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BIOSURFACTANTS FROM BACTERIA ISOLATED FROM COLD CLIMATIC REGIONS AND ITS EVAULATION FOR BIOMEDICAL AND NON MEDICAL APPLICATION

Submitted in partial fulfillment of the requirements for the award of Master

of Technology degree in Biotechnology

by

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INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade “A” by NAAC

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This is to certify that this Project Report is the bonafide work of **ESHWARNATH .V S** (41810004) who carried out the project entitled **"BIOSURFACTANTS FROM BACTERIA ISOLATED FROM COLD CLIMATIC REGIONS AND ITS EVAULATION FOR BIOMEDICAL AND NON MEDICAL APPLICATION"** under our supervision from January 2023 to May 2023.

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I ESHWARNATH .V S (41810004) hereby declare that the Project Report entitled **"BIOSURFACTANTS FROM BACTERIA ISOLATED FROM COLD CLIMATIC REGIONS AND ITS EVAULATION FOR BIOMEDICAL AND NON MEDICAL APPLICATION"** done by me under the guidance of **Dr. R. Thyagarajan (Internal)** and **Dr. M. Radhakrishnan (External)** in Centre for Drug Discovery and Development at Sathyabama Institute of Science and Technology is submitted in partial fulfillment of the requirements for the award of Master of Technology degree in Biotechnology.

DATE: 5/5/2023

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ABSTRACT

Due to industrialization and urbanization, there is a growing global demand for oil. Oil is a crucial source of energy for humanity, but it also has a significant negative impact on the environment. Biosurfactants are widely employed in the agriculture, food, cosmetics, and pharmaceutical industries as well as in microbial-enhanced oil recovery (MEOR). In the present study, soil samples were collected from Kashmir, India. 27 morphologically different bacterial colonies were obtained from the soil sample using Starch casein agar, ISP2, NA, AIA medium at 4^{o}C. Bacterial cultures were screened for biosurfactant production by different assays such as haemolytic activity, oil displacement method, emulsification index and phenol sulphuric acid method. The strain KM14, which showed positive results in biosurfactant production in all the screening methods. Effect of medium components on biosurfactant production was done using different carbon source, nitrogen source. Among the carbon and nitrogen sources tested starch and Glucose showed higher result. The results of phenotypic and 16s rRNA sequence analysis showed that the strain KM14 showed 98% close similarity with *Stenotrphomonas rhizophila.* Further purification and characterization of biosurfactant from the potential strains will pave the way for its industrial and biomedical applications.

KEY WORDS: Bacteria, crude oil, degradation, Biosurfactant

Chapter 1
INTRODUCTION

The challenges of food security and environmental management are made more difficult by the world's fast industrialization and growing population. A greater population from the developed countries depend upon the chemical pesticides, in the soaps and detergent industries as emulsifiers, detergents, dispersants (Kumar et al.,2021, Marchut-Mikołajczyk *et al.,* 2021). The chemical surfactants have various and important roles in a diverse segments like petroleum industry, environmental pollution abatement, soaps and detergent industry, and even in the food and beverage industry (Singh *et al.,* 2019). Oil spills at sea are frequently treated using chemical dispersants as a first-step response method. Organo-chemical synthesis is used to produce the dispersants that have been licensed and are kept in stockpiles across the world in case of an oil spill (Nikolova *et al.,*2021). The synthetic surfactants (organo-chemical) that have been utilized in the present are of high risk to the environment as they are derived from the petroleum sources which are non-renewable and a finite source (de Oliveira Schmidt *et al.,*2021).

Another one such consideration is where the scientists are extremely concerned about the growing threat that toxic heavy metal pollution of soil poses to the ecosystem due to its toxicological manifestations and adverse impacts across the world (Mishra *et al.,*2021). Using materials to the fullest extent possible, such as oil hydrocarbons (such as saturated, unsaturated, polyaromatic, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCBs), and heavy metals such as cadmium, iron, titanium, thallium, copper, zinc, mercury, and arsenic. insecticides, herbicides, air pollutants (carbon monoxide, nickel), particulate pollution, ozone, acid rain, and volatile organic compounds), bisphenol, sulphonamides, nitroaromatic chemicals, organophosphorus compounds, trichloroethylene, perchloroethylene, solvents, and chlorinated. Hydrocarbons have the potential to do great harm by being extremely toxic that have direct effects to humans (Jimoh *et al.,*2019). However, it is imperative to implement remediation solutions for such dangerous contaminants. The use of remedial techniques such soil washing, pumping, aeration, oxidation, and incineration is rather rare. The production of

additional secondary pollutants, which is economically unfeasible, is one of the many downsides of these remediation techniques. In order to address these issues, microbial bioremediation is regarded as one of the sustainable and cost-effective methods for the removal of environmental toxins (Sharma *et al.,*2021).

Chemical cleanup methods employing chelating agents and other chemical surfactants, such as sodium lauryl sulphate or acetyl trimethyl ammonium bromide, as complexing agents are one approach. As a result, finding natural alternatives to artificial surfactants and using them responsibly are essential (Eras-Muñoz *et al.,*2022). Numerous microorganisms are known to produce a range of surface-active substances for a variety of natural purposes, including the ability to adapt and flourish on different substrates (Singh *et al.,*2021).One of the most recently discovered microbially produced/synthesized bimolecular molecules that are termed as biosurfactants is becoming increasingly popular due to their exceptional benefits over synthetic ones. small toxicity (Mohanty et al.,2021, Yun *et al.,*2021).

The word "biosurfactant" refers to surface-acting substances that can enhance surface-surface interactions by generating micelles that are derived from natural sources including plants, microorganisms, and animals (Yun *et al.,*2021). Highly recommended for the treatment of environmental toxins without the creation of any secondary pollutants or harmful consequences, bioremediation is a natural, sustainable, and ecologically beneficial method. Outstanding microbial products known as biosurfactants, also known as biologically active chemicals, have been effectively used in the detoxification and/or removal of harmful heavy metals (Mishra *et al.,*2021).The benefits of green surfactants (biosurfactants made from microorganisms) over synthetic surfactants have been emphasized in a number of papers. In comparison to synthetic surfactants, biosurfactants are low or nontoxic, biodegradable, exhibit outstanding surface activity, have high specification, are effective in harsh environments, and may also be recycled through regeneration (Sachdev *et al.,*2013). Biosurfactants are generated by bacteria, yeasts, and filamentous fungus. They are classified as glycolipids, phospholipids and fatty acids, lipopeptides and lipoproteins, polymeric surfactants, and particulate surfactants (Domínguez *et al.,*2019). Biosurfactants are a class of secondary metabolites which are secreted either extracellularly into the culture broth or affixed to the cell surface

before being released (Ravinder *et al.,*2022). Microbial surfactants are a common occurrence in human lifestyles nowadays and are a major component of their everyday items like detergents, food additives, and cosmetics. Additionally, they are extensively utilized in the petroleum, healthcare, pharmaceutical, agricultural, and environmental fields (Pardhi *et al.,*2022). They are used commercially as cheaper manufacturing alternatives to chemical surfactants, particularly in the pharmaceutical industry (Sajid *et al.,*2020). A biosurfactant is a substance that has tensioactive properties because of the hydrophilic and hydrophobic components of its structure, which include long-chain fatty acids, hydroxy fatty acids, and -alkyl-hydroxy fatty acids (carbohydrates, amino acids, cyclic peptides, phosphate groups, and alcohol, among other things) (ibeiro *et al.,*2020). There are already more than 2000 different biosurfactant structures that have been identified, including chemically separate families of compounds as well as groups of congeners, or structurally similar compounds with slight structural changes (Kubicki *et al.,*2019). Biosurfactants may dissolve in both polar and non-polar solvents due to their amphiphilic nature. The capacity of a surfactant to reduce the surface tension (ST) and interfacial tension (IFT) between two immiscible phases is what determines the surfactant's efficacy.(Nikolova *et al.,*2021)

Since these natural surfactants are found to be utilized as carbon sources by soil-dwelling bacteria, they can replace the harsh surfactants now employed in the pesticide industry. This accounts for the biological elimination of biosurfactants from agricultural soil (Sachdev *et al.,*2013). Due to their accessibility and affordability, agricultural waste and food processing byproducts can be used as a carbon source for the production of microbial biosurfactants (Marchut-Mikołajczyk *et al.,*2021).

1.1 Impact of sustainable agriculture

There is a need to avoid the degradation of land as it is high time for us to preserve the land due to the increase in human population and subsequent increase in the food consumption. To provide food security for a growing population need, this entail the implementation of sustainable land use techniques and protection of any degraded or margined soil (Ahmad *et al.,*2018). The micronutrient deficiencies in soil must also be addressed in order to fulfil the crops increasing demands.

Increasing the use nutrients availability by a biosurfactant, which is a multifunctional microbial metabolite may be a suitable strategy to increase the agricultural output (Singh *et al.,*2018). Numerous potential for the sustainable agriculture have been provided by bio control techniques involving the use of biomaterials and biomolecules. Due to their biocompatabilty, the green approach makes use of multifunctional biomolecules such as biosurfactants, chitosan, and chitosan derived nanoparticles (Karamchandani *et al.,*2022). Since surfactants of chemical origin have number of adverse effects on the environment, such as toxicity towards lesser forms of life, soil contamination, etc. Biocontrol strategies have been adopted (Sangwan *et al.,*2022). Most of the biosurfactants are obtained using pricey culture media and purification procedures, which restrict their usage in the industry. Due to this their manufacturing process is been carried out by inexpensive substrates that can be discovered among the agricultural and food wastes and by products to contribute for the sustainability (Janek *et al.,*2021). Affordable feedstocks are used in the bioeconomy and circular economy of the biosurfactants, which supports the utilization of waste converted into useful products. New affordable, renewable health-grade biosurfactants are sustained by waste reduction, reuse, and recycling in an integrated green economy bioprocess (Mgbechidinma *et al.,*2022).

Chapter 2
REVIEW OF LITERATURE

2.1 MICROBIAL BIOSURFACTANT CLASSIFICATIONS

Microbial Biosurfactnts are classified into various types that is depending on its diverse properties. But are mainly classified based on it Molecular weight. Low-molecular-weight surface-active molecules of considerable industrial relevance include microbial biosurfactants due to their chemical characteristics and environmental resilience. The low and high molecular weight molecules are the two main groups into which biosurfactants fall. Generally, high molecular weight biosurfactants, often referred to as bioemulsifiers or bioemulsans, may stabilize emulsions and powerfully attach to surfaces, whereas low molecular weight surfactants effectively lower surface and interface tension.The first class consists of lipopeptides and glycolipids, whereas the second class is made up of proteins, polysaccharides, and lipoproteins. Efficiency is assessed using the critical micelle concentration (CMC), which can range from 1 to 2,000 mg/L. The CMC is the lowest concentration needed to achieve the lowest surface or interface tension or the solubility of a surfactant in an aqueous solution (Ravinder *et al.,*2022). Surface and interface tension, which may reach levels below 30, is connected to effectiveness (Moutinho *et al.,*2021).

2.1.1 Glyclolipds

For use in biotechnology, glycolipid biosurfactants have gained a lot of interest. Typically they were composed of a fatty acid attached to a carbohydrate moiety ny a glycosidic connection (Mnif *et al.,*2018).Glycolipids A carbohydrate moiety is joined to a fatty acid chain to form glycolipid molecules. The Rhamnolipids, trehalolipids, mannosylerythritol lipids (MELs), and cellobiose lipids are examples of the class of biosurfactants. It has been demonstrated that several glycolipids have the capacity to create holes and weaken cellular membranes. It has been examined the antibacterial, antifungal, anticancer, and anti-biofilm activities of glycolipid biosurfactants (Paraszkiewicz *et al.,*2021). Because they contain both hydropjilic

glycosyl and the lipophilic lipid residues, simple gylcolipids are amphiphilic molecules (Abdel-Mawgoud and Stephanopoulos 2018). Certain antiviral, antibacterial and antifungal properties are exhibited by gycolipid surfactants (Jezierska *et al.,*2018).The fermentation conditions, strain selection, culture media and growth conditions are what led to the various structures of glycolipid biosurfactants (Dardouri *et al.,*2021, Sekhar *et al.,*2018). Studies have been conducted where the glycolipid can be used as a feasible bioplastics (Fukuoka *et al.,*2018).

2.1.2 Rhamnolipids

Rhamnolipids are equally stable and effective in emulsifying as the widely used anionic surfactant sodium dodecyl sulphate (Salek *et al.,*2022).Rhamnolipids are extracellular secondary metabolites that are released by different Pseudomonas strains, primarily by the opportunistic pathogen *Pseudomonas aeruginosa*. These bacteria use them at different phases of the biofilm building process. Due to their capacity to expedite the removal of various organic and inorganic contaminants, rhamnolipids are effectively used in environmental technologies, particularly in water and soil remediation processes Rhamnolipids, on the other hand, have anti-adhesive and disruptive properties when it comes to biofilms produced by certain pathogenic microbes (Paraszkiewicz *et al.,*2021). One of the glycolipid-type biosurfactants, which are produced mainly by *Pseudomonas aeruginosa* and are the most frequently studied because of their effective surface activity and high yields of production (Shu *et al.,*2021). Biosurfactant produced by *Klebsiella* species are identified to be monorhamnolipid (Ahmad 2021).By utilizing a variety of carbon sources and different organism can affect the supply of basic precursors for the biosynthesis of rhamnolipids , due to which different *Pseudomonas aeruginosa* strains produce variants of rhamnolipid (Thakur *et al.,* 2021). Due to its adverse effects severe foaming is not experienced while rhamnolipid undergo fermentation (Gong *et al.,* 2021). Rhamnolipid biocomplex are biosynthesized by *Pseudomonas* species which is cheaper and environmental friendly, which act as an alternative for purified rhamnolipids (Kłosowska-Chomiczewska *et al.,* 2021).

2.1.3 Sophorolipds

Two glucose rings connected by a 1-2 glycosidic bond make form the sophorose polar group of the glycolipid known as sophorolipds, which also has a hydroxylated fatty acid lipid tail (Salek *et al.,* 2022). Sophorolipids are intriguing substitutes for surfactants made of petrochemicals due to all these characteristics.They have little foaming, quick wetting,and low toxicity, as well as strong surface activity (Liwarska-Bizukojc *et al.,*2018).Candida bombicola and C. apicola are two examples of non-pathogenic yeast that produce sophorolipids, which are biosurfactants that include the fatty acid and the sugar sophorose bound together.Examined the antimicrobial qualities and biofilm disruption activity of sophorolipid biosurfactants against both Gram-negative and Gram-positive microorganisms.The data obtained showed that sophorolipids at a concentration of 5% v/v inhibited the growth of Cupriavidus necator ATCC 17699 and B. subtilis BBK006 bacteria and also disrupted biofilms of single B. subtilis BBK006 and S. aureus ATCC 9144 cultures as well as of mixed B. subtilis BBK006 and B. subtilis BBK006 cultures (Paraszkiewicz *et al.,*2021).

2.1.4 Lipopeptides

A physically varied family of extracellular compounds produced by bacteria and fungi is called lipopeptides.The most widely used lipopeptide biosurfactants include substances from the surfactin, iturin, and fengycin families generated by various Bacillus strains and Pseudomonas lipopeptides (divided into four primary groups: viscosin, amphisin, tolaasin, and syringomycin). Recent research identified the marine bacterial strain Pontibacter korlensis SBK47's production of pontifactin, a new biosurfactant, as a lipopeptide comprising palmitic acid and a brief heptapeptide with a Ser-Asp-Val-Ser-Ser sequence (Paraszkiewicz *et al.,*2021). Microorganisms that usually undergo environmental stress produce Antibiotic lipopeptides (Vazquez *et al.,*2018).One of the most well-known lipopeptides is fengycin from *Bacillus subtilis*.*Bacillus*-related lipopeptides, *Pseudomonas*-related lipopeptides, other bacterial-related lipopeptides, *actinomycete*-related lipopeptides, and fungal-

related lipopeptides are the different categories of lipopeptides (Mnif and Ghribi 2015).

2.2 Properties of biosurfactants

Many prokaryotic and eukaryotic microorganisms create biosurfactants, which are either secreted extracellularly or bonded to cell-bound chemicals that reduce the surface and interfacial tension. It is not only possible for biosurfactant to form water-in-oil and oil-in-water emulsions, but also dehydrate emulsions, which is a promising technology in businesses that depend on petroleum (Najmi *et al.,*2018). The asymmetric structure of surfactant molecules causes them to adsorb and micellize. As a result, the surfactant molecule can be separated into hydrophilic (head) and hydrophobic (tail) (Zdziennicka *et al.,*2018). The chemical makeup and characteristics of biosurfactants makes it easier to disperse emulsions in liquids like water (which is an important property in cases of oil spill in natural environments). When we modify their metabolic pathway through rational design, their structures and characteristics may also be changed. Thus, new products with a certain profile can be created (Vazquez *et al.,*2018).

2.2.1 Physicochemical Properties of Biosurfactants

For biosurfactants to be successfully used in practice, one must be aware of their physicochemical characteristics. It is important to gain understanding about how these components interact since the origin and production/purification procedures of biosurfactants have a significant impact on their molecular properties and subsequent interfacial behavior (Jahan *et al.,*2020). The Fatty Acid chain length and isomerism are known to affect the physicochemical property of biosurfactants (Hu *et al.,*2019). The effective use of biosurfactants in various sectors of industries depend upon the understanding their physicochemical characteristics, such as surface tension reduction, dispersion, emulsifying, foaming or, micelle formation (Salek *et al.,*2022). The values of the tail and head's surface tension (a macroscopic characteristic) and the size of specific

molecular components (a microscopic property) determine the surfactants propensity to form micelles at a critical concentration (CMC) and adsorb at the water-air and soil-water interfaces (Zdziennicka *et al.,*2018). They are typically divided into low (such as glycolipids and lipolipides) and high atomic weight (such as polysaccharides, protein, and lipoproteins) surfactant based on their synthetic approach and sub-atomic weight (Abbot *et al.,*2022). In addition, the physicochemical properties of the support that must be chosen in accordance with the desired reaction because they can affect the efficiency of the enzyme. It is hypothesized that an increase amount in the rate of reaction conversions was caused by a decrease in the hydrophilicity of the support (Zago *et al.,*2021). The physicochemical characteristics derivatives are analyzed using in-silico method (Hassan *et al.,*2017).

2.2.2 Surface and Interfacial Tension

One of the most important properties of amphiphilic compounds is their capacity to lower surface and interfacial tension. For example, this is necessary for the development of kinetically stabilized emulsions. Due to their dual hydrophobic-hydrophilic nature, amphiphilic compounds, such as biosurfactants, adsorb at interfaces (air/liquid, liquid/liquid, and solid/liquid). Surfactant molecules efficiently lower intermolecular interactions between solvent molecules when they replace water or oil molecules at the contact, hence reducing surface or interfacial tension (Jahan *et al.,*2020). Surfactants are added to solution to lower the surface tension of the (air/water) and (oil/water) interfacial tension, which promotes the deposition of solid phase (adsorption state) oil contaminants and enhances their ability to migrate into the aqueous phase or their contact efficiency with aqueous phase remediation agents of microorganisms (Liu *et al.,*2021). Surface tension and critical micelle concentration places an important role in the interface (da Rocha Junior *et al.,*2019). The biosurfactant demonstrate emulsification and surface tension stability over a wide range of pH (4-10) and temperature range (up to 100 °C) (Giri *et al.,*2019). The dynamic surface tension data are used to shed light on the mechanisms that regulates surfactin at air-water interface (Onaizi 2018).

Indicating a strong potential for biotechnological applications, the *Serratia* genus which demonstrated greater emulsification capacity and created a more significant surface tension drop (Oliveira *et al.,*2021). In fact, even at a low concentration surfactin was able to reduce the water surface tension by about 2.5 times (Iglesias-Fernández, *et al.,*2015).

2.2.3 Air-water interface

Air water surface tension is how well the surfactants are packed at the air-water interface, with the hydrocarbon chain facing air and phydrophilic groups facing bulk water. The air-water interface configuration causes a low degree of surfactin immersion in the aqueous phase, making it more hydrophopbic nanoparticle and enabling to create a very compact surface layer that is denser than that of ordinary amphiles (Otzen 2017). Hydrophobic chains group together to avoid water molecules, whereas surfactin molecule tend to assemble near the interface with polar residues facing the water phase. The peptidic residues of surfactin is typically found parallel to the water/air boundary, and the flexibility end of the fatty acyl chains (Iglesias-Fernández, *et al.,*2015). β-aescin has a great propensity to enrich at the air-water interface due to its amphiphlic nature (Geisler *et al.,*2019).

At the air-water interface, the surface adsorption characteristics of the saponin, escin (two common nonionic surfactants) are been studied using neutron reflectivity and surface tension (Tucker et,al 2020). Escin adsorption layers at the air-water interface display very high surface elastic moduli (>1100mN/m) and surface viscosities (about 130 N/m) (Tsibranska *et al.,*2017). The molecular orientation of adsorbed monorhamnolipids at the air-water interface is investigated using polarization modulation-infrared reflection absorption spectroscopy (PMIRRAS) (Wang *et al.,*2013).There are several isotherms that have been used to characterise biosurfactant adsorption at the air/water interface, including Frumkin, Gibbs, Gibbs-Szyszkowski, and Gibbs for ionic molecules (Paraszkiewicz *et al.,*2021).

2.2.4 Antibioflm property

The way that bacteria express themselves can change in response to their environment. The ability of bacteria to carry out quorum sensing activity, this capability are related to the formation of biofilms (Abbot *et al.,*2022).Biofifilm production has been seen to be inhibited by biosurfactants. They are able to change the surface's physico-chemical characteristics to lessen adhesion (Janek et al. 2012). *Pseudomonas aeruginosa* and *Staphylococcus aureus* are two of the dangerous biofilm forming bacteria that are frequently found in industrial and hospital waste Biosurfactant coated with nanoparticles could be effective for preventing harmful biofilms. Gram-negative bacteria (*Salmonella enteritids*), Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus pumilus* and *Listeria monocyotgens*) and fungal strains (*Yarrowia lipolytica*) possess antimicrobial and antibiofilm properties (Khalid *et al.,*2019). Due to its biodegradable nature, low cytotoxicity, anti-microbial and antibiotic properties and their ability to disperse microbial biofilms, biosurfatants have attracted increasing interest in the clinical and sanitary fields (Cheffi *et al.,*2021).

Additionally, several biosurfactants suppress the expression of bacterial genes essential for the production of biofilms. Noted that the presence of biosurfactants purified from Lactobacillus reuteri suppressed the production of glucosyltransferases (gtfs) and fructosyltransferase (ftf) genes, which are essential for Streptococcus mutans' initial adherence to the tooth surface (Paraszkiewicz *et al.,*2021). Because of their inherent surface activity and the possibility that they could be used to prevent the formation of biofilms, biosurfactants have antibacterial, antibiofilm, and antiadhesive properties (Abdollahi *et al.,*2020). Microbial surfactant have received more attention recently (Giri *et al.,*2020)

2.3. APPLICATIONS

Biosurfactants have multiple uses in a variety of industries, including agriculture, biomedicine, construction, the pulp and paper sector, metal, textile, pharmaceutical, and cosmetics (Moutinho *et al.,*2021).(Figure 1)

2.3.1 Cosmetic industry

Because of their high added value products, high specificity, and skin compatibility, biosurfactants are crucial to the beauty industry. Given their significant qualities, including detergency, foaming, wetting, emulsifying, solubilizing, and dispersing, biosurfactants are widely wanted for usage in cosmetics. The glycolipids group, which includes the rhamnolipids, sophorolipids, and mannosylerythritol lipids, is the largest and most diversified biosurfactant group utilised in the cosmetic and personal care sector because to its physicochemical qualities, biological activity, and biocompatibility (Moutinho *et al.,*2021). The choice of a biosurfactant for a definite cosmetic product is a delicate task that depends of several factors based on the requirement of the product (Bezerra *et al.,*2018).

2.3.2 Petroleum industry

Biosurfactants are essential in the petroleum industry as well. For instance, the Microbial Enhanced Oil Recovery (MEOR) technology employs the addition of microorganisms to the reservoir rock that are then stimulated to generate polymers and surfactants to lower the surface tension of the oil rock and enable the transportation of the oil through the rock's pores (Moutinho *et al.,*2021) Additionally, biosurfactants lessen the viscosity of crude oil residues that have been left behind and deposited at the bottom of oil storage reservoirs (Moutinho *et al.,*2021).

2.3.3 Medical industry

In the past 10 years, there has been a surge in the possible uses of biosurfactants in the medical industry. Several biosurfactants were shown to have biological properties, including antibacterial, antifungal, antiviral, anticancer activity,

suppression of clot formation and hemolysis, anti-adhesiveness, and creation of ion channels in membranes, which encouraged their usage in the biomedical industry (Jahan *et al.,*2020).

2.3.4 Other Applications

The marine and shipping industries suffer severe issues and financial losses as a result of biofouling. Marine bacteria with the potential to produce biosurfactants can be a great choice when looking for new antifouling agents because they have the amphipathic surface-active property that confers antibacterial and antibiofilm actions (Alemán-Vega *et al.,*2020). In food industries these factors play an advantageous role where the biosurfactants possess specific esistance to variations in temperature, acidity and salinity,and allows biomolecules to maintain their original characteristics, which can positively influence the quality of the final product (Ribeiro *et al.,*2020).

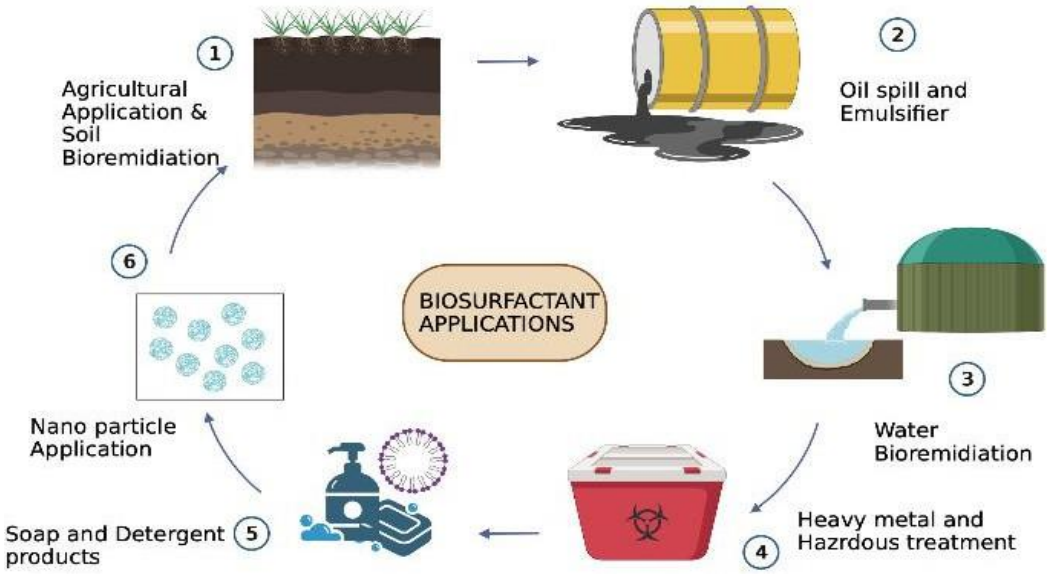


Figure 2.1*: *The various industrial application in which biosurfactants are being applied in the recent decades (Mohanty et. al 2021, Mishra et al.,2021, Domínguez et al.,2019).

2.4. SOURCES OF BIOSURFACTANT

The Microbial biosurfactants are been derived from various species, though there are n number of variants these are few of the species that are been listed (Table1) (Moutinho *et al.,*2021). *Planococcus* is a gram positive bacteria is a pioneer marine resource for the biosurfactant production and even other secondary metabolites *(*Waghmode *et al.,*2020). Several other phyla like Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Euryarchaeota, Ascomycota, and Basidiomycota have been utilized for the production of diverse biosurfactants (Alemán-Vega c2020). Pony Lake from Ross Island, Antarctica is a source for the *Psychrobacter arcticus* Strain that are used to isolate biosurfactants and The Cotton Glacier stream in Victoria Land rich source of *Janthinobacterium svalbardens* which come under the classes of sophorolipids and di-rhamnolipids (Trudgeon *et al.,*2020).

Table 2.1: The table lists out some of the strain from which the specific biosurfactants are been derived (Moutinho et,al 2021, Malviya *et al.,*2020).

S.No	Source Organism	Lipopeptide Class
1	*Actinoplanes friuliensis*	Friulimicin
2	*Arthrobacter* spp. MIS38	Arthrofactin
3	*Bacillus subtilis*	Iturin A, Bacillomycin
4	*B. subtilis* HC8	Surfactin, Fengycin A
5	*B. subtilis* K1	Fengycin A and B, Fengycin A2 and B2
6	*B. subtilis* GA1	Iturins, Fengycins, Surfactins
7	*B. subtilis* and *B. amyloliquefaciens*	Surfactins, Bacillomycin
8	*Pseudomonas chlororaphis,Pseudomonas putida and Burkholderia thailandensis*1	Glycolipid

Researchers have discovered and isolated biosurfactant-producing bacteria from various marine environments of the Canadian Arctic; they noted that the most common species were of the genus *Rhodococcus*, followed by *Bacillus* and antibiofilm activities and biosurfactants at 4 °C by the genera *Pseudomonas*, *Pseudoalteromonas* and *Rhodococcus*, were collected from Antarctic and Arctic polar environments (Schultz and Rosado 2020).Studies have concluded the antimicrobial activity of biosurfactants extract that were obtained from the enduring stream of the corn-milling industry (Rodríguez-López *et al.,*2020). The lactic acid bacteria such as *Lactobacillus agilis*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, and *Lactobacillus pentosus* are the most researched glycolipopeptides and glycopeptide microbial biosurfactants (Moldes *et al.,*2020). *Serratia* species have been shown to create two biosurfactants: lipopeptides and glycolipids. These species include *Serratia marcescens, Serratia rubidaea, and Serratia surfactantfaciens* (Clements *et al.,*2019).

2.5. AGRICULTURAL IMPACT OF BIOSURFACTANTS

2.5.1 Anti-phytopathogenic activity

Through the prevention of the growth of phytopathogenic fungi, sophorolipids, cellobiose lipids, and mannosyl-erythriol-lipids are implicated in plant protection. Sclerotinia sclerotiorum and Phomopsis helianthi are phytopathogenic fungi that are inhibited from growing by the cellobiose lipid of Pseudozyma fusiformata and Cryptococcus humicola. The phytopathogenic fungus Sphaerotheca fuliginea was resistant to the antifungal action of cellobiose lipids produced from Pseudozyma flocculosa. Recent studies on the effects of sophorolipids and their derivatives on plant diseases have provided more support for the microbial biosurfactants' capacity to control plant diseases. Researchers found that mixtures of sophorolipid derivatives and individual sophorolipid derivatives significantly inhibited the growth of 18 plant fungal diseases (Mnif and Ghribi 2016). Because they can be produced at scale for commercial purposes, have low toxicity, and have a high biodegradability, biosurfactants produced by bacteria, yeast, and fungi are promising molecules for a wide range of applications (Crouzet *et al.,*2020).

2.5.2 Plant Defence

Huge economic losses come from plants infections that causes a significant agricultural damage, that range from 10-40% depending on the crops before and after harvest (Savary *et al.,*2019). However, to overcome this scenario chemical pesticides were in implementation. But they can be destructive to human and environmental health that led to the development and optimization of alternative strategies to reduce the chemical pesticide utilization for crop protection is becoming a necessity (Berg *et al.,*2017).A immune response is been initiated by rhamnolipids that also aids in local resistance against *B. cinerea* and the hemibiotrophic fungus *Leptosphaeria maculans* in *B. napus* (Crouzet *et al.,*2020).

Recent research has also demonstrated that some rhamnolipids can protect plants from phtopathogenic fungus and bacteria through activation of the plant immune system. It has been shown that A P. aeruginosa rhamnolipid activates defense genes in Arabidopsis thaliana, wheat, and tobacco (Mnif and Ghribi 2016). One such biosurfactant is Rhamnolipids which due to its innovative structures, adaptable biological roles, lesser toxicity, increased biodegradability, and manufacturing from renewable resources, rhamnolipids have lately come to be recognized as potential bioactive compounds. Rhamnolipids are desirable research targets for a wide range of applications due to their benefits. The area of biomedicine and agriculture may be able to use rhamnolipids as possible antimicrobials, immunological modulators, virulence factors, and anticancer agents to help fulfil the growing need for pharmacological therapy and food safety in the coming years (Chen *et al.,*2017). Because of their potential to generate pores in pathogens, siderophore action, biofilm inhibition, and dislodging activity, as well as their antiviral and other activities, lipopeptides have several applications in plant protection. Lipopeptide-containing microorganisms are effective bio control agents. Investigating these antimicrobial substances may open up new avenues for biological pest management of established and newly emergent plant diseases (Malviya et,al 2020). One of the safer green alternative for the chemical pesticide is mannosylerythritol lipids (Matosinhos *et al.,*2022).

Various pathogens infect *Capsicum* spp (Pepper) which is an important spice, this contribute to economic losses on a global range. Where *Cucumber mosaic virus* (CMV) is the most destructive pathogen (Jones 2016, Mandadi and Scholthof 2013).We face limitation in ehich the commercially available CMV plants that have been developed by breeding technologies and transgenic method face time-limited and environmental issues (Khalid *et al.,*2017). These strain (biocontrol), *Bacillus amyloliquefaciens* PPL exhibits various useful properties, such as antibacterial and antifungal activities against various plant pathogens, also including *Colletotrichum gloeosporioides, Phytophthora capsici, Rhizoctonia solani*, and *Fusarium oxysporum* that also fight against the CMV (Kang *et al.,*2019). *Bacillus* species that produce cyclic lipopeptides that has been reported to exhibit antiviral and antifungal activity in plants (Kang *et al.,*2021).Furthermore, *Brevibacilis brevis* lipopeptides have shown potent antibacterial and antifungal activities. The ability of rhamnolipid to boost plant immunity and decrease plant pathogen infection was demonstrated.*Tenebrio molitor's* immunological response to microbes could be triggered by biosurfactants (Edosa *et al.,*2020).

Sheath blight (ShB) of rice is a pathogenic disease, caused by Rhizoctonia solani, obligates for signifificant yield losses globally. Currently disease is controled through the use of chemical fungicides (Kumar *et al.,*2011). endophytic bacteria have been widely used to produce potent biocontrol agents as they elicit antagonism at the site of infection. Some of the endophytic bacteria strains are endophytic diazotrophic, *Bacillus* subtilis under gnotobiotic conditions can sures the ShB in rice (Shabanamol *et al.,*2017). Biosurfactants are frequently utilised as antagonistic molecules against pests, pathogens, or plant diseases. They have also been used to improve soil quality by breaking down toxic and dangerous contaminants or by making trace nutrients available in the soil for sustainable farming practices (Ali *et al.,*2022). They can penetrate and harm fungal cell membranes, which reduces the likelihood that they will develop resistance, compared to traditional antibacterial treatments or insecticides (Choub *et al.,*2021).

Banded leaf and sheath blight is a plant disease, caused by *Rhizoctonia solani*, where the infection restricts the crop output in climatic situations critically during the

monsoons in India (Singh *et al.,*2020). The effectiveness of a novel biosurfactant extract against *Aspergillus brasiliensis* and *Candida albicans* was tested in a residual stream from the corn-milling sector. It may be possible to use biosurfactants derived from corn steep water as preservatives and antibacterial agents against fungi on agricultural and food goods (López-Prieto *et al.,*2020). Pepper (*Capsicum annuum* L.) is an economically important crop is susceptible to various diseases. Pathogens of the genus *Colletotrichum* can cause severe yield loss (Park *et al.,*2022). Strains of *Bacillus subtilis* and *Bacillus amyloliquefaciens* have been reported to be very effective in several soil-borne plant diseases (Borriss etl.al 2011, Liu *et al.,*2019).

2.5.3 Plant Growth Promotion

Organic and inorganic contaminants that cause abiotic stress in crop plants have an impact on the productivity of agricultural land. Bioremediation is necessary to improve the condition of soil that has been polluted with hydrocarbons and heavy metals. Biosurfactants produced by microorganisms and/or biosurfactants can be utilised to remove heavy metals and hydrocarbons from a solution (Sachdev *et al.,*2013). The need for novel, environmentally friendly bactericides has increased as a result of rising agricultural output and stronger regulations regarding food security. The majority of the bactericides in use today are persistent organic compounds, which pose a threat to both human and environmental health. Lipopeptide biosurfactants made of C16 and C18 fatty acids have been identified as the biosurfactants extracted from CSW that can lower water's surface tension by up to 30 mN/m. Additionally, the extract contains phenolic components (López-Prieto *et al.,*2019). Alkyl polyglucosides (APG), which are generated from plants, have been demonstrated to be natural biosurfactants that are useful in bovine nutrition due to their favourable effects on physiological and production parameters in, for example, ruminants. Enhanced ruminal and intestinal organic matter digestibility and ruminal microbial protein synthesis lead to increased duodenal microbial nitrogen flux (Naughton *et al.,*2019). Many rhizosphere-dwelling microorganisms have the potential to promote plant growth; as a result, they are referred to as plant growth-promoting bacteria (PGPB) or plant growth-promoting rhizobacteria (Ahmad *et al.,*2018).

Rhodococcus erythropolis other the genera *Rahnella, Serratia and Proteus* where some of the these bacteria exhibit features of plant growth-promoting bacteria which increase the biomass of plants under several mechanisms the production of phytohormones, siderophores, 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD), nitrogen fifixation as well as the solubilisation of phosphorus (Pacwa-Płociniczak *et al.,*2016).

When the soil was directly treated with biosurfactant producing bacterium *Bacillus tequilensis* of mazie plants (Chaurasia *et al.,*2020). Lipopeptides from *Bacillus subtilis* under low cost production exhibit great stability range of pH (1–11), salinity (1–8%), temperature (20–121°C) even after autoclaving. Which act as a potent plant growth-promoting agent that signifcantly increases seed germination and plant growth promotion of chili pepper, lettuce, tomato, and pea maximum with maximum concentration added to the soil (Umar *et al.,*2020). Streptomyces has the ability to produce bioactive metabolites that expresses plant growth promoting agents. They show traits of plant growth initiation of chilli under greenhouse conditions, where *Streptomyces puniceus , Streptomyces mediolani* showed signifificant biosurfactant and plant growth promoting activity (Ravinder *et al.,*2022).

Biocontrol agents from Endophytic fungi can act as growth promoters, a defenders against predators, and competitors of microbial pathogens (Scannerini *et al.,*2001). By a variety of screening techniques, as well as through its antagonistic activity against phytopathogens including *Fusarium oxysporum* and *Aspergillus niger, Tuja plicata* was assessed for its capacity to produce biosurfactants and plant growth-promoting properties (Adnan *et al.,*2018). Biosurfactant producing Pseudomonas sp. That utilize petroleum as carbon source showed great plant growth potential in plants under various petroleum concentration with hight values of high values for all the parameters studied namely germination, shoot length, root length, fresh and dry weight and pigments (Das *et al.,*2016). *Pseudomonas aeruginosa* L10 isolated from the roots of a reed (*Phragmites australis*) from Yellow River Delta, Shandong, China, showed great plant-growth-promoting, and endophytic avtivity (Wu *et al.,*2018).

Chapter 3
AIM AND OBJECTIVES

AIM

To isolate and characterize biosurfactant from bacteria isolated from cold climatic region.

OBJECTIVES

- Isolation of bacteria from soil sample collected from cold climatic region.
- Primary and secondary screening of bacterial cultures for biosurfactant production
- Selection and characterization of potential biosurfactant producing bacterial culture.
- Production and optimization of biosurfactant from potential culture
- Extraction, purification and characterization of biosurfactant
- In vitro evaluation of biosurfactant for biomedical (antimicrobial, anti-TB, anticancer) and environmental (dye and oil degradation) applications

Chapter 4

MATERIALS AND METHODS

4.1 Isolation and characterization of biosurfactant from bacteria isolated from the cold climatic region

4.1.1 Soil sample collection

Soil sample were collected from area of **Kashmir,India (lat 34.37108 long 74.37690).** Samples were collected from 10 cm depth in soil and brought to the laboratory in sterilized containers and preserved in cold temperature.

4.1.2 Pre-treatment of the soil sample

The collected soil samples were kept for drying for about 3-5 days which is done as a primary method of eliminating the bacterial content. The dried sample was then heat treated at at 55ºC for 5 minutes in order to enhance the bacterial growth and to suppress the growth of other organisms during isolation (Radhakrishnan *et al.,* 2007)

4.1.3 Isolation of bacteria

Bacteria were isolated by standard spread plate method using Starch casein agar, ISP2, NA, AIA medium.About 10 g of pre-treated soil sample was added into 100 ml of sterile distilled water in 250 ml conical flask and kept in rotary shaker with 95 rpm for proper mixing. The soil suspension was serially diluted up to 10^{-5} dilutions using sterile distilled water blank. A 100µl of aliquots each from 10^{-3}, 10^{-4} and 10^{-5} dilutions were taken and spread over agar plate using sterile L-rod. All the plates were incubated at 28±2ºC for 2 days. Colonies with bacterial morphology were selected(Radhakrishnan *et al.,* 2014).

4.1.4. Characterization of bacterial strains

Cultural characterization of bacterial strains were studied using ISP2 agar medium. ISP2 agar was prepared and sterilized by autoclaving at 121°C for 15 minutes and poured into sterile petriplates under aseptic condition and allowed to solidify. Then all the bacterial cultures were inoculated into ISP2 agar plates and incubated at 28°C for 2 days. After incubation, all the plates were observed for growth, consistency, aerial mycelia colour (Shirling and Gottileb, 1966).

4.2 Screening of bacterial strains from biosurfactant production

4.2.1 Hemolytic activity

Hemolytic activity is a primary screening method to detect the biosurfactant producers. Hemolytic activity was performed in ISP2 medium amended with 5% (v/v) human blood. Plates were examined for hemolysis after incubation at 37ºC for 24 h. The plates were inspected for zone of clearance around the colony. The presence of clearing zone served as an indicator of biosurfactant producing microorganism (Hassanshahian, 2014).

4.2.2 Oil displacement method

Oil spreading technique is the most common method to identify the biosurfactant producers. Briefly, 30 ml of distilled water was added to the Petri plate followed by addition of 100 ml of olive oil to the surface of the water. Then, 20µlfollow the cell-free culture broth was dropped on the crude oil surface. The clear zone on the oil surface was measured and compared to 10 µL of distilled water as a negative control (Sharma *et al.,* 2014).

4.2.3 Emulsification activity (E24)

The emulsification activity is the method used to quantify the production of biosurfactant (Varjani *et al.,* 2014). About 2 ml of kerosene and 2 ml of cell-free medium (supernatant) were inoculated to a test tube and homogenized by vortexing at high speed for 2 min. After 24 h, the emulsification activity was calculated using following formula:

E24 (%) = total height of the emulsified layer/total height of the liquid layer X 100

4.2.4 Phenol sulphuric acid method

Biosurfactant producing strains selected from above screening methods were inoculated in ISP2 broth and incubated at 28°C on rotary shaker for 4-5 days. After incubation, broth was centrifuged at 10,000 rpm for 15 minutes and supernatant was collected while pellet was discarded. One ml of collected supernatant was mixed with 1ml of 5% phenol then 5ml of con.H2So4 was added in drop wise manner. Presence of biosurfactant in supernatant produces orange color from yellow color indicated the presence of glycolipids(Ellaiah *et al.,* 2012).

4.2.5 CTAB method

The CTAB agar plate method is a semi-quantitative assay for the identification of extracellular glycolipids or other anionic surfactants. Wagner and Siegfried created it. The target bacteria are grown on a plate of light blue mineral salts agar that also contains the basic dye methylene blue and the cationic surfactant cetyltrimethylammonium bromide. If the bacteria on the plate release anionic surfactants, they combine with cetyltrimethylammoniumbromide and methylene blue to form a dark-blue, insoluble ion pair. As a result, luminous blue halos surround productive colonies.

4.2.6 Drop collapse test

Screening of biosurfactant production was performed using the qualitative drop-collapse test described by Bodour and Maier in 1998 [28, 29]. Crude oil was used in this test. Two microlitres of oil was applied to the well regions delimited on the covers of 96- well micro plates and these were left to equilibrate for 24 hours. Five of the 48 hours culture, was transferred to the oil-coated well regions and drop size was observed after 1 min . The result was considered positive for biosurfactant production when the drop gets collapsed and those cultures that gave rounded drops were scored as negative, indicative of the lack of biosurfactant production

4.2.7 Phenol red method

Lipolytic Enzyme Assay Using Olive Oil with Phenol Red Agar. The serial diluted bacterial samples were also plated on phenol red agar and incubated at 37∘ C overnight. The phenol red agar plates were prepared by incorporating phenol red (0.01% w/v), olive oil (0.1% v/v), CaCl2 (0.1% w/v), and agar (2% w/v) [24]. Phenol red has an end point at pH 7.3-7.4, where a slight decrease in pH will turn its color from pink to yellow. The change in color of phenol red was used as an indicator for lipase activity, where lipase producing bacteria will turn the dye into yellow color.

4.3 OPTIMIZATION OF BIOSURFACTANT PRODUCTION FROM POTENTIAL ACTINOBACTERIA USING AGRICULTURAL AND OTHER SUBSTRATES

To attain higher biosurfactant production, carbon source, nitrogen source, pH was considered as suitable physic-chemical parameters and BS production were quantified by % emulsification activity (E24) described byNaliniand Parthasarathi (2017).

4.3.1 Effect of Carbon, nitrogen sources and Minerals

For the production of biosurfactant ninety-seven ml of minerals salts medium g l^{-1} (NaNO3 (2.5); K2HPO4 (1.0); KH2PO4 (1.0); KCl(0.1); MgSO4.7H2O (0.5); CaCl2 (0.01); FeSO4 7H2O (0.01) and yeast extract (0.1) prepared with different carbon sources like glucose, starch, mannitol and lactose and nitrogen sources yeast extract, malt extract, potassium nitrate and peptone. After sterilization bacterial strain was (**KM14**) transferred to each flask kept for incubation at 30°C.Control flasks were also maintained without inoculum. The samples were drawn aseptically at 24 hrs intervals and centrifuged at 10000 rpm for 15 min and the cell's free supernatant was analyzed for quantification of BS production by method described above. All the experiments were performed in triplicates and mean and SD was calculated (Nalini and Parthasarathi, 2013).

4.4. Production of biosurfactant

The inoculum of biosurfactant bacterial strain **KM14** was inoculated in 600ml soya bean broth and incubated at 28 °C with shaking at 150 rpm for 24 hours. The fermenter was sterilized with water at 121°C at allowed to cool down until it reaches room temperature, and the water is drained out. Once the fermenter is at room

temperature the 6ltr soya bean broth is poured and sterilized at 121°C. It is then allowed to cool down until it reached 30°C-35°C and the 600ml inoculum is inoculated. The fermenter is allowed to run for 2 days and the broth is collected

4.5 Extraction of the biosurfactant

After 2 days of fermentation, the culture broth was centrifuged at 10000 rpm at 4°C for 30 min. The supernatant was transferred to the separation funnel, and an equal volume of ethyl acetate was added to it for the extraction of biosurfactants. The solvent was evaporated to concentrate the biosurfactant. (Aparna *et al.,*2012).

4.6 Molecular Characterization of potential organism

4.6.1 16sRNA Sequencing

Also called as Sanger's sequencing is a method that's used obtain the DNA sequence of any microorganism. Using the sequence obtained one can understand the genetic structure of an organism. 16s rRNA sequencing is a culture-free method to identify and compare bacterial diversity from complex microbiomes or environments that are difficult to study. It is commonly used to identify bacteria present within a given sample down to the genus and/or species level.In general, the comparison of the 16S rRNA gene sequences allows differentiation between organisms at the genus level across all major phyla of bacteria, in addition to classifying strains at multiple levels, including what we now call the species and subspecies level.

4.7 Characterization of biosurfactant

4.7.1 Fourier Transform Infrared Spectroscopy (FT-IR) analysis

The extracted biosurfactant was subjected to Fourier transform infrared spectroscopy (FT-IR) analysis to identify the chemical bonds or the functional groups present. Two milligrams (freeze-dried) purified biosurfactant was ground with 100 mg KBr pellet and pressed with 7500 kg for 30 s to obtain a translucent pellet and scan wave number range of 4000–400 cm^{-1}. The analysis of IR spectra was carried out by using Thermo software. All the measurements consisted of 500

scans and a KBr pellet was used as a background reference **(Nalini and Parthasarathi,, 2014).**

4.7.2 HRMS LC-MS

Crude extract was analyzed by LC-HRMS and LC-HRMS/MS using a Thermo LTQ Orbitrap XL/ mass spectrometer (Thermo Fisher Scientifific Spa, Rodano, Italy) coupled to an Agilent model 1100 LC system (Agilent Technologies, CernuscosulNaviglio, Italy) equipped with a solvent reservoir, an in-line degasser, a binary pump, and a refrigerated autosampler. The spectrum was recorded by infusion into the ESI source using MeOH as the solvent. A 5m Kinetex C18 column (50 mm 2.1 mm),µ maintained at 25 ◦C, was operated using a gradient elution of H2O and MeOH both with 0.1% formic acid, running at 200 µL/min. The gradient program was as follows: 10% MeOH for 3 minutes, 10–90% µL/ MeOH over 30 minutes, and 90% MeOH for 3 min. Data dependent acquisition mode MS spectra was recorded in positive ion mode with a spray voltage of 5 kV, a capillary temperature of 230 ◦C, a sheath gasrate of 12 units N2 (ca. 120 mL/min), and an auxiliary gas rate of 5 units N2 (ca. 50 mL/min). HRMS/MS scans were obtained for selected ions with collision-induced dissociation (CID) fragmentation, isolation width of 2.0, normalized collision energy of 35, activation Q of 0.250, and activation time of 30 ms.

An Agilent 1290 Infifinity II UHPLC System (Santa Clara, CA, USA) combined with a Bruker ImpactII ultra-high-resolution Qq-TOF mass spectrometer (Bruker Daltonics,GmbH, Bremen, Germany) equipped with an electron spray ionization (ESI) source was used for metabolite analysis. A Kinetex™ 1.7 µm UHPLC (C18) column (50 2.1 mm)× µ was used for chromatographic separation. MS spectra were acquired in positive ionization mode from m/z 50–2000 Da. Metabolic extracts of the Streptomyces strain were resuspended in 1 mL methanol (LC-MS grade) and directly analyzed. After injection with 10 µL of the µ metabolic extract, it was separated using a gradient of water (A) and acetonitrile 100% (B)with 0.1% formic acid and at a flflow rate of 0.5 mL/min throughout the run. The gradient elution was initiated at 5% solvent B for 3 minutes and then at a linear gradient of 5% to 50% B over 5 min and held at 50% B for 2 min followed by a linear gradient of 50% to 100%

B over 5 min and held at 100% B for 3 min. The column was then re-equilibrated to 5% B for 1 min.

4.8 EVALUATION OF BIOSURFACTANT FOR BIOMEDICAL AND NON-MEDICAL APPLICATIONS

4.8.1 Biomedical application of biosurfactant

4.8.1.1 Antibacterial activity

Antibacterial activity of biosurfactant was tested by agar well diffusion method against bacterial pathogens, *viz. S. aureus* ATCC 29213 and *E. coli* 25922. Muller Hinton agar medium was prepared and poured into the sterile Petri plates and allowed to solidify. Then wells (7 mm) were made in the medium using sterile well cutter. Each well was filled of biosurfactant (dissolved in 10%DMSO) and 10%DMSO served as control. The plates were incubated at 37 °C for 24 h and the plates were observed for formation of clear inhibition zone around the well and zone of inhibition was measured (Singh *et al.,* 2014).

Chapter 5
RESULTS AND DISCUSSION

5.1 Soil sample collection

Soil samples were collected from Kashmir, India **(lat 34.37108 long 74.37690)** for the isolation of bacteria.

Figure 5.1: Region of soil isolation

5.2 Isolation of bacteria

Totally 27 morphologically different bacterial strains were isolated from soil samples. Both the isolates are recovered and stored in AIA and ISP2 plate stored in 4T he cultures showed good growth on AIA agar.

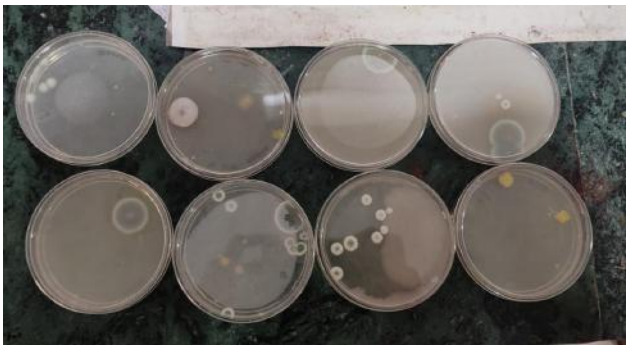


Figure 5.2: Isolation of bacteria on AIA agar plate.

5.3 Screening of bacterial strain for biosurfactant production

5.3.1 Hemolytic activity

The bacterial strain KM14 only showed hemolytic activity on blood agar (Fig 5.3). Hence the strain KM13 was selected for the secondary screening methods for further confirmation. Previously Korayem *et al.,* (2015)reported the biosurfactant producing bacteria were selected by primary screening method like blood hemolysis assay.

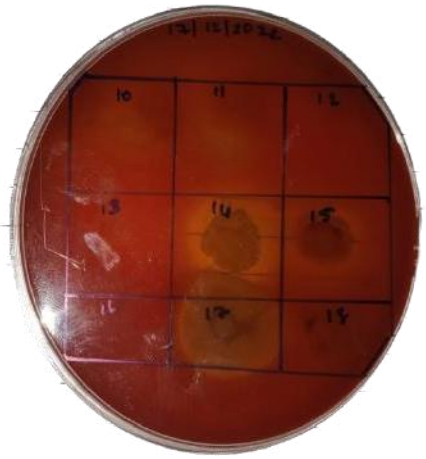


Figure 5.3: Blood Agar Hemolysis

5.3.2 Oil displacement activity

In oil spread assay, a 25 mm zone was observed after adding the culture supernatant (Fig 5.4). It confirmed the production of biosurfactant by the strain KM14. Khopade *et al.,* (2012) investigated the bacteria for biosurfactant production screened by oil displacement activity with the zone formation in surface of the oil

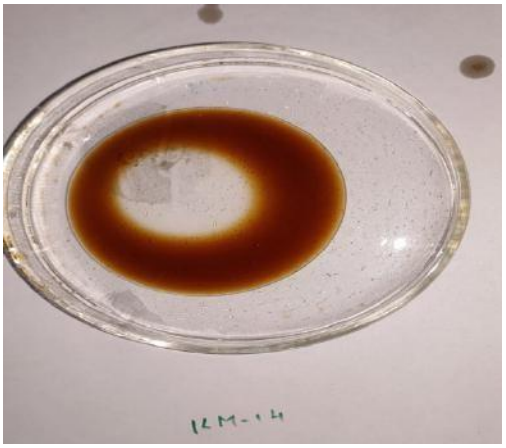


Figure 5.4: Oil displacement assay of KM14

5.3.3 Emulsification activity

Bacterial strain KM14 showed positive results (Fig 5.5) and was tested for its ability to emulsify crude oil and in this study, kerosene was used for the emulsification assay. The test was done by adding 2 ml of supernatant and kept overnight. After the results were recorded. Previously, Loganathan and Karthik., (2010) reported the bio emulsifing activities of bacteria isolated from different ecosystems for the production of biosurfactants.

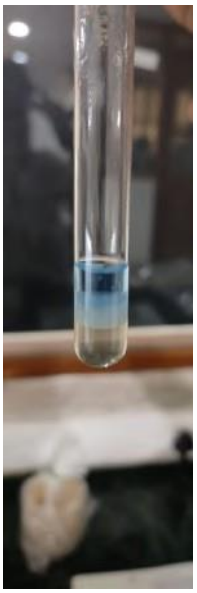


Figure 5.5: Emulsification of KM14

5.3.4 Phenol sulfuric acid method

In phenol –sulfuric acid method yellow colour has been changed to red color(Fig 5.6) when phenol and concentrated sulfuric acid were added. The results conform the production of biosurfactant by the bacterial strain KM14. A similar type of study has been reported by Kalyani *et al.,* (2014)in which color change has been observed when the solution added with cell free supernatant from *Streptomyces sp.*

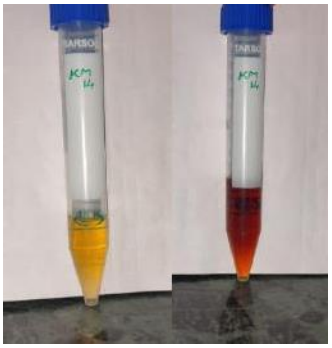


Figure 5.6: Colour changes in Phenol sulphuric acid method

5.3.5 CTAB method

A semi-quantitative technique for the identification of extracellular glycolipids or other anionic surfactants is the CTAB agar plate method. Wagner and Siegmund came up with it. The KM14 biosurfactant isolate showed productive colonies encircled by pale blue haloes.

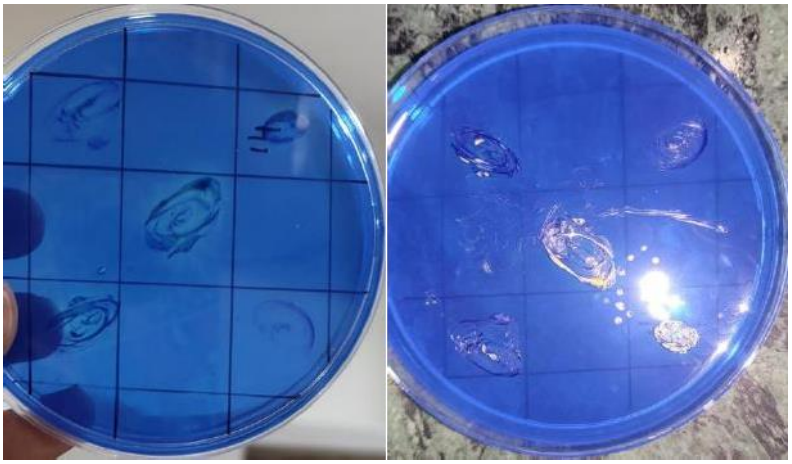


Figure 5.7: Confirmation activity on CTAB agar plate.

5.3.6 Drop collapse test

The drop collapse test findings of the wetting activity investigation showed that km14 was capable of creating biosurfactant. The drop collapsing assay depends on surfactants' ability to make liquid droplets on an oily surface less stable. Its surface tension and spreading tension are related to this capability. Surface tension of the oil, surface tension of the surfactant solution, and interfacial tension between the oil and the surfactant solution all affect how easily the surfactant solution spreads over the surface of the oil. The oil surface tension was fixed because the same oil was utilised. As a result, drops on oily surfaces will collapse if their spreading tension is higher or their surface tension is lower. However, drops with higher surface or lower spreading tension will not spread.

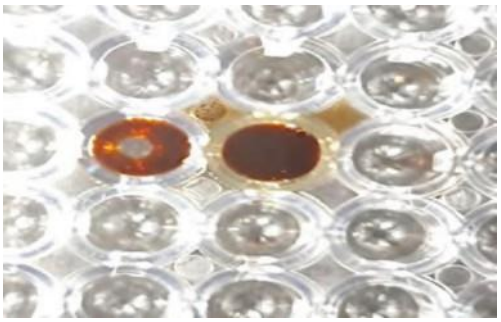


Figure 5.8: Drop collapse activity of potential strain(KM14).

5.3.7 *Phenol red method*

The potential strain KM14 has shown positive results by forming light pink colour around the culture spots. This proves that the satin has biosurfactant activity.

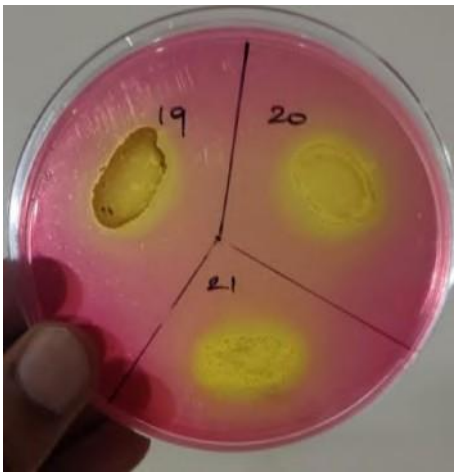


Figure 5.9: Screening of microbial lipase production on Phenol red agar plate

5.4 OPTIMIZATION OF BIOSURFACTANT PRODUCTION FROM POTENTIAL BACTERIA USING AGRICULTURAL AND OTHER SUBSTRATES

5.4.1 Effect of carbon, nitrogen sources and Minerals on the production of biosurfactant

In order to select the best carbon, nitrogen sources and minerals for the production of biosurfactant by potential bacterial strain KM14 in the mineral salt medium. Among the carbon and nitrogen sources tested starch and yeast extract is the most preferred source and emulsification activity respectively and low-level activity was observed in lactose and potassium nitrate and emulsification activity.

Strain KM14 shows well growth in all the mineral sources. However, a maximum emulsification activity biosurfactant production was observed at Glucose(Fig 5.7). Previously, Nalini and Parthasarathi, (2013) reported mannitol and yeast extract are the best carbon and nitrogen sources of emulsification activity, respectively. Ashish and Mira Debnath, (2018) also reported a maximum emulsification index of 62 % when the biosurfactant is produced in Glucose.

Figure 5.10: **Media optimization**

5.5 Production of biosurfactant

Km14 strain was introduced into 6 litres soya bean broth . 5 litres of the supernatant were extracted once the fermentation process completed and centrifuged.

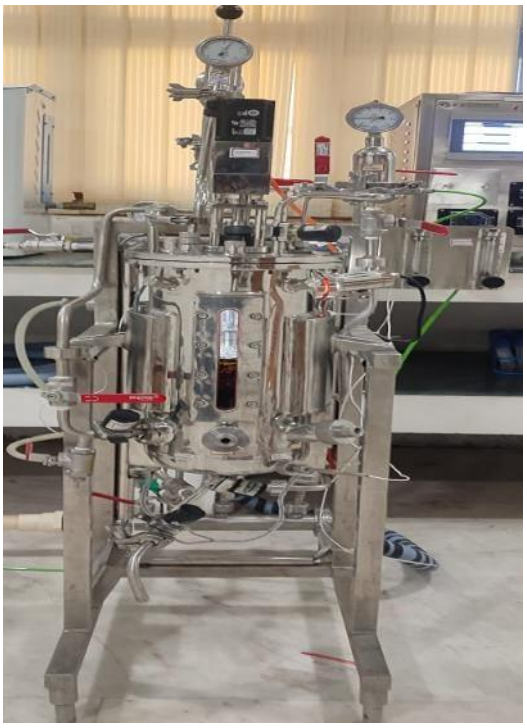
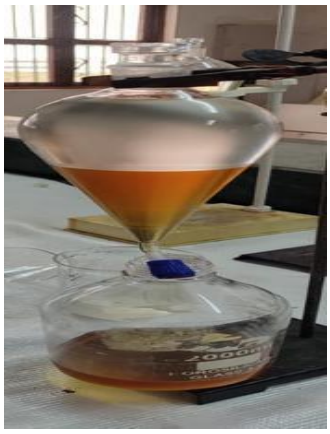


Figure 5.11 Fermentation of KM14 strain.

5.6 Extraction of the biosurfactant

5 litres of supernatant was extracted using the liquid-liquid extraction method using ethyl acetate as the solvent for extraction. It was mixed and allowed to separate overnight. The solvent is then transferred to a sterile beaker after separation. It was then allowed to dry completely. 100 µl ethyl acetate is added to each of the beakers to scrape out the crude extract. The crude was then transferred to a 50ml falcon tube. Approximately 2gm of extract was obtained.



(a)

(b)

(C)

Figure 5.12: (a) Liquid-liquid extraction. (b) Drying process (c) Crude extract

5.7 Molecular characterization of potential organism

5.7.1 16sRNA Sequencing

16sRNA gene of *Stenotrophomonas rhizophila* KM14 was sequenced at IIT-Bombay. The BLAST result of the sequence 16sRNA gene showed that the isolate exhibit 93% similarity to *Stenotrophomonas rhizophila* B903/16. The strain has been uploaded to Genbank and the accession number is **SUB13186139** *Stenotrophomonas* **OQ874562.**

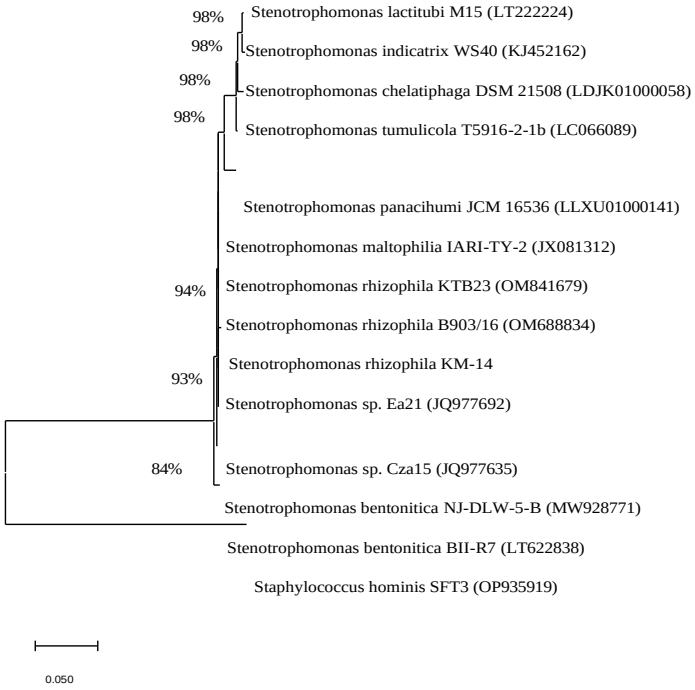


Figure 5.13 Phylogenic tree of the bacterial strain KM14

5.8 Characterization of biosurfactant

5.8.1 Fourier Transform Infrared Spectroscopy (FT-IR) analysis

The FTIR analysis was conducted on the KM14 strain in Ocean research, Sathyabama Institute of Science and Technology, Tamil Nadu, India. The highest peak was observed at 1649.34699 with an intensity of 23.80347 which has a C=C stretching group of alkene compound class.

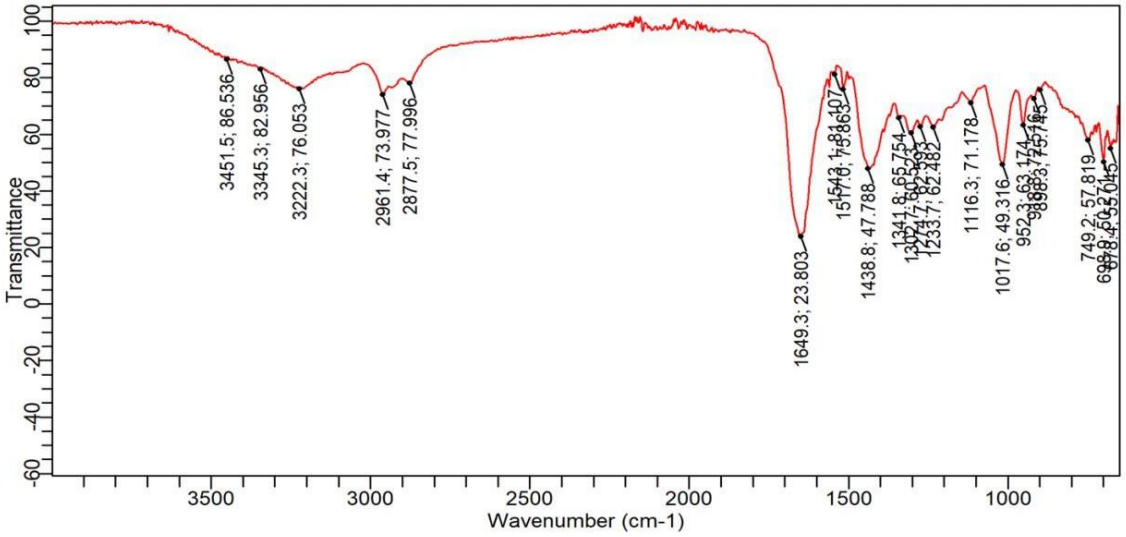


FIGURE 5.14: FTIR analysis conducted on the KM14 strain

Table 5.1 : FTIR chracterization

Peak Number	Wavenumber (cm^{-1})	Intensity
1	678.37549	55.04458
2	698.87584	50.27121
3	749.19491	57.81886
4	898.28842	75.74484
5	918.78878	72.51628
6	952.33482	63.17448
7	1017.56323	49.31625
8	1116.33768	71.17751
9	1233.74882	62.48158
10	1274.74954	62.59275
11	1302.70457	60.52332
12	1341.84162	65.75446

13	1438.75241	47.78788
14	1517.02650	75.86307
15	1543.11786	81.10688
16	1649.34699	23.80347
17	2877.50481	77.99568
18	2961.36991	73.97654
19	3222.28356	76.05316
20	3345.28571	82.95593
21	3451.51484	86.53569

5.8.2 HRMS LC-MS

The spectrum was recorded by infusion into the ESI source using MeOH as the solvent. A 5m Kinetex C18 column (50 mm 2.1 mm),µ maintained at 25 ◦C, was operated using a gradient elution of H2O and MeOH both with 0.1% formic acid, running at 200 µL/min. The gradient program was as follows: 10% MeOH for 3 min, 10–90% µL/ MeOH over 30 minutes, and 90% MeOH for 3 minutes. Data dependent acquisition mode MS spectra was recorded in positive ion mode with a spray voltage of 5 kV, a capillary temperature of 230 ◦C, a sheath gasrate of 12 units N2 (ca. 120 mL/min), and an auxiliary gas rate of 5 units N2 (ca. 50 mL/min). HRMS/MS scans were obtained for selected ions with collision- induced dissociation (CID) fragmentation, isolation width of 2.0, normalized collision energy of 35, activation Q of 0.250, and activation time of 30 ms.

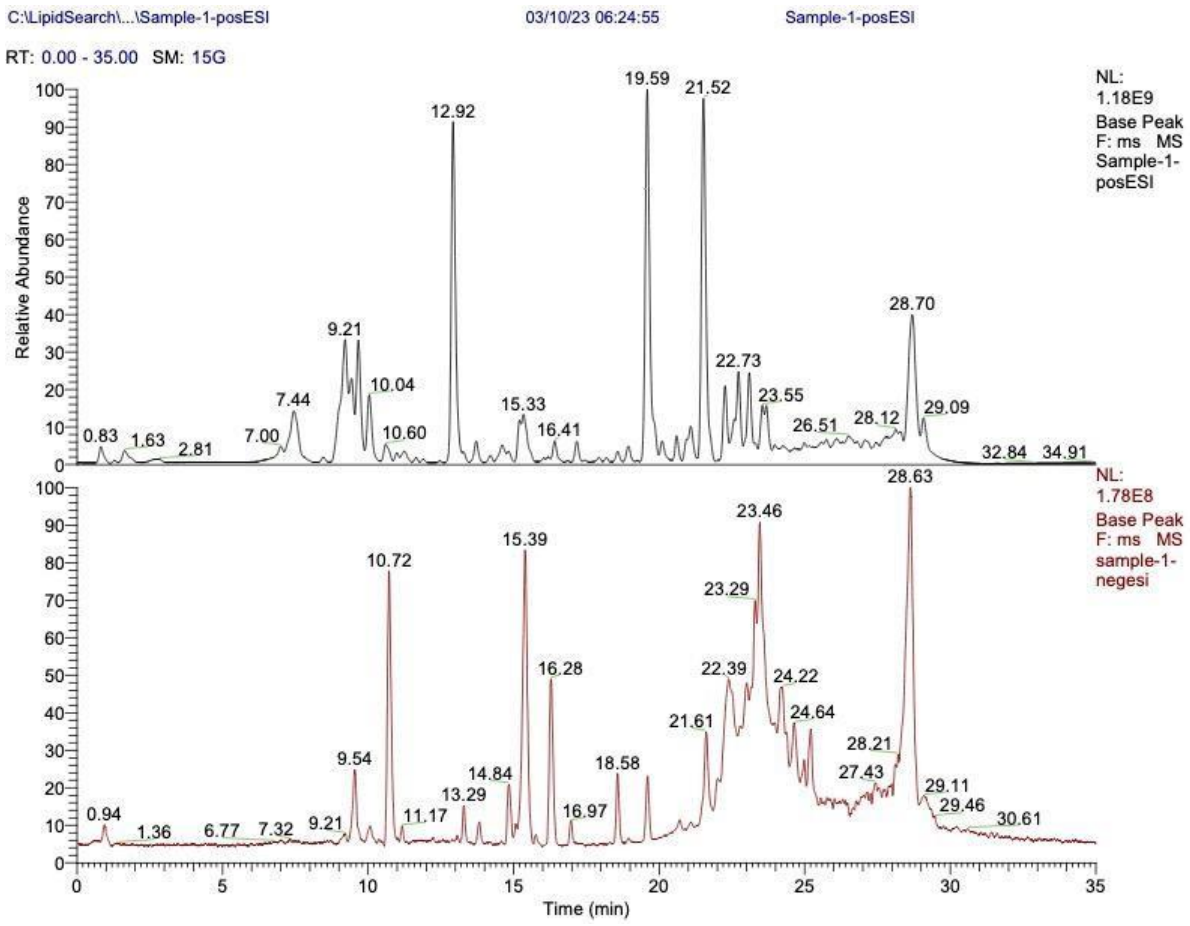


FIGURE 5.15: HRMS LC-MS chromatogram for KM14.

Table 5.2: List of suspected compounds from potential KM14 identified through HRMS LC-MS at negative ESI mode

NAME	FORMULA	MOLECULAR WEIGHT
Glycolic acid	C2 H4 O3	76.0160
4- Hydroxybenzaldehyde	C7 H6 O2	122.0368
Benzoic acid	C7 H6 O2	122.0368
2- Hydroxybenzothiazole	C7 H5 NOS	151.0092
Genistein	C15 H10 O5	270.0528
Salicylic acid	C7 H6 O3	138.0317
Apigenin	C15 H10 O5	270.0528
Phenol	C6 H6 O	94.0419
L-(+)-Lactic acid	C3 H6 O3	90.0317
Azelaic acid	C9 H16 O4	188.1049
DL-Malic acid	C4 H6 O5	134.0215

Daidzein	C15 H10 O4	254.0579
Chrysin	C15 H10 O4	254.0579
Luteolin	C15 H10 O6	286.0477
NP-008915	C15 H24 O3	252.1725
Myristic acid	C14 H28 O2	228.2089
Pyruvic acid	C3 H4 O3	88.0160
Stearic acid	C18 H36 O2	284.2715
α,α-Trehalose	C12 H22 O11	342.1162
Linoleic acid	C18 H32 O2	280.2402
4-Methylphenol	C7 H8 O	108.0575
trans-10- Heptadecenoic acid	C17 H32 O2	268.2402
2-[(1S,2S,4aR, 8aS)-1-hydroxy-4amethyl-8- methylidene decahydronaphthal en-2yl prop-2-enoic acid	C15 H22 O3	250.1569
Oleic acid	C18 H34 O2	282.2559
trans-Petroselinic acid	C18 H34 O2	282.2559
NP-002089	C15 H22 O3	250.1569
NP-014839	C19 H22 O3	298.1569
Pentadecanoic acid	C15 H30 O2	242.2246
16- Hydroxyhexadecanoic acid	C16 H32 O3	272.2351
NP-004987	C15 H22 O5	282.1467
Hexadecanedioic acid	C16 H30 O4	286.2144
Xanthine	C5 H4 N4 O2	152.0334
NP-012534	C15 H24 O5	284.1624
NP-005196	C15 H22 O5	282.1467
Pyridoxine	C8 H11 N O3	169.0739
NP-020632	C16 H20 O4	276.1362
L-Saccharopine	C11 H20 N2 O6	276.1321
Butylparaben	C11 H14 O3	194.0943
NP-022394	C15 H22 O3	250.1569
(±)9(10)-DiHOME	C18 H34 O4	314.2457

(+/-)9,10-dihydroxy- 12Z-octadecenoic Acid	C18 H34 O4	314.2457
(+/-)9-HODE	C18 H32 O3	296.2351
NP-020521	C18 H32 O3	296.2351
NP-006255	C17 H26 O4	294.1831
Erucic acid	C22 H42 O2	338.3185
(+/-)9(10)-EpOME	C18 H32 O3	296.2351
(±)9-HpODE	C18 H32 O4	312.2301
N-Acetyl-L-cysteine	C5 H9 N O3 S	163.0303
NP-001596	C16 H30 O4	286.2144
13(S)-HOTrE	C18 H30 O3	294.2195
2- Hydroxyphenylacetic acid	C8 H8 O3	152.0473
3-Hydroxypicolinic acid	C6 H5 N O3	139.0269
2- Mercaptobenzothiazole	C7 H5 N S2	166.9863
6-Hydroxynicotinic acid	C6 H5 N O3	139.0269
(+/-)12(13)- DiHOME	C18 H34 O4	314.2457
Tenofovir	C9 H14 N5 O4 P	287.0783
NP-004020	C17 H14 O6	314.0790
Arachidic acid	C20 H40 O2	312.3028
(1R,3S,4S,5R,7R)-4-(3 hydroxybutyl)-5-methyl-10- methylidene-8-oxatricyclo [5.3.0.0Âl, âµ]decan-9-one	C15 H22 O3	250.1569
Mono(2-ethylhexyl) phthalate (MEHP)	C16 H22 O4	278.1518
(+/-)13-HODE	C18 H32 O3	296.2351
3-Hydroxybutyric acid	C4 H8 O3	104.0473
Sucrose	C12 H22 O11	342.1162
NP-021701	C15 H22 O4	266.1518

Nordiazepam	C15 H11 Cl N2 O	270.0560
D-(-)-Mannitol	C6 H14 O6	182.0790
NP-005166	C30 H28 O6	484.1886
Caprylic acid	C8 H16 O2	144.1150
Deoxycorticosterone 21-glucoside	C27 H40 O8	492.2723
Pyrrole-2-carboxylic acid	C5 H5 N O2	111.0320
Gentisic acid	C7 H6 O4	154.0266
N-Acetyl-DL-tryptophan	C13 H14 N2 O3	246.1004
3,5- Dihydroxybenzoic acid	C7 H6 O4	154.0266
Arachidonic acid	C20 H32 O2	304.2402
NP-014789	C26 H30 O13	550.1686
4-Hydroxybenzoic acid	C7 H6 O3	138.0317
L-Iditol	C6 H14 O6	182.0790
Desalkylflurazepam -d4	C15 H6 [2]H4 Cl F N2 O	292.0717
4-Aminobenzoic acid	C7 H7 N O2	137.0477
Dulcitol	C6 H14 O6	182.0790
3-Hydroxybenzoic acid	C7 H6 O3	138.0317
NP-008993	C18 H34 O4	314.2457
2-Hydroxycinnamic acid	C9 H8 O3	164.0473
2-Anisic acid	C8 H8 O3	152.0473
Protocatechuic acid	C7 H6 O4	154.0266
Ethylmalonic acid	C5 H8 O4	132.0423
13,14-dihydro-15- keto-tetranor Prostaglandin D2	C16 H26 O5	298.1780
Dodecanedioic acid	C12 H22 O4	230.1518
Norepinephrine	C8 H11 N O3	169.0739
NP-020515	C13 H20 O5	256.1311
DL-α-Aminocaprylic acid	C8 H17 N O2	159.1259
α-Lactose	C12 H22 O11	342.1162

Citrinin	C13 H14 O5	250.0841
3,14-dihydro-15- keto-tetranor Prostaglandin E2	C16 H26 O5	298.1780
Indole-3-acrylic acid	C11 H9 N O2	187.0633
N-Acetylanthranilic acid	C9 H9 N O3	179.0582
2,4- Dihydroxybenzoic acid	C7 H6 O4	154.0266
3-Pyridylacetic acid	C7 H7 N O2	137.0477
4-Coumaric acid	C9 H8 O3	164.0473
Estriol	C18 H24 O3	288.1725

Table 5.3: List of suspected compounds from potential KM14 identified through HRMS LC-MS at posESI mode

NAME	FORMULA	MOLECULAR WEIGHT
Hexadecanamide	C16 H33 N O	255.2562
3-Pyridinol	C5 H5 N O	95.0371
2- Hydroxybenzothiazole	C7 H5 N O S	151.0092
Adenosine	C10 H13 N5 O4	267.0968
2- Mercaptobenzothiazole	C7 H5 N S2	166.9863
8-Hydroxyquinoline	C9 H7 N O	145.0528
5,6- Dimethylbenzimidazole	C9 H10 N2	146.0844
Prunin	C21 H22 O10	434.1213
1-Methyladenine	C6 H7 N5	149.0701
C-8 Ceramide-1- phosphate	C26 H52 N O6 P	505.3532
3-Methyladenine	C6 H7 N5	149.0701
7-Methyladenine	C6 H7 N5	149.0701
Crotonic acid	C4 H6 O2	86.0368
NP-013736	C10 H16 N2 O2	196.1212
Stearoyl ethanolamide	C20 H41 N O2	327.3137
N6-Methyladenine	C6 H7 N5	149.0701
2'-Deoxyadenosine	C10 H13 N5 O3	251.1018
Norharman	C11 H8 N2	168.0687
3-(propan-2-yl)-	C10 H16 N2 O2	196.1212

octahydropyrrolo [1,2-a]pyrazine-1,4- dione		
Flavin mononucleotide (FMN)	C17 H21 N4 O9 P	456.1046
Adenine	C5 H5 N5	135.0545
3-(2-methylpropyl)-octahydropyrrolo [1,2-a]pyrazine-1,4- dione	C11 H18 N2 O2	210.1368
2-Amino-6-methylmercaptopurine	C6 H7 N5 S	181.0422
Nootkatone	C15 H22 O	218.1671
Oleamide	C18 H35 N O	281.2719
N-(5- acetamidopentyl) acetamide	C9 H18 N2 O2	186.1368
4-Methoxycinnamic acid	C10 H10 O3	178.0630
Nicotinuric acid	C8 H8 N2 O3	180.0535
6-Methylquinoline	C10 H9 N	143.0735
Cordycepin	C10 H13 N5 O3	251.1018
(1R,2R,6R,9R)- 2,11,11-trimethyl-3-oxotricyclo [4.3.2.0Âa, âµ]undecane-9- carboxylic acid	C15 H22 O3	250.1569
NP-007077	C15 H16 N2 O4	288.1110
Guanine	C5 H5 N5 O	151.0494
NP-011220	C11 H18 N2 O2	210.1368
2-Methyl-S benzothiazole	C8 H7 N S2	181.0020
Kynurenic acid	C10 H7 N O3	189.0426
Monobutyl phthalate	C12 H14 O4	222.0892
N-(2- hydroxyphenyl) acetamide	C8 H9 N O2	151.0633
Pyridoxine	C8 H11 N O3	169.0739
2- (Methylthio) benzothiazole	C8 H7 N S2	181.0020

2,3-dihydroxypropyl 12-Methyltridecanoate	C17 H34 O4	302.2457
Mono(2-ethylhexyl) phthalate (MEHP)	C16 H22 O4	278.1518
N- Methylnicotinamide	C7 H8 N2 O	136.0637
D-Sphingosine	C18 H37 N O2	299.2824
3-Methylhistidine	C7 H11 N3 O2	169.0851
1-Methylhistidine	C7 H11 N3 O2	169.0851
2-Phenylglycine	C8 H9 N O2	151.0633
NP-022231	C6 H13 N O4	163.0845
α-Methyl-DL-histidine	C7 H11 N3 O2	169.0851
2-(3,4-dihydroxyphenyl) acetamide	C8 H9 N O3	167.0582
NP-004713	C15 H24 O2	236.1776
3-(3,4,5- trimethoxyphenyl) propanoic acid	C12 H16 O5	240.0998
N,N-Dimethylsphingosine	C20 H41 N O2	327.3137
Betaine	C5 H11 N O2	117.0790
Indirubin	C16 H10 N2 O2	262.0742
NP-021868	C15 H14 O4	258.0892
NP-021781	C19 H36 O5	344.2563
HET0016	C12 H18 N2 O	206.1419
Ohmefentanyl	C23 H30 N2 O2	366.2307
Coumestrol	C15 H8 O5	268.0372
QQH	C16 H25 N7 O6	411.1866
Epicatechin	C15 H14 O6	290.0790
Ecgonine	C9 H15 N O3	185.1052
Catechin	C15 H14 O6	290.0790
Tenofovir	C9 H14 N5 O4 P	287.0783
Estrone	C18 H22 O2	270.1620
NP-001501	C16 H17 N3 O2	283.1321
Deisopropylatrazine	C5 H8 Cl N5	173.0468
Caffeine	C8 H10 N4 O2	194.0804

5.9 EVALUATION OF BIOSURFACTANT FOR BIOMEDICAL AND NON-MEDICAL APPLICATIONS

The antibacterial activity of biosurfactant was tested by agar well diffusion method against bacterial pathogens, viz. S. aureus , E. coli, Candida 10 and 30 ,klebsiella . Muller Hinton agar medium was prepared and poured into the sterile Petri plates and allowed to solidify. Then wells (7 mm) were made in the medium using a sterile well cutter. Each well was filled with biosurfactants. The plates were incubated at 37 °C for 24 h and the plates were observed for the formation of a clear inhibition zone around the well and zone of inhibition was measured.

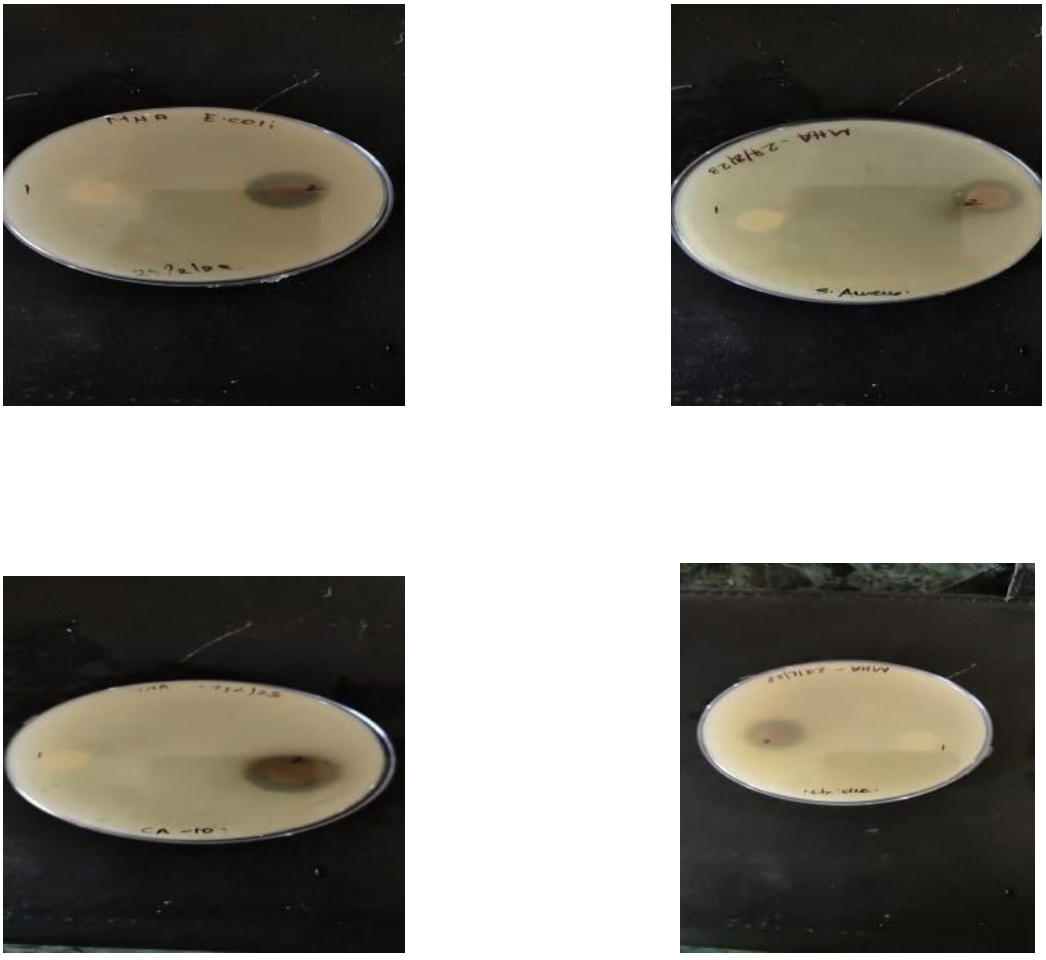


FIGURE 5.16: Antibacterial activity of KM14 strain.

Chapter 6

SUMMARY AND CONCLUSION

There are several uses for surfactants in the agrochemical and agricultural sectors. Biosurfactants, which are more environmentally friendly, are only sometimes used. The precise role of surfactants in assisting other systems as biocontrol agents is still poorly known and calls for further research. These investigations will aid in the transition to eco-friendly surfactants from harsh chemical ones. To obtain net economic benefit from the application of biosurfactants in agriculture as well as other industries, it is necessary to work on the production cost of green surfactants. A more serious consideration is also needed for the overproduction of biosurfactants from agricultural waste. By altering the production process, the chemical compositions of biosurfactants that have been identified as powerful biocontrol agents can be changed. This strategy could result in the biosynthesis of highly targeted green surfactants.

Gram-negative *Stenotrophomonas rhizophila* bacteria are members of the Xanthomonadaceae family. The current investigation focuses on understanding how bacteria isolated from soil samples produce biosurfactants. specifically *Stenotrophomonas rhizophila*, which is thought to be a powerful generator of biosurfactants. *Stenotrophomonas rhizophila,* which produces biosurfactants, was screened using the drop collapse test, haemolysis, emulsification index, oil displacement, phenol red agar, CTAB technique, and phenol sulphuric acid.Using glucose as their primary source of production, growth was optimised at 7pH. Results from molecular characterisation, FTIR, HRMS, and LC-MS were also published.

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ANNEXURE

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Harnessing microbial biosurfactant for agricultural applications

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Abstract

Biosurfactant refers to surface-acting substances that can enhance surface-surface interactions by generating micelles that are derived from natural sources including plants, microorganisms, and animals. Biosurfactants are a structurally diverse group of secondary metabolites with lots of potential to serve mankind. The structural and compositional diversity of biosurfactants is unambiguously substrate-dependent. Microbial surfactants are environment-friendly alternatives to synthetic surfactants. Biosurfactants are surface-active agents produced by microorganisms that have higher efficiency and stability, lower toxicity and higher biocompatibility and biodegradability than chemical surfactants. The differences between biosurfactant production can be attributed to the different compositions of the hydrolyzates.

Keywords: Biosurfactant, Agriculture, Sustainability, Plant defence, biodegradability.

1. Introduction

The challenges of food security and environmental management are made more difficult by the world's fast industrialization and growing population. A greater population of developed countries depend upon chemical pesticides, in the soaps and detergent industries as emulsifiers, detergents, and dispersants (Kumar *et al.,*2021, Marchut-Mikołajczyk *et al.,* 2021). Oil spills at sea are frequently treated using chemical dispersants as a first-step response method. Organo-chemical synthesis is used to produce the dispersants that have been licensed and are kept

in stockpiles across the world in case of an oil spill (Nikolova *et al.,* 2021). The synthetic surfactants (organo-chemical) that have been utilized in the present are of high risk to the environment as they are derived from non-renewable petroleum sources and a finite source (de Oliveira Schmidt *et al.,* 2021). Another such consideration is where that scientist are extremely concerned about the growing threat that toxic heavy metal pollution of soil poses to the ecosystem due to its toxicological manifestations and adverse impacts across the world (Mishra *et al.,* 2021). Using materials to the fullest extent possible, such as oil hydrocarbons and heavy metals such as cadmium, iron, titanium, copper, zinc, mercury, insecticides, herbicides, air pollutants (carbon monoxide, nickel), particulate pollution, ozone, acid rain, and volatile organic compounds), bisphenol, sulphonamides, nitroaromatic chemicals, organophosphorus compounds, trichloroethylene, perchloroethylene, solvents, and chlorinated. However, it is imperative to implement remediation solutions for such dangerous contaminants. The use of remedial techniques such as soil washing, pumping, aeration, oxidation, and incineration is rather rare. The production of additional secondary pollutants, which is economically unfeasible, is one of the many downsides of these remediation techniques. To address these issues, microbial bioremediation one of the sustainable and affordable techniques for removing environmental contaminants is bioremediation. (Sharma *et al.,* 2021).

As a result, finding natural alternatives to artificial surfactants and using them responsibly is essential (Eras-Muñoz *et al.,* 2022). One of the most recently discovered microbially produced/synthesized bimolecular molecules are termed biosurfactants which are becoming more and more popular due to their exceptional benefits over synthetic ones (Mohanty *et al.,* 2021, Yun *et al.,* 2021).

The word "biosurfactant" refers to surface-acting substances that can enhance surface-surface interactions by generating micelles that are derived from natural sources including plants, microorganisms, and animals (Yun *et al.,* 2021). Bioremediation is a natural, sustainable, and environmentally advantageous technology for treating environmental contaminants without prod any secondary pollutants or adverse effects. Outstanding microbial products known as biosurfactants, also known as biologically active chemicals, have been effectively

used in the detoxification and removal of hazardous heavy metals (Mishra *et al.,* 2021). The benefits of green surfactants (biosurfactants made from microorganisms) over synthetic surfactants have been emphasized in several papers. In comparison to synthetic surfactants, biosurfactants are low or nontoxic, biodegradable, exhibit outstanding surface activity, have a high specification, are effective in harsh environments, and may also be recycled through regeneration (Sachdev *et al.,* 2013). Microbial surfactants are a common occurrence in human lifestyles nowadays and are a major component of everyday items like detergents, food additives, and cosmetics (Pardhi *et al.,* 2022). They are used commercially as cheaper manufacturing alternatives to chemical surfactants, particularly in the pharmaceutical industry (Sajid *et al.,* 2020). There are already more than 2000 different biosurfactant structures that have been identified, including chemically separate families of compounds as well as groups of congeners, or structurally similar compounds with slight structural changes (Kubicki *et al.,* 2019). The effectiveness of a surfactant is determined by its ability to reduce surface tension (ST) and interfacial tension (IFT) between two immiscible phases. (Nikolova *et al.,* 2021).

Since these natural surfactants are found to be utilized as carbon sources by soil-dwelling bacteria, they can replace the harsh surfactants now employed in the pesticide industry (Sachdev *et al.,* 2013). Agricultural waste and leftovers of food processing can be used as a carbon source for the creation of microbial fuels because they are readily available and inexpensive. biosurfactants (Marchut-Mikołajczyk *et al.,* 2021).

1.1 Impact of sustainable agriculture

There is a need to avoid the degradation of land as it is high time for us to preserve the land since there is an increase in human population and subsequent raise in food consumption. Providing food security for a growing population need, entails the implementation of sustainable land use techniques and the protection of any degraded or margined soil (Ahmad *et al.,* 2018). The micronutrient deficiencies in the soil must also be addressed to fulfil the crops increasing demands. Increasing the use of nutrient availability by a biosurfactant, which is a multifunctional microbial

metabolite may be a suitable strategy to increase agricultural output (Singh *et al.,* 2018). Numerous potentials for sustainable agriculture have been provided by biocontrol techniques involving the use of biomaterials and biomolecules. The green strategy uses multifunctional biomolecules including biosurfactants, chitosan, and nanoparticles synthesized from chitosan because of their biocompatibility. (Karamchandani *et al.,* 2022). Since surfactants of chemical origin have a number of adverse effects on the environment, such as toxicity towards lesser forms of life, soil contamination, etc. Biocontrol strategies have been adopted (Sangwan *et al.,* 2022). Affordable feedstocks are used in the economy and circular economy of biosurfactants, which supports the utilization of waste converted into useful products. Waste reduction, reuse, and recycling are supported by novel, inexpensive, renewable health-grade biosurfactants in an integrated green economy bioprocess. (Mgbechidinma *et al.,* 2022).

2. Microbial biosurfactant classification

Microbial Biosurfactnts are classified into various types that are depending on their diverse properties. But are mainly classified based on their Molecular weight. The low and high molecular weight molecules are the two main groups into which biosurfactants fall. Generally, high molecular weight biosurfactants often referred to as bioemulsifiers or bioemulsans, may stabilize emulsions and powerfully attach to surfaces, whereas low molecular weight surfactants effectively lower surface and interface tension. The first class consists of lipopeptides and glycolipids, whereas the second class is made up of proteins, polysaccharides, and lipoproteins. (Ravinder *et al.,* 2022). Surface and interface tension, which may reach levels below 30, is connected to effectiveness (Moutinho *et al.,* 2021).

2.1 Glyclolipds

For use in biotechnology, glycolipid biosurfactants have gained a lot of interest (Mnif *et al.,* 2018). Glycolipids A carbohydrate moiety is joined to a fatty acid chain to form glycolipid molecules. The Rhamnolipids, trehalolipids, mannosylerythritol lipids (MELs), and cellobiose lipids are examples of the class of biosurfactants. It has been demonstrated that several glycolipids can create holes and weaken cellular

membranes. The antibacterial, antifungal, anticancer, and anti-biofilm properties of glycolipid biosurfactants have been investigated. (Paraszkiewicz *et al.,* 2021). Because they contain both hydrophilic glycosyl and lipophilic lipid residues, simple glycolipids are amphiphilic molecules (Abdel-Mawgoud and Stephanopoulos 2018). Certain antiviral, antibacterial and antifungal properties are exhibited by glycolipid surfactants (Jezierska *et al.,* 2018). The fermentation conditions, strain selection, culture media and growth conditions are what led to the various structures of glycolipid biosurfactants (Dardouri *et al.,* 2021, Sekhar *et al.,* 2018). Studies have been conducted where the glycolipid can be used as a feasible bioplastic (Fukuoka *et al.,* 2018).

2.2 Rhamnolipids

Rhamnolipids are equally stable and effective in emulsifying as the widely used anionic surfactant sodium dodecyl sulphate (Salek *et al.,* 2022). They are extracellular secondary metabolites that are released by different Pseudomonas strains, primarily by the opportunistic pathogen *Pseudomonas aeruginosa*. These bacteria use them at different phases of the biofilm-building process. Rhamnolipids are useful in environmental technology, especially in water and soil remediation procedures, because they can remove different organic and inorganic contaminants more quickly. On the other hand, have anti-adhesive and disruptive properties when it comes to biofilms produced by certain pathogenic microbes (Paraszkiewicz *et al.,* 2021). Biosurfactants produced by *Klebsiella* species are identified to be monorhamnolipid (Ahmad 2021). Due to its adverse effects, severe foaming is not experienced while rhamnolipid undergoes fermentation (Gong *et al.,* 2021). Rhamnolipid biocomplex are biosynthesized by *Pseudomonas* species which is cheaper and environmental friendly and acts as an alternative for purified rhamnolipids (Kłosowska-Chomiczewska *et al.,* 2021).

2.3 Sophorolipds

Two glucose rings connected by a 1-2 glycosidic bond make form the sophorose polar group of the glycolipid known as sophorolipds, which also has a hydroxylated fatty acid lipid tail (Salek *et al.,* 2022). Sophorolipids are intriguing substitutes for

surfactants made of petrochemicals due to all these characteristics. They have little foaming, quick wetting,and low toxicity, as well as strong surface activity (Liwarska-Bizukojc *et al.,* 2018). Candida bombicola and C. apicola are two examples of non-pathogenic yeast that produce sophorolipids, which are biosurfactants that include the fatty acid and the sugar sophorose bound together. Examined the antimicrobial qualities and biofilm disruption activity of sophorolipid biosurfactants against both Gram-negative and Gram-positive microorganisms (Paraszkiewicz *et al.,* 2021).

2.4 Lipopeptides

A physically varied family of extracellular compounds produced by bacteria and fungi is called lipopeptides. The most widely used lipopeptide biosurfactants include substances from the surfactin, iturin, and fengycin families generated by various Bacillus strains and Pseudomonas lipopeptides (divided into four primary groups: viscosin, amphisin, tolaasin, and syringomycin) (Paraszkiewicz *et al.,* 2021). Microorganisms that usually undergo environmental stress produce Antibiotic lipopeptides (Vazquez *et al.,* 2018). One of the most well-known lipopeptides is fengycin from *Bacillus subtilis*. *Bacillus*-related lipopeptides, *Pseudomonas*-related lipopeptides, other bacterial-related lipopeptides, *actinomycete*-related lipopeptides, and fungal-related lipopeptides are the different categories of lipopeptides (Mnif and Ghribi 2015).

3. Properties of biosurfactant

Numerous prokaryotic and eukaryotic microorganisms produce biosurfactants, which are molecules that lower surface and interfacial tension and are either produced extracellularly or linked to compounds that are associated with cells. It is not only possible for biosurfactants to form water-in-oil and oil-in-water emulsions, but also dehydrate emulsions, which is a promising technology in businesses that depend on petroleum (Najmi *et al.,* 2018). The asymmetric structure of surfactant molecules causes them to adsorb micelles (Zdziennicka *et al.,* 2018). When we modify their metabolic pathway through rational design, their structures and

characteristics may also be changed. Thus, new products with a certain profile can be created (Vazquez *et al.,* 2018).

3.1 Physicochemical Properties of Biosurfactants

For biosurfactants to be successfully used in practice, one must be aware of their physicochemical characteristics (Jahan *et al.,* 2020). The Fatty Acid chain length and the isomerism are known to affect the physicochemical property of biosurfactants (Hu *et al.,* 2019). The values of the tail and head's surface tension (a macroscopic characteristic) and the size of specific molecular components (a microscopic property) determine the surfactant's propensity to form micelles at a critical concentration (CMC) and adsorb at the water-air and soil-water interfaces (Zdziennicka *et al.,* 2018). They are typically divided into low and high-atomic-weight surfactants based on their synthetic approach and sub-atomic weight (Abbot *et al.,* 2022). In addition, the physicochemical properties of the support must be chosen by the desired reaction because they can affect the efficiency of the enzyme. It is hypothesized that an increasing amount in the rate of reaction conversions was caused by a decrease in the hydrophilicity of the support (Zago *et al.,* 2021).

3.2 Surface and Interfacial Tension

One of the most crucial characteristics of amphiphilic substances is their capacity to lower surface and interfacial tension. For example, this is necessary for the development of kinetically stabilized emulsions. Due to their dual hydrophobic-hydrophilic nature, amphiphilic compounds, such as biosurfactants, adsorb at interfaces. Surfactant molecules efficiently lower intermolecular interactions between solvent molecules when they replace water or oil molecules at the contact, hence reducing surface or interfacial tension (Jahan *et al.,* 2020). Surfactants are added to the solution to lower the surface tension of the (air/water) and (oil/water) interfacial tension, which promotes the deposition of solid phase (adsorption state) oil contaminants and enhances their ability to migrate into the aqueous phase or their contact efficiency with aqueous phase remediation agents of microorganisms (Liu *et al.,* 2019). Surfactin was able to roughly 2.5 times lower the water surface tension even at low concentrations. (Iglesias-Fernández, *et al.,* 2015).

3.3 Air-water interface

With the hydrocarbon chain facing air and the hydrophilic groups towards bulk water, the air-water surface tension measures how tightly the surfactants are packed at the air-water interface. The air-water interface configuration causes a low degree of surfactin immersion in the aqueous phase, making it a more hydrophobic nanoparticle and enabling it to create a very compact surface layer that is denser than that of ordinary amphiles (Otzen 2017). Hydrophobic chains group together to avoid water molecules, whereas surfactin molecules tend to assemble near the interface with polar residues facing the water phase. The peptidic residues of surfactin are typically found parallel to the water/air boundary, and the flexibility end of the fatty acyl chains (Iglesias-Fernández, *et al.,* 2015). At the air-water interface, the surface adsorption characteristics of saponin, and escin are been studied using neutron reflectivity and surface tension (Tucker *et al.,* 2020).

3.4 Antibiofilm property

The way that bacteria express themselves can change in response to their environment. The ability of bacteria to carry out quorum-sensing activity, this capability is related to the formation of biofilms (Abbot *et al.,* 2022). Biofilm production has been seen to be inhibited by biosurfactants. They can change the surface's physicochemical characteristics to lessen adhesion (Janek *et al.,* 2012). Gram-negative bacteria (*Salmonella enteritids*), Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus pumilus* and *Listeria monocyotgens*) and fungal strains (*Yarrowia lipolytica*) possess antimicrobial and antibiofilm properties (Khalid *et al.,* 2019). Biosurfactants have gained interest in the therapeutic and sanitary domains due to their biodegradable nature, low cytotoxicity, anti-microbial and antibiotic activities, and capacity to dissolve microbial biofilms. (Cheffi *et al.,* 2021). Because of their inherent surface activity and the possibility that they could be used to prevent the formation of biofilms, biosurfactants have antibacterial, antibiofilm, and antiadhesive properties (Abdollahi *et al.,* 2020). Microbial surfactant have received more attention recently (Giri *et al.,* 2020)

4. Application

Biosurfactants have multiple uses in a variety of industries, including agriculture, biomedicine, construction, the pulp and paper sector, metal, textile, pharmaceutical, and cosmetics (Moutinho *et al.,* 2021).(Figure 1)

4.1 Cosmetic industry

Biosurfactants are essential to the beauty sector because of their high-added-value products, high specificity, and skin compatibility. Given their significant qualities, including detergency, foaming, wetting, emulsifying, solubilizing, and dispersing, biosurfactants are widely wanted for usage in cosmetics. Because of its physicochemical properties, biological activity, and biocompatibility, the glycolipids group, which comprises the rhamnolipids, sophorolipids, and mannosylerythritol lipids, is the largest and most diversified biosurfactant group used in the cosmetic and personal care industry. (Moutinho *et al.,* 2021). The choice of a biosurfactant for a definite cosmetic product is a delicate task that depends on several factors based on the requirement of the product (Bezerra *et al.,* 2018).

4.2 Petroleum industry

Biosurfactants are essential in the petroleum industry as well. For instance, the Microbial Enhanced Oil Recovery (MEOR) technology employs the addition of microorganisms to the reservoir rock that are then stimulated to generate polymers and surfactants to lower the surface tension of the oil rock and enable the transportation of the oil through the rock's pores (Moutinho *et al.,* 2021) Additionally, biosurfactants lessen the viscosity of crude oil residues that have been left behind and deposited at the bottom of oil storage reservoirs (Moutinho *et al.,* 2021).

4.3 Medical Industry

The number of potential applications for biosurfactants in the medical sector has increased significantly during the last ten years. Several biosurfactants were shown to have biological properties, including antibacterial, antifungal, antiviral, anticancer activity, suppression of clot formation and hemolysis, anti-adhesiveness, and

creation of ion channels in membranes, which encouraged their usage in the biomedical industry (Jahan *et al.,* 2020).

4.4 Other Applications

The marine and shipping industries suffer severe issues and financial losses as a result of biofouling. Marine bacteria with the potential to produce biosurfactants can be a great choice when looking for new antifouling agents because they have the amphipathic surface-active property that confers antibacterial and antibiofilm actions (Alemán-Vega *et al.,* 2020). In food industries these factors play an advantageous role where the biosurfactants possess specific resistance to variations in temperature, acidity and salinity, and allow biomolecules to maintain their original characteristics, This may have a favourable impact on the end product's quality (Ribeiro *et al.,* 2020).

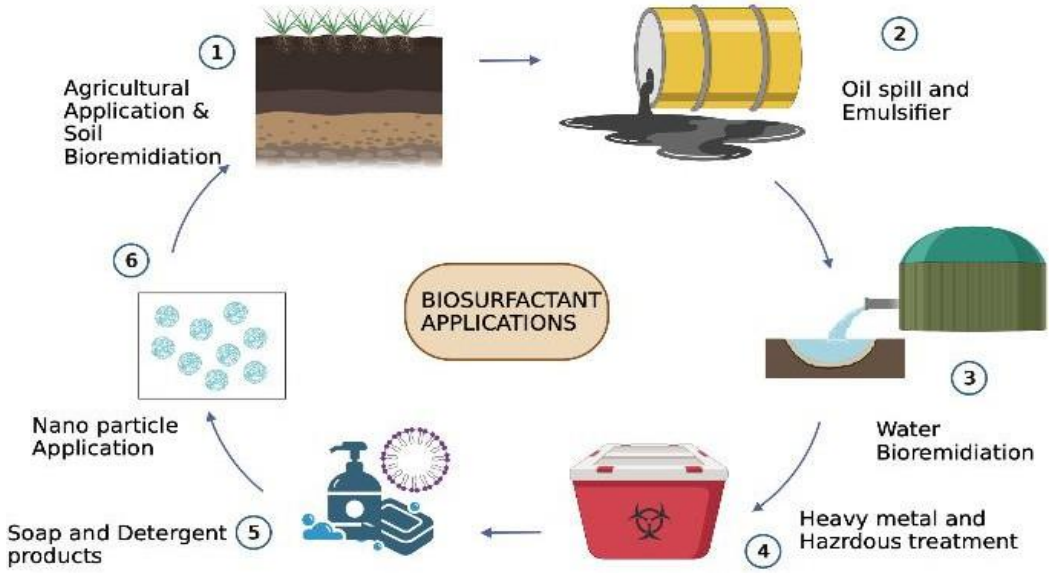

Figure 1: The various industrial application in which biosurfactants are being applied in the recent decades (Mohanty et. al 2021, Mishra et al.,2021, Domínguez *et al.,* 2019).

5. Sources of biosurfactant

The Microbial biosurfactants are been derived from various species, though there is n number of variants these are a few of the species that are been listed (Table 1) (Moutinho *et al.,* 2021). *Planococcus* a gram-positive bacteria is a pioneer marine resource for biosurfactant production and even other secondary metabolites *(*Waghmode *et al.,* 2020). Several other phyla like Actinobacteria, Firmicutes, Proteobacteria, Ascomycota, and Basidiomycota have been utilized for the production of diverse biosurfactants (Alemán-Vega *et al.,* 2020). Pony Lake from Ross Island, Antarctica is a source for the *Psychrobacter arcticus* Strain that is used to isolate biosurfactants The Cotton Glacier stream in Victoria Land rich source of *Janthinobacterium svalbardens* which come under the classes of sophorolipids and di-rhamnolipids (Trudgeon *et al.,* 2020).

Table 1: The table lists some of the strains from which the specific biosurfactants are been derived (Moutinho *et al.,* 2021, Malviya *et al.,* 2020).

S.No	Source Organism	Lipopeptide Class
1	*Actinoplanes friuliensis*	Friulimicin
2	*Arthrobacter* spp. MIS38	Arthrofactin
3	*Bacillus subtilis*	Iturin A, Bacillomycin
4	*B. subtilis* HC8	Surfactin, Fengycin A
5	*B. subtilis* K1	Fengycin A , and B, Fengycin A2
6	*B. subtilis* GA1	Iturins, Fengycins, Surfactins
7	*B. subtilis* and *B. amyloliquefaciens*	Surfactins, Bacillomycin
8	*Pseudomonas chlororaphis, Pseudomonas putida and Burkholderia thailandensis*	Glycolipid
9	*Bacillus pumilus, Bacillus subtilis*3, *Brevibacilis brevis*	Lipopeptide

Researchers have discovered and isolated biosurfactant-producing bacteria from various marine environments of the Canadian Arctic; they noted that the most common species were of the genus *Rhodococcus*, followed by *Bacillus* and antibiofilm activities and biosurfactants at 4 °C by the genera *Pseudomonas*,

Pseudoalteromonas and *Rhodococcus*, were collected from Antarctic and Arctic polar environments (Schultz and Rosado 2020). Studies have concluded the antimicrobial activity of biosurfactant extract that was obtained from the enduring stream of the corn-milling industry (Rodríguez-López *et al.,* 2020). The lactic acid bacteria such as *Lactobacillus agilis*, *Lactobacillus Plantarum*, *Lactobacillus paracasei*, and *Lactobacillus pentosus* are the most researched glycolipopeptides and glycopeptide microbial biosurfactants (Moldes *et al.,* 2020). *Serratia* species have been shown to create two kinds of biosurfactants, namely lipopeptides and glycolipids. These species include *Serratia marcescens, Serratia rubidaea, and Serratia surfactantfaciens* (Clements *et al.,* 2019).

6. Agriculture impacts of biosurfactants

6.1 Anti-phytopathogenic activity

Sophorolipids, cellobiose lipids, and mannosyl-erythritol-lipids are involved in plant protection by inhibiting the growth of phytopathogenic fungi. Sclerotinia sclerotiorum and Phomopsis helianthi are phytopathogenic fungi that are inhibited from growing by the cellobiose lipid of Pseudozyma fusiformata and Cryptococcus humicola. The phytopathogenic fungus Sphaerotheca fuliginea was resistant to the antifungal action of cellobiose lipids produced by Pseudozyma flocculus (Mnif and Ghribi 2016). Because they can be produced at scale for commercial purposes, have low toxicity, and have a high biodegradability, Biosurfactants are promising compounds for a variety of uses that are created by bacteria, yeast, and fungi. (Crouzet *et al.,* 2020).

6.2 Plant Defence

Huge economic losses come from plant infections that cause significant agricultural damage, that range from 10-40% depending on the crops before and after harvest (Savary *et al.,* 2019). However, to overcome this scenario chemical pesticides were in implementation. However, they have the potential to be harmful to both human and environmental health, which has prompted the creation and improvement of alternate crop protection measures. (Berg *et al.,* 2017). Recent research has also

demonstrated that some rhamnolipids can protect plants from phytopathogenic fungi and bacteria through the activation of the plant immune system. It has been shown that P. aeruginosa rhamnolipid activates defence genes in Arabidopsis thaliana, wheat, and tobacco (Mnif and Ghribi 2016). The area of biomedicine and agriculture may be able to use rhamnolipids as possible antimicrobials, immunological modulators, virulence factors, and anticancer agents to help fulfil the growing need for pharmacological therapy and food safety in the coming years (Chen *et al.,* 2017). Because of their potential to generate pores in pathogens, siderophore action, biofilm inhibition, and dislodging activity, as well as their antiviral and other activities, lipopeptides have several applications in plant protection. Lipopeptide-containing microorganisms are effective biocontrol agents. Investigating these antimicrobial substances may open up new avenues for biological pest management of established and newly emergent plant diseases (Malviya *et al.,* 2020). One of the safer green alternatives for the chemical pesticide is mannosylerythritol lipids (Matosinhos *et al.,* 2022).

Various pathogens infect *Capsicum* spp (Pepper) which is an important spice, this contributes to economic losses on a global range. Where the *Cucumber mosaic virus* (CMV) is the most destructive pathogen (Jones 2016, Mandadi and Scholthof 2013). We face limitations in which the commercially available CMV plants that have been developed by breeding technologies and transgenic method face time-limited and environmental issues (Khalid *et al.,* 2017). This strain (biocontrol), *Bacillus amyloliquefaciens* PPL exhibits various useful properties, such as antibacterial and antifungal activities against various plant pathogens, also including *Colletotrichum gloeosporioides, Phytophthora capsici, Rhizoctonia solani*, and *Fusarium oxysporum* that also fight against the CMV (Kang *et al.,* 2019). *Bacillus* species that produce cyclic lipopeptides have been reported to exhibit antiviral and antifungal activity in plants (Kang *et al.,* 2021).

Sheath blight (ShB) of rice is a pathogenic disease, caused by Rhizoctonia solani, which obligates significant yield losses globally. Currently, chemical fungicides are used to control the disease. (Kumar *et al.,* 2011). Endophytic bacteria have been widely used to produce potent biocontrol agents as they elicit antagonism at the site of infection. Some of the endophytic bacteria strains are endophytic diazotrophic,

and *Bacillus* subtilis under gnotobiotic conditions can suppress the ShB in rice (Shabanamol *et al.,* 2017). Biosurfactants can penetrate and harm fungal cell membranes, which reduces the likelihood that they will develop resistance, compared to traditional antibacterial treatments or insecticides (Choub *et al.,* 2021).

Banded leaf and sheath blight is a plant disease, caused by *Rhizoctonia solani*, where the infection restricts the crop output in climatic situations critically during the monsoons in India (Singh *et al.,* 2020). An economically significant crop, pepper (Capsicum annuum L.), is subject to several illnesses. Pathogens of the genus *Colletotrichum* can cause severe yield loss (Park *et al.,* 2022). Bacillus *subtilis* and Bacillus *amyloliquefaciens* strains have reportedly been particularly successful in treating several soil-borne plant illnesses. (Borriss *et al.,* 2011, Liu *et al.,* 2019).

6.3 Plant Growth Promotion

Organic and inorganic contaminants that cause abiotic stress in crop plants have an impact on the productivity of agricultural land. Bioremediation is necessary to improve the condition of the soil that has been polluted with hydrocarbons and heavy metals. Biosurfactants produced by microorganisms and/or biosurfactants can be utilised to remove heavy metals and hydrocarbons from a solution (Sachdev *et al.,* 2013). The majority of the bactericides in use today are persistent organic compounds, which pose a threat to both human and environmental health (López-Prieto *et al.,* 2019). Alkyl polyglucosides (APG), which are generated from plants, have been demonstrated to be natural biosurfactants that are useful in bovine nutrition due to their favourable effects on physiological and production parameters in, for example, ruminants. Increased duodenal microbial nitrogen flux results from improved ruminal and intestinal organic matter digestion and ruminal microbial protein synthesis (Naughton *et al.,* 2019). Many rhizosphere-dwelling microorganisms have the potential to promote plant growth; as a result, they are also known as plant growth-promoting rhizobacteria or plant growth-promoting bacteria (PGPB). (Ahmad *et al.,* 2018). *Rhodococcus erythropolis* other to the genera *Rahnella, Serratia and Proteus* where some of these bacteria exhibit features of plant growth-promoting bacteria which increase the biomass of plants under several mechanisms (Pacwa-Płociniczak *et al.,* 2016). Lipopeptides from

Bacillus subtilis under low-cost production exhibit a great stability range of pH (1–11), salinity (1–8%), and temperature (20–121°C) even after autoclaving. Which acts as a potent plant growth-promoting agent that significantly increases seed germination and plant growth promotion of chilli pepper, lettuce, tomato, and pea maximum with maximum concentration added to the soil (Umar *et al.,* 2020). Streptomyces show traits of plant growth initiation of chilli under greenhouse conditions, whereas *Streptomyces puniceus, and Streptomyces median* showed significant biosurfactant and plant growth-promoting activity (Ravinder *et al.,* 2022). Biosurfactant-producing Pseudomonas sp. that utilize petroleum as a carbon source showed great plant growth potential in plants under various petroleum concentrations with high values of high values for all the parameters studied namely germination, shoot length, root length, fresh and dry weight and pigments (Das *et al.,* 2016).

7. Conclusion

There are several uses for surfactants in the agrochemical and agricultural sectors. Biosurfactants, which are more environmentally friendly, are only sometimes used. The precise role of surfactants in assisting other systems as biocontrol agents is still poorly known and calls for further research. These investigations will aid in the transition to eco-friendly surfactants from harsh chemical ones. Working on the manufacturing cost of green surfactants is required if the use of biosurfactants in agriculture and other industries is to yield a net economic gain. More serious consideration is also needed for the overproduction of biosurfactants from agricultural waste. By altering the production process, the chemical compositions of biosurfactants that have been identified as powerful biocontrol agents can be changed. This strategy could result in the biosynthesis of highly targeted green surfactants.

7. Reference

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