbons. Example: *Pseudomonas* sp, *Bacillus* sp., and *Acinetobacter* sp.

Probiotic biosurfactant is preferred in many applications in recent years owing to the property of the probiotic strain than other pathogenic ones (Sharma et al. 2016). Their antimicrobial and antiadhesive property makes them more effective in the biomedical application aspect. The antiadhesive property of *Lactobacillus plantarum* and *Lactococcus lactis* preventing the microbial colonization has been demonstrated in the study of Fatena *et al.* (2016) and Rodrigues *et al.* (2004).

There are certain factors that determine the efficacy of the biosurfactant- producing probiotic strain, which include acid and bile tolerance, proteolytic resistance, and antagonist properties against pathogenic microbes, providing a better environment for the beneficial microbes. Typically, lactic acid bacteria (probiotic strain) have established a substantial interest for their potential use in establishing the competitive environment towards pathogenic microbiota. However, *Lactobacillus species* shows evidence of antioxidative activity, thereby decreasing the risk of accruing reactive oxygen species on food products (Kullisaar *et al.* 2002).

6.6.2 Biosurfactant Extending the Shelf Life of Food Products:

In recent times, biosurfactant has been established in numerous food products for extending its shelf life, thereby accounting for profitability in the food industries and better health of consumers The rhamnolipids are currently approved for use in the food industry by the US Environment Protection Agency (EPA). The rhamnolipids in synergistic action with niacin

extends the shelf life of UHT (ultrahigh temperature) soymilk by inhibiting hemophilic spores. Furthermore, the similar combinations of niacin with rhamnolipids have also been implemented in salads as well as cottage cheese to increase its shelf life and to inhibit the growth of mold, bacteria and spore formers as stated by Haesendonck et al. (2004).

6.6.3 Potential of Biosurfactants in Pesticide Industries:

Surfactants act as adjuvant with fungicides, insecticides, and herbicides. The synthetic surfactant presently used in pesticides industries act as emulsifying, dispersing, spreading and wetting agent and enhance the efficiency of pesticides. The synthetic surfactants are considered as potent organic pollutants in soil, bacteria belonging to *Pseudomona* sp. And *Burkholderia* sp. from paddy field to degrade surfactants. Pesticides formed with the assistance of biosurfactant can be widely used on agricultural fields.

6.6.4 Microbes from Soil Environment as Producers of Biosurfactants:

Many rhizospheres and plant associated microbes are known to produce biosurfactant indicating the potential role of biosurfactant in plant-microbe interaction and further application of biosurfactant in agriculture. The prevalence of biosurfactant producing bacteria in environment and applications in agriculture and related industries as they play vital role in soil remediation, plant pathogen elimination and by increasing the bioavailability of nutrient for beneficial plant associated microbes.

6.6.5 Plant Pathogen Elimination:

Biosurfactants facilitates biocontrol mechanism of plant growth promoting microbes such as parasitism, antibiosis, competition, induced systemic resistance, and hypovirulence. Surfactants producing *Pseudomonas putid* promotes growth that can cause lysis off zoospores of the oomycete pathogen *Phytophthora capsici*, causative agent of root rot of capsicum. The lipopeptide biosurfactant produced by strains of *Bacillus* exhibits growth inhibition of phytopathogenic fungi like *Fusarium* spp., *Aspergillus* spp., and *Biopolaris sorokiniana.*

6.6.6 Plant–Microbes Interactions and their Importance to Biocontrol:

Fluorescent *Pseudomonas spp*. are well-studied plant-associated bacteria, which include several plants– pathogen strains as well as a range of isolates with biocontrol abilities. *Bacillus* comes second after *Pseudomonas,* with bio-control abilities. *Pseudomonads* and *Bacilli* mainly produce cyclic lipopeptide (CLP) type biosurfactants, which are made up of a cyclized oligopeptide lactone ring coupled to a fatty acid tail.

6.6.7 Plant–Pseudomonas Interactions:

Pseudomonas aeruginosa, an opportunistic pathogen, cause serious infections in susceptible individuals also exhibit plant growth promoting effects. Most strains of this species produce rhamnolipid type biosurfactants. 60% of fluorescent *Pseudomonas* isolates

from the sugar-beet rhizosphere in a sandy soil produced biosurfactants, whereas only 6% of sugar beet rhizosphere isolates from a loamy soil were able to produce biosurfactants.

Table 6.b: Anti-Phytopathogenic Properties of Rhamnolipids.

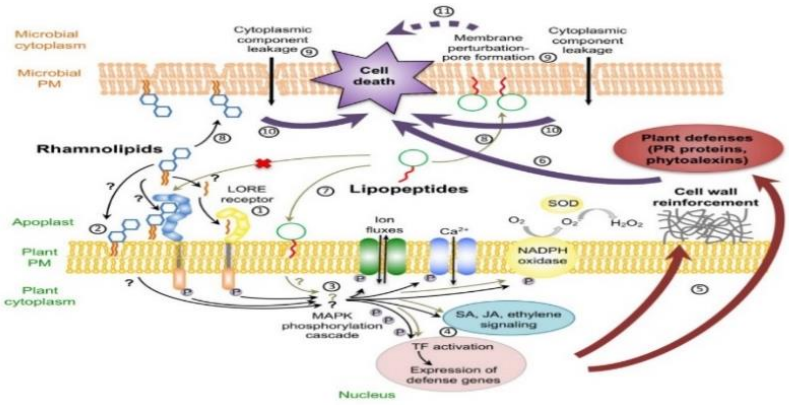
Compositions	**Source organisms**	**Sensitive hytopathogens**	**Effect**
Rhamnolipids	*Pseudomonas spec.DSM 2874*	*Glomerella cingulata*	Conidial germination inhibition, Growth inhibition (MIC) Lang *et al*., 1989
Rha-Rha-C_{10}-$C_1$0, Rha-C_{10}-C_{10}	*Pseudomonas aeruginosa*	*Phytophthora capsici, Pythium aphanidermatum, Plasmopara lactucae-radicis*	Zoospore lysis (Stanghellini and Miller, 1997)
Rha-Rha-C_{10}-C_{10}	*Pseudomonas aeruginosa B5*	*Cercospora kikuchii, Cladosporium cucumerinum, Colletotrichum orbiculare, Cylindrocarpon destructans, Magnaporthe grisea, Phytophthora capsici*	Zoospore lysis, spore germination and hyphal growth inhibition (Kim *et a*l., 2000)
Rha-Rha-C_{10}-C_{10}, Rha-C_{10}-C_{10}, Rha-Rha-C_{10}-C_{12}, Rha-C_{10}-C_{12}, Rha-$C_{12:1}$-C_{10}, Rha-$C_{12:2}$, Rha-$C_{8:2}$	*Pseudomonas aeruginosa AT10*	*Botrytis cinerea, Rhizoctonia solani, Colletotrichum gloeosporioides, Fusarium solani, Penicillium funiculosum*	Growth- inhibition (MIC) Abalos *et al.*, 2003
Rha-Rha-C_8-C_{10}, Rha-C_{10}-C_8/Rha-C_8-$C_1$0, Rha-Rha-C_8-$C_{12:1}$, Rha-Rha-C_{10}-$C_{10,}$ Rha-Rha-C_{10}-$C_{12:1}$, Rha-C_{10}-C_{10}, Rha-Rha-$C_{10}C_{12}$/Rha-Rha-C_{12}-C_{10} , Rha-C_{10}-$C_{12:1}$/Rha-$C_{12:1}$-C_{10},	*Pseudomonas aeruginosa 47T2*	*Penicillium funiculosum, Fusarium solani, Botrytis cinerea, Rhizoctonia solani*	Growth inhibition (MIC) (Benincasa *et al.*, 2004)

Compositions	**Source organisms**	**Sensitive hytopathogens**	**Effect**
Rha-Rha-$C_{12:1}$-C_{12}, Rha-Rha-C_{10}-$C_{14:1}$, Rha-C_{10}-C_{12}/Rha-C_{12}-C_{10}			
Rha-Rha-C_{10}-C_{10}, Rha-C_{10}-C_{10}, Rha-Rha-C_{10}-$C_{12:1}$, Rha-C_{10}-$C_{12:1}$, Rha-Rha-C_{10}-C_{12}, Rha-C_{10}-C_{12}	*Pseudomonas aeruginosa LBI*	*Penicillium funiculosum, Alternaria alternata*	Growth inhibition (MIC) (De Jonghe *et al*., 2005)
Biosurfactant PRO1 (formulation of 25% RLs) Plant support (the Netherlands)	*Pseudomonas aeruginosa*	*Phytophthora cryptogea*	Zoospore lysis, reduction of sporangia formation (Yoo *et al.*, 2005)
Mono- and di-rhamnolipids	*Pseudomonas aeruginosa IGB 83*	*Phytophthora capsici, Phytophthora nicotianae, Phytophthora cactorum, Phytophthora infestans, Pythium aphanidermatum, Pythium ultimum*	Motility inhibition, zoospore lysis, mycelial growth inhibition (Parneel *et al*., 2008)
Rha-Rha-C_{10}-C_{10}, Rha-C_{10}-C_{10} (Jeneil Biosurfactant Company JBR599)	*Pseudomonas aeruginosa*	*Botrytis cinerea*	Spore germination and mycelial growth inhibition (Varnier *et al*., 2009); (Monnier et al., 2018)
Rha-Rha-C_{10}-C_{10}, Rha-C_{10}-C_{10}	*Pseudomonas aeruginosa ZJU211*	*Phytophthora infestans, Phytophthora capsici, Botrytis cinerea, Fusarium graminearum, Fusarium oxysporum*	Mycelial growth Inhibition (sha *et al.*, 2012)

Compositions	**Source organisms**	**Sensitive hytopathogens**	**Effect**
Mono- and di-rhamnolipids	*Pseudomonas aeruginosa ZJU-211*	*Alternaria alternata*	Spore germination and mycelial growth inhibition (Goswami *et al*., 2014)
Rha-C_{10}-C_{10}, Rha-Rha-C_{10}-C_8, Other Rha or Rha-Rha: -C_{10}-C_{10}, -C_8-C_{10}, -C_{10}-C_{12}, -C_{12}-C_{12}, -C_{14}-C_{10}, -C_{10}-C_{16}	*Serratia rubidaea SNAU02*	*Fusarium oxysporum, Colletotrichum gloeosporioides*	Mycelial growth Inhibition (Nalini and Parthasarathi., 2014)
Rha-C_{10}-C_{10}, Rha-Rha-C_{10}-C_{10}	*Pseudomonas aeruginosa KVD-HM52*	*Fusarium oxysporum*	Mycelial growth and fungal biomass accumulation inhibition (Deepika *et al*., 2015)
Rha-$C_{8:2}$, Rha-$C_{8:1}$, Rha-C_{10}, Rha-$C_{12:1}$, Rha-Rha-$C_{10:1}$, Rha-C_{10}-$C_{10:1}$/Rha-$C_{10:1}$-C_{10}	*Pseudomonas aeruginosa DS9*	*Colletotrichum falcatum*	Spore germination and mycelial growth inhibition (Goswami *et al*., 2015)
Rha-C_8, Rha-C_{10}-C_{10}	*Pseudomonas aeruginosa SS14*	*Fusarium verticillioides*	Spore germination and mycelial growth inhibition (Borah *et al*., 2016)
Rha-Rha-C_{10}, Rha-Rha-C_8-C_{10}, Rha-Rha-C_{10}-C_{10}	*Pseudomonas aeruginosa DR1*	*Sclerotium rolfsii, Fusarium oxysporium, Phytophthora nicotianae, Macrophomina phaseolina*	Mycelial growth inhibition (Sathi Reddy *et al*., 2016)
Rha-Rha-C_{10}-C_{10}, Rha-C_{10}-C_{10}	*Pseudomonas aeruginosa ZJU211*	*Verticillium dahliae*	Spore germination and mycelial growth inhibition (Sha and Meng, 2016)
Rha-C_{10}-C_8, Rha-C_{10}-C_{10}, Rha-C_{10}-$C_{12:1}$, Rha-C_{10}-C_{12},	*Pseudomonas aeruginosa #112*	*Aspergillus carbonarius*	Mycelial growth inhibition (Rodrigues *et a*l., 2017)

Compositions	**Source organisms**	**Sensitive hytopathogens**	**Effect**
Rha-Rha-C_8-C_{10}, Rha-Rha-C_{10}-C_{10}, Rha-Rha-C_{10}-$C_{12:1}$, Rha-Rha-C_{10}-C_{12}			
Semipurified rhamnolipid mixture (RL90-A, AGAE Technologies, Corvalis, United States) and RL90-N, NatSurFact, Fairfax, United States)	*Pseudomonas aeruginosa*	*Leptosphaeria maculans*	Mycelial growth inhibition (Monnier *et al.*, 2020)

Dual Effects of Rhamnolipids and Lipopeptides on Antimicrobial Activities and Plant Defense Induction:



Courtesy: Jerome Crouzet et al 2020 Frontiers in Bioengineering and Biotechnology (https:// doi.org/10.3389/fbioe.2020.01014)

1. mc-3-OH-acyl building block of rhamnolipids is perceived by plant through the LORE receptor.
2. Rhamnolipid could be sensed through their direct insertion in plasma membrane.
3. Recognition of rhamnolipids leads to early signaling events like ion fluxes (Ca2+), reactive oxygen species production (H_2O_2) and MAPK phosphorylation cascade.
4. These early responses trigger defense gene expression, probably through activation of transcription factors (TF) and hormonal signaling pathways.
5. This leads to defense mechanisms like cell wall reinforcement and PR protein accumulation.

6. Triggering the resistance to the microbes.
7. Plant immunity due to lipopeptides does not involve a protein receptor and rely on interaction between lipopeptides and the plant membrane.
8. Both rhamnolipids and lipopeptides can also have direct antimicrobial effects through direct insertion into the microbial plasma membrane.
9. These insertions trigger loss of cell morphology leading to pore formation.
10. 10.The pore formation causes cellular component leakage triggering microbial cell death µ. 11. Cell death due to lipopeptides can also be indirectly due to the inhibition or activation of microbial cell functions.

6.7 Application in Agriculture:

Various biosurfactants from microorganisms have antimicrobial activity against phytopathogens and proven to be a promising biocontrol molecule for achieving sustainable agriculture.

The purified mono- and dirhamnolipid were found to highly effective against three zoosporic plant pathogens, *Pythium aphanidermatum, Phytophthora capsici* and Plasmopara lactucae-radicis at a concentration ranging from 5 to 30 mg/L, which caused lysis of the entire zoospore population in less than 1 min (Stanghellini and Miller 1997).

Rhamnolipid mixture obtained from *P. aeruginosa AT10* exhibited inhibitory activity against the bacteria, namely, *Escherichia coli, Micrococcus luteus* and *Alcaligenes faecalis*, at the concentration of 32 mg/ml whereas *Serratia marcescens* and *Mycobacterium phlei* at the concentration of 16 mg/ml.

An excellent antifungal activity was displayed against *Chaetomium globosum, Penicillium chrysogenum* and *Aureobasidium pullulans* at a concentration of 32 mg/ml and *Aspergillus niger* at 16 mg/ml, respectively (Abalos *et al*. 2001). Nielsen and Sorensen (2003)

6.7.1 Biocontrol Activity Against *Phytophthora Infestans:*

All the isolates from Eastern, Northern, Southern part of India, were tested for their biocontrol activity against *P. infestans* using dual culture method. Bacterial cell suspension was prepared and 5 µl aliquots of the bacterial suspension were placed around 5 mm plug of *P. infestans* at four places at equal distance in full grown plates of *P. infestans* on rye A media and kept at 20ºC. Out of 95 bacterial isolates, only five isolates namely *P. aeruginosa-1, P. aeruginosa-2, P. aeruginosa -3, P. aeruginosa-4,* and *P. aeruginosa-5* exhibited best biocontrol activities when tested in dual culture method. These isolates showed 62.22%, 38.33%, 46.22%, 32.66%, and 35.33% inhibition, respectively.

6.7.2 The Biosurfactant Produced by *P. Putida 267:*

The putisolvin-like CLPs, provides excellent biocontrol activity against *Phytophthora* damping-off cucumber (Raaijmaker *et al.*, 2006).

6.7.3 The Biosurfactant Produced by Pseudomonas Koreensis:

The crude extract used against the oomycetes *Pythium ultimum* in hydroponic tomato cultivation (Hultberg *et al.,* 2009). The ability of biosurfactant to bring about a reduction in plant disease caused by *P. infestans* has reported by Tran *et al*. (2007), in a study focusing on tomato late blight.

6.8 Biosurfactant from Pseudomonas as Biocontrol against Fungal Plant Pathogens:

6.8.1 The treatment with Pseudomonas fluorescens SS101:

The biosurfactant induced resistance against *P. infestans* in tomato, both systemically and locally, Tran *et al.* (2007). The biosurfactant used in a grapevine study by Varnier (2009) showed the ability to induce resistance to *Botrytis cinerea* (necrotrophic fungus). Rhamnolipid biosurfactant plays a key role in triggering the plant defense responses.

6.8.2 The treatment with Pseudomonas koreensis 2.74:

Study based on detached leaf assays, shows suppression of late blight, when biosurfactant were applied to potato leaflets *(Hultberg et al. 2010).* The biosurfactant forms the channels in the cell wall and perturbations of the cell surface of the pathogen *(Raaijmakers et al., 2006).* The best-known biosurfactants in biological control are rhamnolipids eg. *P. aeruginosa* PNA1 produces rhamnolipids *(Perneel, 2006).* Malin Hultberg *et al.* (2011) found zoospore-producing oomycetes pathogens of particular in hydroponic systems. They tried three ways of supplying a biosurfactant-producing strain to a recirculating hydroponic cultivation system infected with a zoospore-producing plant pathogen.

The strain *Pseudomonas koreensis* 2.74 was added as washed cells, in a minimal medium adapted from the nutrient solution, and compared with control treatments. A significant reduction in disease with up to 50% was achieved when a high concentration of washed cells was added weekly to the plant cultivation system. The disease suppression obtained through addition of washed cells equaled the effect achieved when the purified biosurfactant was used.

6.8.3 Biosurfactant Producing -Fluorescent Pseudomonads:

Hultberg et al. (2008) Inhibit the growth of fungal pathogens such as *Pythium ultimum* (causative agent of damping off and root rot of plants) *Fusarium oxysporum* (causes wilting in crop plants). *Phytophthora cryptogea* (causes rotting of fruits and flowers).

6.8.4 Pseudomonas Fluorescens Strain Pfg32r:

The antifungal activity against *Fusarium oxysporum f. sp. radicis-lycopersici* and *Fusarium graminearum* was inactivated by mutation in GacS/GacA two-component regulatory system *(Susanta and Takikawa 2006).*

6.8.5 Pseudomonas Sp GR3, Isolated from the Central Himalayan Region.

Pseudomonas sp GR3, could effectively control Damping off (*Phythium aphanidermatum* and *Phytophthora nictotianae)*

6.8.6 Mode of Action:

The disease was spread by propagule of the oomycetes, which is particularly sensitive to interaction with surfactant, as it lacks cell wall.

Disruption of the plasma membrane. As rhamnolipid biosurfactant lysis the plasma membrane of the zoospore fungi (Sharma *et al.* 2007).

6.8.7 Pseudomonas Isolates from Different Crop Plants:

Pseudomonas aeruginosa JS29, control of anthracnose disease, caused by fungal pathogen *Colletotrichum capsica* in Chili. Lankar *et al.* (2018) Ozyilmaz and Benlioglu (2013) isolated *Pseudomonas* from different crop plants were screened for *in vitro* growth inhibition of *Phytophthora capsici* and production of biosurfactant.

6.9 Biosurfactant Against Insects:

6.9.1 P. Aeruginosa Lbi 2a1:

Glycolipid biosurfactant as biopesticides for the insects and control of mosquito invasion. The rhamnolipid exhibited the larvicidal potency against *Aedes aegypti* larvae (Silva *et al.* 2014).

6.9.2 Pseudomonas Sp. EP-3:

Kim *et al.* (2011) reported insecticidal activity of rhamnolipid biosurfactant against *Myzus persicae* (green peach aphid).

The application dose- dependent activity against aphid with. 50% mortality at 40 μg/ml and 100% mortality at 100 μg/ml.

6.9.3 Mode of Action: Damages the cuticle membrane.

6.10 Biosurfactant Against Bacterial Pathogen:

6.10.1 P. Aeruginosa AT10:

Rhamnolipid mixture obtained from *P. aeruginosa AT10* exhibited inhibitory activity against the bacteria, ***Escherichia coli, Micrococcus luteus, Alcaligenes faecalis,*** *A*t the concentration of 32 mg/ml

6.10.2 Mode of Action:

Increase the permeability nature of the membrane present in Gram-positive bacteria, while the lipopeptide type of biosurfactant molecule may penetrate the cellular membrane, resulting in the leakage of cytoplasmic material and leading to cell lysis (Naruse *et al*. 1990; Ocheretina and Scheibe 1997).

6.10.3 Biosurfactant from Bacillus as Biocontrol Against Fungal Pathogens:

The major producers of lipopeptide are *B. subtilis, B. cereus, B. licheniformis, B. amyloliquefaciens*.

6.10.4 Mode of Action:

Due to their amphipathic characteristics, lipopeptides can induce the formation of pore and ion channels in lipid bilayer membranes and cause less pathogen resistance compared with traditional antibiotics or fungicides.

6.10.5 Biosurfactant Produced by Bacillus Licheniformis:

Alkadious *et al.* (2019) reported the antifungal activity of a biosurfactant produced by *Bacillus licheniformis* against *Rhizoctonia solani AG-4* that causes root rot in two cultivars of *Vicia faba* (Nubaria 1 & Sakha 1). Treatment with biosurfactant decreased the disease incidence; Nubaria 1 shows the disease reduction ranging from 20.00 to 62.11%, Sakha 1 shows the disease reduction ranging from 16.51 to 38.93%

6.10.6 Biosurfactant Producing Bacillus Subtilis:

The *Colletotrichum gloeosporioides*, causes anthracnose on papaya leaves is reported to be controlled by biosurfactant producing *Bacillus subtilis* isolated from soil (Kim *et al.,* 2010).

6.11 Bacillus Subtilis SPB1:

Mnif et al. (2015) reported *Bacillus subtilis* SPB1 lipopeptides were evaluated as a natural antifungal agent *against Fusarium solani* infestation. *In vitro* antifungal assay showed a minimal inhibitory concentration of about 3 mg/ml. **Treatment by *SPB1* lipopeptides on** *F. solani* will Generate excessive lyses of the mycelium and cause polynucleation and destruction of the related spores together with a total inhibition of spore production. I*n vivo* antifungal activity was proved against the dry rot potato tubers caused by *F. solani*. After 20 days of fungi inoculation, rot invasion was reduced by almost 78%, in comparison to that of non-treated one. Mnif *et al.* (2016) The crude lipopeptide mixture was tested for its inhibitory activity against phytopathogenic fungi.

6.11.1 Fungistatic Mode of Action for Rhizoctonia Bataticola:

- A minimal inhibitory concentration (MIC)- 0.04mg/ml

- Inhibitory concentration at 50% (IC50%)- 0.012mg/ml
- Inhibitory concentration at 90% (IC90%)- 0.02 mg/ml

6.11.2 Fungistatic Mode of Action for Rhizoctonia Solani:

- A minimal inhibitory concentration (MIC)- 4mg/ml
- Inhibitory concentration at 50% (IC50%)- 0.25mg/ml
- Inhibitory concentration at 90% (IC90%)- 3.3 mg/ml.

6.12 Biosurfactant Against Insects:

6.12.1 Bacillus Subtilis SPB1:

Ghribi *et al.* (2011) evaluated the insecticidal activity of this biosurfactant against the Egyptian cotton leaf worm (*Spodoptera littoralis*) and Displayed toxicity with an LC_{50} of 251 ng/cm^2.

6.12.2 Mode of Action:

The histopathological changes occurred in the larval midgut of *S. littoralis* treated with *SPB1* biosurfactant were formation of vesicle in the apical region, cellular vacuolization, necrosis and damage of epithelial cells.

6.12.3 The Bacterial Strain Bacillus Subtilis V26:

Ben et al. (2020) elucidated antifungal and insecticidal activities, they provide protection against gray mold caused by *Botrytis cinerea*, in grapes and the tomato leaf miner *Tuta absoluta. In vitro* antifungal assay showed a minimal inhibitory concentration of about 2 mg mL$^{1.}$ The biosurfactant V26 also exhibited remarkable insecticidal activity against *Tuta absoluta* larvae (LC_{50} = 278.78 ng cm^2).

6.12.4 Mode of Action:

Act by binding to the receptors located in the brush boarder membrane vesicles of the larvae.

6.12.5 The Lipopeptide Produced by Bacillus Amyloliquefaciens Q-426:

Biocontrol activity against *Curvularia lunata Boed,* exhibits significant inhibitory activity even at extreme temperature, pH and salinity condition and also could grow well in the presence of Fe^{2+} ions below 0.8 ML^{-1} *(Zhao et al. 2013).*

6.12.6 The Bacillus Subtilis Strain NCD-2:

This strain is strongly antagonistic toward phytopathogenic fungi, and functions as an excellent biocontrol agent for cotton soil-borne diseases (Guo *et al.*, 2014).

Strongly inhibited the growth of *Rhizoctonia solani* in vitro. The lipopeptides of strain NCD-2 were separated by Fast Protein Liquid Chromatography (FPLC). The antifungal compound was identified as a cluster of fengycin homologs analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. A fengycin-deficient mutant was obtained by in-frame deletion of the fengycin synthetase gene in *B. subtilis* NCD-2. Compared with the wild-type strain, this mutant showed decreased abilities to inhibit the growth of *R. solani* in vitro and to suppress cotton damping-off disease *in vivo*. *R. solani* in the cotton rhizosphere colonized by the fengycin-deficient mutant was twice that in the cotton rhizosphere colonized by the NCD-2 wild-type strain. This study demonstrated that fengycin-type lipopeptides are the main antifungal active compounds produced by *B. subtilis* NCD-2.

6.12.7 Bacillus Sp. CS30 from the Cold Seep in Deep Sea:

Wu et al. (2019) isolated *Bacillus* sp. CS30 from the cold seep in deep sea, exhibited strong growth inhibition against *Magnaporthe grisea*. From mass spectrometry (MS) and tandem MS analysis, two purified antifungal agents were determined to be the member of surfactin family, they are Surfactin CS30-1 and Surfactin CS30-2, The Surfactin CS30-1 shows the higher Antifungal activity. Surfactin CS30-2. Shows lower Antifungal activity.

6.12.8 Mode of Action:

Both of them induce the increased generation of reactive oxygen species (ROS) and caused serious damage to the cell wall and cytoplasm, thus leading to the cell death of *M. grisea*.

6.12.9 Bacillus Mojavensis A21:

Ayed et al. (2019) formulated an economic media for lipopeptides production by *Bacillus mojavensis A21* for application as for plant disease treatment. The inhibitory activity of *A21* lipopeptides against the phytopathogenic *Fusarium sp* was investigated. The *in vitro* test showed a minimal inhibitory concentration of about 0.3 mg/ml.

6.12.10 Mode of Action:

The microscopic examination, of the treated *Fusarium* revealed a natural fungicides excessive lysis of the mycelia ultrastructure with destructed spores. *A21* lipopeptides are effective in decreasing by about 78.26% and 60.68%

6.12.11 Bacillus Cereus BS14:

Bacillus cereus BS14 on fungal growth under in vitro experiments and showed in vivo reduction of disease severity in pulse crop *Vigna mungo* L. They are Plant Growth-Promoting Rhizobacterium (PGPR) based on abilities of production of phytohormone and HCN, phosphate solubilization and biocontrol of *Macrophomina phaseolina*. *B. cereus BS14* proved its efficiency for the growth promotion of *Vigna mungo L* and significantly reduced disease severity index. The purified biosurfactant from *BS14* inhibited the fungal growth by arresting radially growing mycelia.

6.12.12 Serratia Rubidaea SNAU02, A Ramnolipid Producer:

Nalini and Parthasarathi *(2014)* reported *Serratia rubidaea* SNAU02, a rhamnolipid producer, as a biocontrol agent against plant phytopathogens. *Phytophthora sojae* is the main damaging oomycete pathogen of soybean. In pot culture study, application of rhamnolipid biosurfactant from *strain SNAU02* at the concentration of 250 µg/ml was effective against Fusarium wilt of eggplant, which minimize its yield loss and completely inhibit its disease severity. This investigation revealed that application of rhamnolipid biosurfactant from *SNAU02* could be a promising biocontrol agent (Nalini and Parthasarathi 2018).

6.13 Conclusion:

Surfactants have several applications in agriculture and agrochemical industries. However, there is rare use of biosurfactants which are more environmentally friendly. *Pseudomonas* and *Bacillus* as producers of biosurfactants indicating that only limited genera have been studied till date. Most of the disinfectant currently used is synthetic chemicals, which are replaced by biosurfactant in disinfectant formulation for improved cleaning and disinfection of food processing environment. Hence, it can be concluded that a cumulative input by researchers from various fields such as molecular biology, biochemistry, microbiology, computational biology, environmental science is indispensable.

6.14 Important Reference:

1. M. Heyd & A. Kohnert & T.-H. Tan & M. Nusser & F. Kirschhöfer & G. Brenner-Weiss & M. Franzreb & S. Berensmeier. 2008. Development and trends of biosurfactant analysis and purification using rhamnolipids as an example. Springer-Verlag.
2. Srinivasan Nalini, Rengasamy Parthasarathi, and Dhinakarasamy Inbakanadan.2020. Environmental Biotechnology Vol. 2. Chapter 4: Biosurfactant in Food and Agricultural Application.Eric Lichtfouse.,80-89.
3. P. Saminathan & P. Rajendran. 2016. Molecular Identification and Characterization of the Biosurfactant Produced by *Pseudomonas aeruginosa*-PSPA15 from the Oil Contaminated Soil. *International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706* Volume 5 Number 8 (2016) pp. 708-720.
4. R.Parthasarathi and S.Nalini 2021. Antifungal activity of biosurfactants from *Pseudomonas* sp. and *Bacillus* sp. With a potential to control phyto pathogens.
5. Lan Hee Kim., Yongmoon Jung., Sung-Jo Kim., Chang-Min Kim., Hye-Weon Yu., Hee-Deung Park & In S. Kim. 2016. Use of rhamnolipid biosurfactant for membrane biofouling prevention and cleaning. Biofouling; *The Journal of Bio adhesion and Biofilm Research*. Vol. 31, no. 2, 211–220
6. Sonica Tomar., B.P. Singh., Mehi Lal., M.A. Khan., Touseef Hussain., Sanjeev Sharma., S.K. Kaushik and Sathish Kumar. 2013. Screening of Noval microorganisms for biosurfactant and biocontrol activity against *phytophthora infestans. Journal of Environmental biology,* Vol. 35, 893-899.
7. Juan C. Mata-Sandovala., Jeffrey Karnsb., Alba Torrents. 1999. High-performance liquid chromatography method for the characterization of rhamnolipid mixtures

produced by *Pseudomonas aeruginosa* UG2 on corn oil. *Journal of Chromatography A, 864* (1999) 211–220.

8. Jolien D'aes., Katrien De Maeyer., Ellen Pauwelyn and Monica Höfte. 2009. Biosurfactants in plant–*Pseudomonas* Interactions and their importance to biocontrol. Environmental Microbiology Reports (2010) **2(**3), 359–372.
9. Jérôme Crouzet, Anthony Arguelles-Arias, Sandrine Dhondt-Cordelier, Sylvain Cordelier, Jelena Pršić, Gregory Hoff, Florence Mazeyrat-Gourbeyre, Fabienne Baillieul1, Christophe Clément, Marc Ongena and Stéphan Dorey .2020. Biosurfactants in Plant Protection Against Diseases: Rhamnolipids and Lipopeptides Case Study.