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Biosurfactant: A potent antimicrobial agent

Abstract

Surfactant is an important class of chemicals with wide domestic and industrial application. Synthetic surfactant, however, used to increase the contaminant solubility are often toxic, representing an additional source of contamination. Microbially produced surface active compounds, i.e., biosurfactants are less toxic, biodegradable and have potential uses in large oil recovery operations, removal of oil spills, biodegradation of hydrocarbons from the soil, production of detergent, agro industries, manufacture of pharmaceutical products, etc. One of potential areas in which the usefulness of biosurfactants has been studied is its antimicrobial activity, which is catching good attention worldwide. The biosurfactants acts as an effective antimicrobial agent against a number of pathogenic organisms. In the present research work, a systematic isolation and screening program was under taken for obtaining biosurfactant producing bacteria. After sampling in mineral salt medium, a total of 31 isolates were screened from the samples collected from Uran sea coast, Mumbai and local Garage and Petrol pump. After primary and secondary screening procedures like hemolysis, emulsification index, oil spread technique and surface tension measurement, 2 isolates, namely 21Y and 14X were selected.

The emulsification index for the 2 isolates was 100% in petrol and diesel in 24 hrs. The isolates reduced surface tension of the MS medium as follows: 21Y: 52.5 Dynes/cm to 39.1 Dynes/cm and 14X: 51.9 Dynes/cm to 41.2 Dynes/cm in a period of 96 hrs. The MS medium was optimized by Plackett- Burman design for the 2 isolates. The biosurfactants was purified by acid precipitation method. The antimicrobial activity tested for the purified surfactant was effective against *E. coli* (NCIM 2065), *B. subtilis* (NCIM 2063), and *S. aureus* (NCIM 5021).

Keywords: biosurfactants, surface tension, antimicrobial activity

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Introduction

A surfactant is soluble compound that reduces surface tension of liquids, or reduces the interfacial tension between two liquids or a liquid and a solid. Surfactants are amphipathic molecules with both hydrophilic and hydrophobic moieties that partition preferentially at the interface between fluid phase with different degrees of polarity and hydrogen bonding such as oil/water or air/water interfaces. These properties render surfactants capable of reducing surface and interfacial tension and forming micro emulsion where hydrocarbons can solubilize in water or water can solubilize in hydrocarbons. Such a characteristic confer excellent detergency, emulsification, foaming, and dispersing traits, which make surfactants some of the most versatile process chemicals. Synthetic surfactants appear relatively recently; there unique properties revolutionize both housekeeping and industry. Synthetic surfactants are environmentally hazardous.

Biosurfactant

However, microbially produced surfactants, i.e. biosurfactants are less toxic, less allergenic, biodegradable, and therefore less detrimental to the environment. They can be produced from various substrates, including industrial waste. They are diverse in chemical structure and properties as the producer microbes. Another reason for the search for biological surfactant is depletion of oil resources required for production of synthetic surfactants. This makes microbial surfactants even more promising.

Advantages: There are many advantages of biosurfactants compared to their chemically synthesized counterparts, including the following:

i. Biodegradability.
ii. Generally low toxicity.
iii. Biocompatibility and digestivity, which allows their application in cosmetics, pharmaceuticals and as a functional food additives.
iv. Availability of raw materials: biosurfactants can be produced from cheap raw materials, which are available in large quantities. The carbon source may come from hydrocarbons, carbohydrates, and/or lipids, which may be used separately or in combination with each other.
v. Acceptable production of production economics: depending on the application, biosurfactants can also be produced from industrial waste and byproducts and this is of particular interest for bulk production.
vi. Use in environmental control: biosurfactants can be effectively used in handling industrial emulsions, control of oil spills, biodegradation, and detoxification or industrial effluents, and bioremediation of contaminated soil.
vii. Specificity: Biosurfactants, because they are complex organic molecules with specific functional groups are often specific in their action. This is of particular interest in detoxification of specific pollutants, de-emulsification of industrial emulsions, and specific cosmetic, pharmaceuticals, and food applications.
viii. Effectiveness at extreme temperature, pH, and salinity.

Their properties of interest regard changing surface active phenomenon such as lowering of surface and interfacial tensions, wetting and penetrating actions, spreading, hydrophilicity and hydrophobicity, emulsification and de-emulsification, detergency, gelling, foaming, flocculating actions, microbial growth enhancement, metal sequestration, and antimicrobial action. In any heterogeneous system, boundaries are of fundamental importance to the behavior of the system as a whole. It is therefore not surprising that microorganism having large surface-to-volume ratio, produce a variety of surface active agents, biosurfactants shortly. Biosurfactants alter the conditions prevailing at interfaces. They interact with interfaces. An interface is defined as a phase boundary between two phases in heterogeneous system. For all interfacial systems, it is known that organic molecules from the nonsolid phase immobilize at a solid interface. There they eventually form a film know as conditioning film, which changes the properties of original surface. Those organic molecules conditioning the surface change the wettability and surface charge on the original surface as determined via contact angle and free surface energy measurement, respectively.

Biosurfactants produced by bacteria interact with interfaces and affect the adhesion and de-adhesion of bacteria. The reason that biosurfactants concentrate at interfaces is that they are amphipathic, i.e. they contain both hydrophobic and polar groups. The hydrophobic group, usually a hydrocarbon chain, tends to be expelled by the water, while the polar group, which is hydrophilic, tends to remain in the water. This gives rise to amphipathic absorption in which the hydrophobic groups are oriented away from the water, the polar groups towards it. Biosurfactants can be divided into low molecular mass molecules, which efficiently lower the surface and interfacial tensions. Eg. Glycolipids such as trehalose lipids, sophorolipids and rhamnolipids, or lipopeptides such as surfactin, gramicidins, and polymyxin. And high molecular mass molecules which are more effective at stabilizing oil in water emulsions, i.e. bind tightly to surfaces. E.g. amphipathic polysaccharides, proteins, lipoproteins, lipids or complex mixture of these biopolymers. Chemically synthetized surfactants are usually classified according to the nature of the polar group – cationic, anionic, and non-anionic.

Microbiologically derived surfactants are more conveniently discussed in terms of:

1. The biochemical nature of the surfactants, protein, lipids, polysaccharides, or complexes containing 2 or more types of biomolecules and
2. The producing microbial species.

There are number of methods that measure directly or indirectly the surface activity of biosurfactant like blood agar hemolysis, oil spread technique, drop collapse assay, glass slide test, emulsification index, surface tension measurement (Tables 1–4) (Figure 1). Out of these we have selected the following screening tests:

i. Blood agar hemolysis
ii. Oil spread technique
iii. Emulsification index
iv. Surface tension measurement.

Table 1 Results of Blood agar hemolysis

Isolates	Blood hemolysis	Result
7 Z II	Positive	++
14 X	Positive	++++
14 Z I	Positive	+
21 Y	Positive	++++
28 Z I	Positive	+++

The zone of clearance was scored as follows:

-, No hemolysis; ++ Incomplete hemolysis; +++ Complete hemolysis; ++++, Complete hemolysis (diameter above 10mm).

Table 2 Results of Oil spread technique

Isolates	Observations after 24 hrs.
7 Z II	+
14 X	++
14 Z I	+
21 Y	++
28 Z I	++

+ > 3, seconds; ++, Within 2 seconds.

Table 3 Results of Emulsification index

Isolates	EI-24 in petrol (48 hrs)	EI-24 in diesel (48 hrs)
7 Z II	100%	100%
14 X	100%	100%
14 Z I	100%	100%
21 Y	100%	100%
28 Z I	20%	19%

Table 4 Results of Surface tension measurement

Isolates	ST of cell free broth (dynes/cm)	ST of broth (dynes/cm)			
		24 hrs	48 hrs	72 hrs	96 hrs
7 Z II	61.3	56.1	51.8	51.3	51
14 X	61.3	51.9	49.1	42.3	41.2
14 Z I	61.3	53.4	45.4	44.2	44
21 Y	61.3	52.5	41.2	38.6	35.1
28 Z I	61.3	53.6	53.2	45.4	44.4

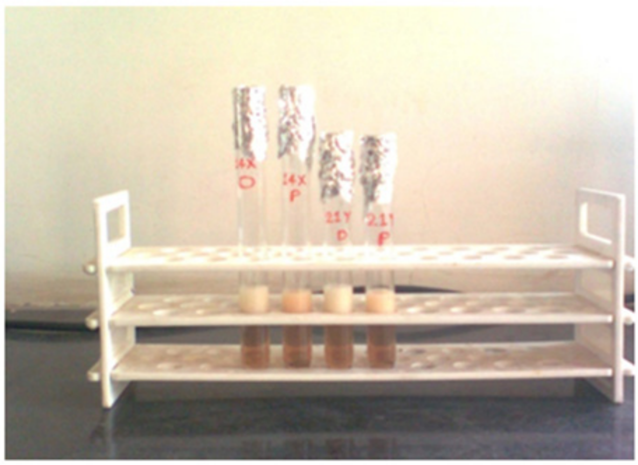

Figure 1 Emulsification Index of 21 Y and 14 X isolates in Diesel (D) and Petrol (P).

Antimicrobial activity

Microbial compounds that exhibit pronounced surface and emulsifying activities are classified as biosurfactants. Biosurfactants comprise a wide range of chemical structures such as glycolipids, lipopeptides, polysaccharide–protein complexes, phospholipids, fatty acids and neutral lipids. It is therefore, reasonable to expect diverse properties and physiological functions for different groups of biosurfactants. Moreover, these molecules can be tailor-made to suit different applications by changing the growth substrate or

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growth conditions. Although most biosurfactants are considered to be secondary metabolites, some may play essential roles for the survival of biosurfactant-producing microorganisms through facilitating nutrient transport or microbe–host interactions or by acting as biocide agents. Biosurfactant roles include increasing the surface area and bioavailability of hydrophobic water-insoluble substrates, heavy metal binding, bacterial pathogenesis, and quorum sensing and biofilm formation. Most work on biosurfactant applications has been focused on their use in environmental applications owing to their diversity, environmentally friendly nature, suitability for large-scale production and selectivity. Despite their potential and biological origin only a few studies have been carried out on applications related to the biomedical field. Some biosurfactants are suitable alternatives to synthetic medicines and antimicrobial agents and may be used as safe and effective therapeutic agents. The use and potential commercial application of biosurfactants in the medical field has increased during the past decade. Their antibacterial, antifungal and antiviral activities make them relevant molecules for applications in combating many diseases and as therapeutic agents. In addition, their role as anti-adhesive agents against several pathogens indicates their utility as suitable anti-adhesive coating agents for medical insertional materials leading to a reduction in a large number of hospital infections without the use of synthetic drugs and chemicals. Microbial surfactants have several advantages over chemical surfactants such as lower toxicity; higher biodegradability and effectiveness at extreme temperatures or pH values. Many of the potential applications that have been considered for biosurfactants depend on whether they can be produced economically; however, much effort in process optimization and at the engineering and biological levels have been carried out. Biosurfactant production from inexpensive waste substrates, which decreases their production cost, has been reported. In addition, legal aspects such as stricter regulations concerning environmental pollution by industrial activities and health regulations will also strongly influence the chances of biodegradable biosurfactants replacing their chemical counterparts.

Biosurfactants are microbial amphiphilic polymers and polyphilic polymers that tend to interact with the phase boundary between two phases in a heterogeneous system, defined as the interface. For all interfacial systems, it is known that organic molecules from the aqueous phase tend to immobilize at the solid interface. There they eventually form a film known as a conditioning film, which will change the properties (wettability and surface energy) of the original surface. In an analogy to organic conditioning films, Biosurfactants may interact with the interfaces and affect the adhesion and detachment of bacteria. In addition, the substratum surface properties determine the composition and orientation of the molecules conditioning the surface during the first hour of exposure. After about 4 hrs, a certain degree of uniformity is reached and the composition of the adsorbed material becomes substratum independent.

Owing to the amphiphilic nature of biosurfactants, not only hydrophobic but a range of interactions are involved in the possible adsorption of charged biosurfactants to interfaces. Most natural interfaces have an overall negative or, rarely, positive charge. Thus, the ionic conditions and the pH are important parameters if interactions of ionic biosurfactants with interfaces are to be investigated. Gottenbos et al.,[1] demonstrated that positively charged biomaterial surfaces exert an antimicrobial effect on adhering Gram-negative bacteria, but not on Gram-positive bacteria. In addition, the molecular structure of a surfactant will influence its behavior at interfaces. In describing the surface-active approach, an effort is made to elaborate on the possible theoretical locations and orientations of the biosurfactants. Nevertheless, it must be kept in mind that the situation in natural systems is far more complex and requires the consideration of many additional parameters. As described above, a broad range of chemical structures, such as glycolipids, lipopeptides, polysaccharide–protein complexes, phospholipids, fatty acids and neutral lipids, have been attributed to biosurfactants. Some of these biosurfactants were described for their potential to act as biologically active compounds and applicability in the medical field.

Lipopeptides

Among the several categories of biosurfactants, lipopeptides are particularly interesting because of their high surface activities and antibiotic potential. Lipopeptides can act as antibiotics, antiviral and anti-tumour agents, immunomodulators or specific toxins and enzyme inhibitors. Ahimou et al. reported that lipopeptides profile and bacterial hydrophobicity vary greatly with the strains. Nielsen et al. reported viscosinamide, a cyclic depsipeptide, to be a new antifungal surface-active agent produced by *Pseudomonas fluorescens*, with different properties compared with the biosurfactant viscosin, known to be produced from the same species and shown to have antibiotic activity. Massetolides A–H, also cyclic depsipeptides were isolated from the *Pseudomonas* species, derived from a marine habitat, and found to exhibit in vitro antimicrobial activity against *Mycobacterium tuberculosis* and *Mycobacterium avium-intracellulare*.

Biosurfactants have been found to inhibit the adhesion of pathogenic organisms to solid surfaces or to infection sites; thus, prior adhesion of biosurfactants to solid surfaces might constitute a new and effective means of combating colonization by pathogenic microorganisms. Pre-coating vinyl urethral catheters by running the surfactin solution through them before inoculation with media resulted in a decrease in the amount of biofilm formed by *Salmonella* typhimurium, Salmonella enterica, *E. coli* and *Proteus mirabilis*. Given the importance of opportunistic infections with *Salmonella* species, including urinary tract infections of AIDS patients, these results have great potential for practical applications. In addition, the use of lactobacilli as a probiotic for the prevention of urogenital infections has been widely studied. The role of *Lactobacillus* species in the female urogenital tract as a barrier to infection is of considerable interest. These organisms are believed to contribute to the control of vaginal microbiota by competing with other microorganisms for adherence to epithelial cells and by producing biosurfactants.

Recently the authors also demonstrated that when rinsing flow chambers, designed to monitor microbial adhesion, with a rhamnolipid biosurfactant-containing solution the rate of deposition and adhesion was significantly reduced for a variety of bacterial and yeast strains. We believe that this rhamnolipids may be useful as a biodetergent solution for a number of hospital instruments. The role of surfactants in defence against infection and inflammation in the human body is a well-known phenomenon. The pulmonary surfactant is a lipoprotein complex synthesized and secreted by the epithelial lung cells into the extracellular space, where it lowers the surface tension at the air–liquid interface of the lung and represents a key factor against infections and inflammatory lung diseases.

Biomedical and therapeutic aspects of Biosurfactant

Some biosurfactants are a suitable alternative to synthetic medicines and antimicrobial agents and may be used as safe and effective therapeutic agents. There has been increasing interest in the effect of biosurfactants on human and animal cells and cell lines. Possible applications of biosurfactants as emulsifying agents for drug transport to the infection site, as agents supplementing the pulmonary surfactant and as adjutants for vaccines were suggested by Kosaric et al.,[2] showed that bacterial lipopeptides constitute potent non-toxic and non-pyrogenic immunological adjuvants when mixed with conventional antigens. The cosmetic and healthcare industries use large amounts of surfactants for a wide variety of products, including insect repellents, antacids, acne pads, contact lens solutions, hair color and care products, deodorants, nail care products, lipstick, eye shadow, mascara, toothpaste, denture cleaners, lubricated condoms, baby products, foot care products, antiseptics and shaving and depilatory products. Biosurfactants are known to have advantages over synthetic surfactants such as low irritancy or anti-irritating effects and compatibility with skin. Rhamnolipids in particular are being used as cosmetic additives and have been patented to make some liposomes and emulsions, both of which are important in the cosmetic industry.

A host of interesting features of biosurfactants have led to a wide range of potential applications in the medical field. They are useful as antibacterial, antifungal and antiviral agents and they also have the potential for use as major immunomodulatory molecules and adhesive agents and in vaccines and gene therapy. Biosurfactants have been used for gene transfection, as ligands for binding immunoglobulins, as adjuvants for antigens and also as inhibitors for fibrin clot formation and activators of fibrin clot lysis. Promising alternatives to produce potent biosurfactants with altered antimicrobial profiles and decreased toxicity against mammalian cells may be exploited by genetic alteration of biosurfactants. Furthermore, biosurfactants have the potential to be used asanti-adhesive biological coatings for medical insertional materials, thus reducing hospital infections and use of synthetic drugs and chemicals. They may also be incorporated into probiotic preparations to combat urogenital tract infections and pulmonary immunotherapy.

In spite of the immense potential of biosurfactants in this field, their use still remains limited, possibly due to their high production and extraction cost and lack of information on their toxicity towards human systems. Further investigations on human cells and natural microbiota are needed to validate the use of biosurfactants in several biomedical and health-related areas. Nevertheless, there appears to be great potential for their use in the medical science arena waiting to be fully exploited. The two isolates namely, 21 Y and 14 X, were grown in fresh sterile MS medium incubated for 24 hours at 37°C. Using disc diffusion technique, each isolate was tested for its antimicrobial activity against few test organisms procured from National Centre for Industrial Microorganisms, Pune, India. The other isolates selected from screening methods were also tested for their antimicrobial activity against *Proteus* species. This was done because though the other isolates were proved to be poorly responding to the screening methods, they showed a good antimicrobial activity.

Materials and methods

Materials

Sterile nutrient agar butts

Sterile filter paper discs

Sterile Petri plates

Suspension of the test organisms - *Staphylococcus aureus, E. coli, Bacillus* subtilis

Method

Disc diffusion technique was performed.

1. The MSM inoculated with the isolates (14X and 21Y) was centrifuged at 8000rpm for 20min and the supernatant was assessed for its antimicrobial activity against the said pathogens.
2. The suspension of the test organism was inoculated in sterile nutrient agar butts and mixed properly.
3. The butts were poured onto the sterile empty plates and allowed to solidify.
4. On solidifying, the sterile discs were dipped in the supernatant and were placed aseptically on the solidified agar.
5. Plates were kept for diffusion at 4°C for 30min.
6. After diffusion the plates were incubated at 37°C for 24hrs.

Observation

After 24 hrs of incubation, zones of clearance were observed around the discs (Figures 2–4).

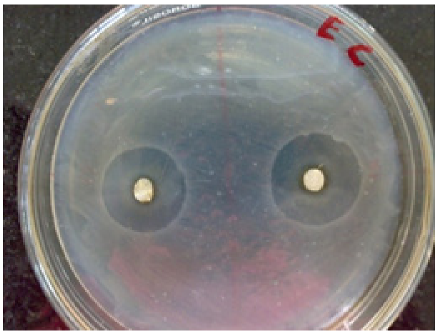

Figure 2 Left: 21Y culture (1.2mm) Right: 14 X (1.6mm) culture against *E. coli*.

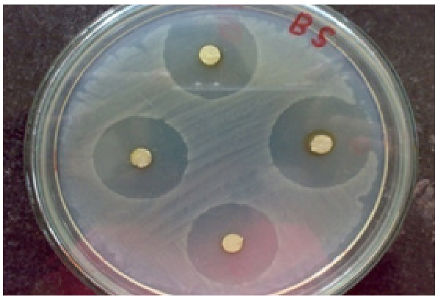

Figure 3 Horizontal: 21 Y culture (1.4mm) Vertical: 14 X (1.6mm) culture against *Bacillus subtilis*.

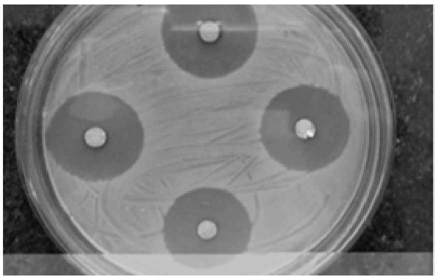

Figure 4 Horizontal: 21 Y culture (1.8mm) Vertical: 14 X (1.8mm) culture against *Staphylococcus aureus*.

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Conclusion

Biosurfactants are surfactants that are produced extracellular or as part of the cell membrane by bacteria, yeast and fungi. The main commercial use of biosurfactants is oil industry, foods, cosmetics, pharmacology and environmental technology because of their ability to stabilize emulsions. The antimicrobial activity of biosurfactants has not been reviewed extensively. The present study shows that biosurfactants, when purified, are a suitable alternative to synthetic medicines and antimicrobial agents and may be used as safe and effective therapeutic agents. The isolated biosurfactant showed the emulsification index as 100% in petrol and diesel in 24 hrs for the 2 isolates. The isolated biosurfactant from 21Y reduced surface tension of the MS medium from 52.5 Dynes/cm to 39.1 Dynes/cm and from 14X isolate the surface tension was reduced from 51.9 Dynes/cm to 41.2 Dynes/cm in a period of 96 hrs. The zones of inhibition were assessed for each of the isolate against the three pathogens. The two isolates were identified by routine biochemical tests and were found to be similar to *Pseudomonas aeruginosa*. It was concluded that the biosurfactant obtained if purified completely may be used in medicines for topical application.

Acknowledgments

None.

Conflicts of interest

Author declares that there is no conflict of interest.

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