CHAPTER II
LITERATURE REVIEW

2.1 Screening of Potential Biosurfactant-Producing Microorganisms

Recent advances in the field of microbial surfactants are largely attributed to the development of quick, reliable methods for screening biosurfactant-producing microbes and assessing their potential. Desai *et al.* (1997) reported that the developed an axisymmetric drop shape analysis (ADSA) by profile for the assessment of potential biosurfactant-producing bacteria. In this technique, drops of culture broth are placed on a fluoroethylene-propylene surface and the profile of the droplet is determined with a contour monitor. Surface tensions are calculated from the droplet profiles by ADSA. Only biosurfactant-producing bacterial suspensions show a reduction in surface tensions. And he described a colorimetric estimation of biosurfactants based on the ability of the anionic surfactants to react with the cationic indicator to form a colored complex. Development of other simple methods include the following: (i) a rapid drop-collapsing test, in which a drop of a cell suspension is placed on an oil-coated surface, and drops containing biosurfactants collapse, whereas non-surfactant-containing drops remain stable; (ii) a direct thin-layer chromatographic technique for rapid characterization of biosurfactant-producing bacterial colonies (iii) colorimetric methods for the screening of rhamnolipid-producing and hydrocarbon-degrading bacteria, respectively; and (iv) estimation of the emulsification index value (*E*-24) by vigorously shaking culture broth samples with an equal volume of kerosene and measuring the percent emulsification after 24 hours, which is most suitable for emulsifying biosurfactants.

Youssef *et al.* (2004) compared three methods to detect biosurfactant production; drop collapse, oil spreading, and blood agar lysis. The result was that the oil spreading technique better predicted biosurfactants production than the drop collapse method. However, the blood agar lysis method is not a reliable method to detect biosurfactant production.

2.2 Estimation of Biosurfactant Activity

Biosurfactant activities can be determined by measuring the changes in surface and interfacial tensions, stabilization or destabilization of emulsions, and hydrophilic-lipophilic balance (HLB). Surface tension at the air/water and oil/water interfaces can easily be measured with a tensiometer. The surface tension of distilled water is 72 mN/m, and the addition of a surfactant lowers this value to 30 mN/m. When a surfactant is added to air/water or oil/water systems at increasing concentrations, a reduction of surface tension is observed up to a critical level, above which amphiphilic molecules associate readily to form supramolecular structures like micelles, bilayers, and vesicles. This value is known as the critical micelle concentration (CMC). CMC is defined by the solubility of a surfactant within an aqueous phase and is commonly used to measure the efficiency of a surfactant. Microbial culture broth or biosurfactants are diluted several fold, surface tension is measured for each dilution, and the CMC is calculated from this value.

An emulsion is formed when one liquid phase is dispersed as microscopic droplets in another liquid continuous phase. Biosurfactants may stabilize (emulsifiers) or destabilize (demulsifiers) the emulsion. The emulsification activity is assayed by the ability of the surfactant to generate turbidity due to suspended hydrocarbons such as a hexadecane-2-methylnaphthalene mixture (Desai *et al.*, 1988) or kerosene (Cooper *et al.*,1987), etc., in an aqueous assay system. The demulsification activity is derived by determining the effect of surfactants on a standard emulsion by using a synthetic surfactant (Rosenberg *et al.*,1986).

The HLB value indicates whether a surfactant will promote water-in-oil or oil-in-water emulsion by comparing it with surfactants with known HLB values and properties. The HLB scale can be constructed by assigning a value of 1 for oleic acid and a value of 20 for sodium oleate and using a range of mixtures of these two components in different proportions to obtain the intermediate values. Emulsifiers with HLB values less than 6 favor stabilization of water-in-oil emulsification, whereas emulsifiers with HLB values between 10 and 18 have the opposite effect and favor oil-in-water emulsification.

2.3 Biosurfactants Classification

Microbial surfactants are commonly differentiated on the basis of their biochemical nature and the microbial species producing them (Healy *et al.*, 1996). Major classes of biosurfactants include:

(1) Glycolipids type.

(2) Phospholipids type.

(3) Lipopeptides and lipoproteins type.

(4) Polymeric type.

2.3.1 Glycolipids

Glycolipids are compounds of a carbohydrate and a lipid; the linkage is by way of either an ether or an ester group. The main glycolipids which are found to occur and are most often investigated are:

(a) rhamnolipids,

(b) mycolates of mono, -di and -trisaccharides,

(c) sophorolipids.

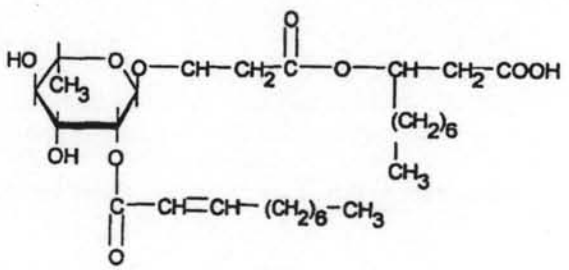

Figure 2.1 A glycolipid produced by a *Pseudomonas* strain.

2.3.2 Phospholipids

Phospholipids are the esters formed between the alcohol groups on a lipid and a phosphate.

2.3.3 Lipopeptides and Lipoproteins

Lipopeptides and lipoproteins consist of a lipid attached to a polypeptide chain.

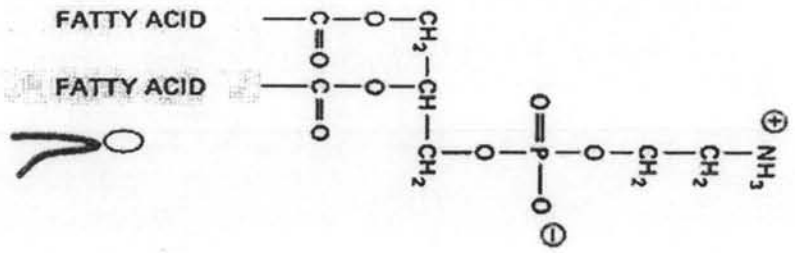

Figure 2.2 A phospholipids and Phosphatidylethanolamine produced by *Acinetobacter.*

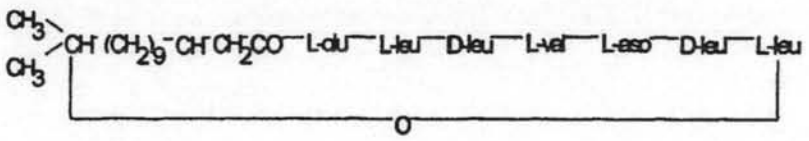

Figure 2.3 A lipopeptide structure (surfactin) produced by *Bacillus subtilis.*

2.3.4 Polymeric

Polymeric types are products again formed between saccharide units and fatty acid residues; but they are polymeric in nature.

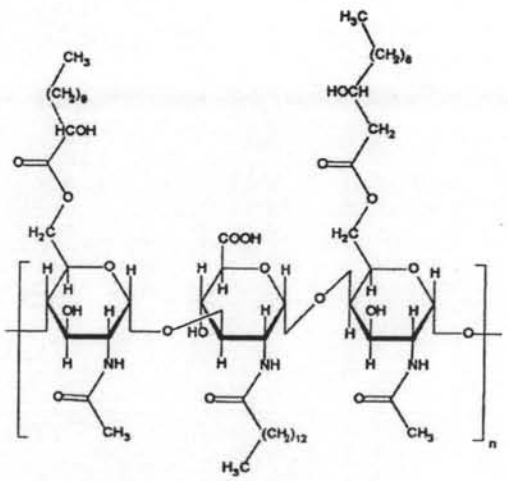

Figure 2.4 Emulsan structure (Gross *et al.,* 2001).

Bognolo *et al.* (1999) concluded that almost all biosurfactants are either non-ionic or anionic, as no studies report biosurfactants with cationic structures.

Morikawa *et al.* (2000) studied arthrofactin (AF) and surfactin (SF) and are the most effective cyclic lipopeptide biosurfactants ever reported. In this study, they reported that both AF and SF expressed higher activity under alkaline conditions than acidic conditions. AF was more resistant to acidic conditions than SF and it maintained high activity even under pH 0.5. Although SF drastically reduced its activity under acidic conditions, surfactin-Asp/Glu-amido ester and surfactin-Asp/Glu-methyl ester retained similar activities irrespective of the pH change. Also, AF with a fatty acid chain length of C10, which was the mail product of the strain, showed the highest activity.

Eliora *et al.* (2002) demonstrated bacteria make low molecular weight molecules that efficiently lower surface and interfacial tensions and high molecular weight polymers that bind tightly to the surface. Therefore, the high molecular weight biosurfactants are less effective in reducing interfacial tension, but are efficient at coating the oil droplets and preventing their coalescence.

2.4 Factors Affecting Biosurfactant Production

2.4.1 Carbon Source

Water-soluble carbon sources such as glycerol, glucose, mannitol, and ethanol were all used for rhamnolipid production by *Pseudomonas* spp. The biosurfactant product, however, was inferior to that obtained with water-immiscible substrates such as *n*-alkanes and olive oil (Robert *et al.*,1989). Desai *et al.* (1997) reported that although different carbon sources in the medium affected the composition of biosurfactant production in *Pseudomonas* spp., substrates with different chain lengths exhibited no effect on the chain lengths of fatty acid moieties in glycolipids. On the other hand, Finnerty and Singer (1985) showed evidence for qualitative variation, reflecting the carbon number of alkane for biosurfactant production in *Acinetobacter* sp. strains H13-A and H01-N.

When *Arthrobacter paraffineus* ATCC 19558 was grown on D-glucose, supplementation with hexadecane in the medium during the stationary growth phase resulted in a significant increase in biosurfactant yield (Duvnjak *et al.*,1982). Desai *et al.* (1997) reported that the presence of large amounts of biosurfactant bound to *Corynebacterium lepus* cells when grown on glucose, and addition of hexadecane facilitated the release of surfactant from the cells. Others observed little biosurfactant production when cells were growing on a readily available carbon source; only when all the soluble carbon was consumed and when water-immiscible hydrocarbon was available was biosurfactant production triggered (Banat *et al.*, 1995). Glycolipid production by *T. bombicola* is stimulated by the addition of vegetable oils during growth on a 10% D-glucose medium, giving a yield of 80 g/l (Cooper, 1986). Davila *et al.* (1992) demonstrated a high yield of sophorose lipids by overcoming product inhibition in *Candida bombicola* CBS 6009 through the addition of ethyl esters of rapeseed oil fatty acids in a D-glucose medium. Using *T. apicola* IMET 43747, Stuwer *et al.* (1987) achieved glycolipid yields as high as 90 g/l with a medium containing D-glucose and sunflower oil. In an interesting study, Lee and Kim (1993) reported that in batch culture, 37% of the carbon input was channeled to produce 80 g of sophorolipid per liter by *T. bombicola*. However, in fed-batch cultures, about 60% of the carbon input was incorporated into biosurfactant, increasing the yield to 120 g/l. Mounting evidence leads to the conclusion that the available carbon source, particularly the carbohydrate used, has a great bearing on the type of biosurfactant produced (Li *et al.*, 1984).

Fox *et al.* (2000) investigated the use of potato substrates as an alternative carbon source to generate biosurfactant from *B. subtilis* ATCC 21332 in shake flask. Results showed that surface tensions dropped from 71.3 ± 0.1 to 28.3 ± 0.3 mN/m (simulated solid potato medium) and to 27.5 ± 0.3 mN/m (mineral salts medium). A critical micelle concentration (CMC) of 0.10 g/l was obtained from a methylene chloride extract of the simulated solid potato medium.

Kitamoto *et al.* (2001) focused their attention on the feasibility of replacing vegetable oils with *n*-alkanes for the production of mannosylerythritol lipids (MEL). Finally, *n*-alkanes ranging from C12 to C18 were converted into glycolipid biosurfactants, MEL, by resting cells of *Pseudozyma* (*Candida*) *antarctica*

T-34. The highest yield (0.87 g / g substrate) was obtained from 6%(v/v) of *n*-octadecane after 7 days reaction. The amount of MEL reached 140 g/l by intermittent feeding of the substrate. Also, based on these results, MEL has significant potential for use as a new material to facilitate the removal and biodegradation of hydrocarbons in oil-contaminated environments.

Cunha *et al.* (2003) selected *Serratia* sp. SVGG16 to produce a biosurfactants compound during growth with ethanol-blended gasoline as a carbon source. The results demonstrated that this strain was able to reduce surface tension of the medium to 34 mN/m, showing potential to be used in bioremediation processes.

2.4.2 Nitrogen Source

Medium constituents other than carbon source also affect the production of biosurfactants. Among the inorganic salts tested, ammonium salts and urea were preferred nitrogen sources for biosurfactant production by *Arthrobacter paraffineus* (Duvnjak *et al.,* 1982), whereas nitrate supported maximum surfactant production in *P. aeruginosa* (MacElwee *et al.,* 1990) and *Rhodococcus* spp. (Abu-Ruwalda *et al.,* 1991). Biosurfactant production by *A. paraffineus* is increased by the addition of L-amino acids such as aspartic acid, glutamic acid, asparagine, and glycine to the medium. The structure of surfactin is influenced by the L-amino acid concentration in the medium to produce either Val-7 or Leu-7 surfactin. Similarly, lichenysin-A production is enhanced two- and fourfold in *B. licheniformis* BAS50 (Yakimov *et al.,* 1996) by addition of L-glutamic acid and L-asparagine, respectively, to the medium. Robert *et al.* (1989) and Abu-Ruwalda *et al.* (1991) observed nitrate to be the best source of nitrogen for biosurfactant production by *Pseudomonas* strain 44T1 and *Rhodococcus* strain ST-5 growing on olive oil and paraffin, respectively. The production started after 30 h of growth, when the culture reached nitrogen limitation, and continued to increase to 58 h of fermentation. In *P.aeruginosa*, a simultaneous increase in rhamnolipid production and glutamine synthetase activity was observed when growth slowed as the culture became nitrogen limiting. Similarly, nitrogen limitation caused increased biosurfactant production in *P. aeruginosa*, *C. tropicalis* IIP-4, and *Nocardia* strain SFC-D reported by Desai *et al.* (1997).

Desai *et al.* (1997) reported that nitrogen limitation not only causes the overproduction of biosurfactants but also changes the composition of the biosurfactants produced. He reported that the maximum rhamnolipid production after nitrogen limitation at a C:N ratio of 16:1 to 18:1 and no surfactant production below a C:N ratio of 11:1, where the culture was not nitrogen limited. According to Hommel *et al.* (1987), it is the absolute quantity of nitrogen and not its relative concentration that appears to be important for optimum biomass yield, while the concentration of hydrophobic carbon source determines the conversion of carbon available to the biosurfactant.

Bednarski *et al.* (2004) evaluated the utilization of fat waste, namely the suitability of soap stock and post-refinery fatty acids as substrates inducing glycolipids biosynthesis by yeast. Results demonstrated that the addition of soap stock to the culture medium or post-refinery fatty acid supplementing the culture medium have a positive effect on the glycolipids synthesis by yeast (*C. antarctica* and *C. apicola*) ranging from 8.2 - 10.3 g/l and 6.5 - 10.4 g/l, respectively.

2.5 Biosurfactant Production

Desai *et al.* (1997) reported that the kinetics of biosurfactant production exhibit many variations among various systems, and only a few generalizations can be derived. However, for convenience, kinetic parameters can be grouped into the following types: (i) growth-associated production, (ii) production under growth-limiting conditions, (iii) production by resting or immobilized cells, and (iv) production with precursor supplementation.

2.5.1 Growth-Associated Production

For growth-associated biosurfactant production, parallel relationships exist between growth, substrate utilization, and biosurfactant production. The production of rhamnolipid by some *Pseudomonas* spp., glycoprotein AP-6 by *P. fluorescens* 378, surface-active agent by *B. cereus* IAF 346, and biodispersan by *Bacillus* sp. strain IAF-343 (Cooper *et al.*, 1987) are all examples of growth-associated biosurfactant production. The production of cell-free emulsan by *A.*

calcoaceticus RAG-1 has been reported to be a mixed growth-associated and non-growth-associated type. An emulsan-like substance accumulates on the cell surfaces during the exponential phase of growth and is released into the medium when protein synthesis decreases.

2.5.2 Production under Growth-Limiting Conditions

Production under growth-limiting conditions is characterized by a sharp increase in the biosurfactant level as a result of the limitation of one or more medium components. A number of investigators have demonstrated an overproduction of biosurfactants by *Pseudomonas* spp. when the culture reaches the stationary phase of growth due to the limitation of nitrogen and iron. Production of bioemulsifier by *Candida tropicalis* IIP-4, of glycolipid by *Nocardia* sp. strain SFC-D, and of water-soluble biosurfactant by *Torulopsis apicola* has also been reported to follow this pattern.

In contrast to the observation in *P. aeruginosa*, low phosphate concentration stimulated bioemulsifier production in a gram-negative bacterium during cultivation on ethanol. Phosphate, iron, magnesium, and sodium were all important elements for a biosurfactant-producing *Rhodococcus sp.*, much more than either potassium or calcium (Abu-Ruwaida *et al.,* 1991). Iron concentration has a dramatic effect on rhamnolipid production by *P. aeruginosa*, resulting in a threefold increase in production when cells were shifted from a medium containing 36 mM iron to a medium containing 18 mM iron. Interestingly, under these conditions, there was no change in the biomass yield.

2.5.3 Production by Resting or Immobilized Cells

Production by resting or immobilized cells is a type of biosurfactant production in which there is no cell multiplication. The cells nevertheless continue to utilize the carbon source for the synthesis of biosurfactants. Several examples of biosurfactant production by resting cells are known. They include production of rhamnolipid by *Pseudomonas* spp. and *P. aeruginosa* CFTR-6, sophorolipid production by *Torulopsis bombicola* and *Candida apicola*, cellobiolipid production by *Ustilago maydis*, trehalose tetraester production by *Rhodococcus erythropolis,*

and mannosylerythritol lipid production by *Candida antarctica.* Biosurfactant production by resting cells is important for the reduction of the cost of product recovery, as the growth and the product formation phases can be separated.

2.5.4 Production with Precursor Supplementation

Many investigators have reported that the addition of biosurfactant precursors to the growth medium causes both qualitative and quantitative changes in the product. For example, the addition of lipophilic compounds to the culture medium of *T. magnoliae, T. bombicola,* and *T. apicola* IMET 43747 (Stuwer *et al.,* 1987) resulted in increased biosurfactant production with yields of about 120 to 150 g/l (Lee *et al.,* 1993). Similarly, increased production of biosurfactants containing different mono-, di-, or trisaccharides was reported to occur in *Arthrobacter paraffineus* DSM 2567, *Corynebacterium* spp., *Nocardia* spp., and *Brevibacterium* spp. through supplementation with the corresponding sugar in the growth medium.

Sung-Chyr *et al.* (1998) demonstrated that a *Bacillus licheniformis* mutant derived by random mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine treatment producing high levels of the lipopeptide biosurfactants was selected by an ion-pair plate assay. HPLC analysis, infrared spectroscopy analysis, and surface tension measurement indicated that the biosurfactants produced by the mutant were identical to those produced by the wildtype strain. The biosurfactants exhibited a low surface tension of 26.5 dyne/cm and a low critical micelle concentration of 10 mg/1.

Sutthivanitchakul *et al.* (1999) proposed to produce a biosurfactants from *Bacillus licheniformis* F2.2, isolated from fermented food, growing in a medium using rice straw hydrolysate as a carbon source, NH_4NO_3 as a nitrogen source, and supplemented with a vitamin solution and trace elements. Finally, the biosurfactant BF2.2 was found to have good properties; its stability over a wide range of pH, temperature and salt concentration.

Roongsawang *et al.* (1999) focused on the isolated biosurfactants *bacillus* sp.strain KP-2 from a culture broth by acid precipitation and characterized by HPLC and MS. Then, preliminary analytical results indicated that biosurfactant kp-2 may be lipopotide-type biosurfactant and were a good surface active agent in comparison with other synthetic surfactants.

Jennings *et al.* (2000) examined the prevalence of biosurfactants producers in uncontaminated soils. Biosurfactant-producing bacteria were found to constitute a significant proportion (up to 35%) of aerobic heterotrophs. Biosurfactant producers were isolated and identified as strains of *Bacillus* and *Pseudomonas.*

Kuyukina *et al.* (2001) proposed methyl tertiary-butyl ether MTBE as a solvent for extraction of biosurfactants from *Rhodococcus* bacterial cultures. After comparison with other well known solvent systems used for biosurfactant extraction, it was found that MTBE was able to extract crude surfactant material with high product recovery (10g/l), efficiency (critical micelle concentration (CMC), 130–170 mg/l), and good functional surfactant characteristics (surface and interfacial tensions, 29 and 0.9 mN/m, respectively). The isolated surfactant complex contained 10% polar lipids, mostly glycolipids possessing maximal surface activity. Ultrasonic treatment of the extraction mixture increased the proportion of polar lipids in crude extract, resulting in increasing surfactant efficiency.

2.6 Potential and Commercial Application

Mulligan *et al.* (2001) showed that the organic phase-associated metals can be removed from sediments by either surfactin or rhamnolipid with sodium hydroxide. Acidic conditions with sophorolipid addition were effective for removing the zinc in the oxide and carbonate phases.

Zouboulis *et al.* (2003) examined an alternative collector for flotation methods (sorptive or adsorbing colloid). For the removal of two representative toxic metals existing in many wastewaters, Cr(VI) and Zn(II) were studied by the application of flotation methods by the addition of biosurfactants (Surfactin-105 and Lichenysin-A). The results showed that Surfactin and Lychenysin biosurfactants can effectively remove goethite in the pH range 4-7 and presented superior collector activity in comparison to dodecylamine and sodium dodecyl sulphate under the experimental conditions. Both biosurfactants can effectively remove Cr(VI), but surfactin was proven to be an effective surfactant for the removal of Zn(II); whereas Lichenysin was proven to be ineffective for the same task.

Rahman *et al.* (2003) investigated possible methods to enhance the rate of biodegradation of oil sludge from a crude oil tank bottom, thus reducing the time usually required for bioremediation. About 10% and 20% sludge contaminated sterile and non-sterile soil samples were treated with a bacterial consortium (BC), rhamnolipid biosurfactant (RL) and nitrogen, phosphorus and potassium (NPK) solution. Also, statistical analysis using the analysis of variance and Duncan's multiple range test revealed that the level of amendments, incubation time and combination of amendments significantly influenced bacterial growth, protein concentration and surface tension at a 1% probability level. All tested additives (BC, NPK, and RL) had significant positive effects on the bioremediation of n-alkane in petroleum sludge.

Edwards *et al.* (2003) performed toxicities comparisons of three synthetic surfactants and three microbiologically produced surfactants which were determined and compared in this study for the estuarine epibenthic invertebrate, *Mysidopsis bahia* and the inland silverside, *Menidia beryllina*. Results were specific to the surfactant, response parameter and test species. The LC50 values (nominal concentrations) for *M. bahia* ranged from 3.3 mg/l (Triton X-100) to >1000 mg/l (PES-61) and 2.5 mg/l (Triton X-100) to 413.6 mg/l (PES-61) for *M. beryllina.* Chronic first-effect concentrations (mg/l) for the six surfactants ranged from 2.3 to 465.0 (*M. beryllina*) and 1.0 to >1000.0 (*M. bahia*) based on reductions in growth and fecundity. Few generalizations could be made concerning the results due to their variability, but *M. bahia* was generally the more sensitive species and the toxicities of the biosurfactants were intermediate to those of the synthetic surfactants.

Wei *et al.* (2004) studied a biosurfactant that was used to clean used oil from used polypropylene nonwoven sorbents. Washing parameters tested included sorbent type, washing time, surfactant dosage and temperature. It was found that with the biosurfactant washing, more than 95 % removal of the oil from the sorbents was achieved, depending on the washing conditions.

Calvo *et al.* (2004) studied the growth, biosurfactant activities and petroleum hydrocarbon compounds utilization of strain 28-11 isolated from a solid waste oil. The isolate was identified as *Bacillus pumilus*. It grew well in the presence

of 0.1 % w/v of crude oil and naphthalene under aerobic conditions and utilized these substances as the carbon and energy sources.

Table 2.1 Some biosurfactants and their applications

Type of biosurfactants	Sources	Applications
Glycolipids:		
1. Rhamnolipids	Bacterial (e.g.*Pseudomonas, Cornyebacterium. Arthobacter and Norcardia* sp.)	Bioremediation, emulsifying agent, enhanced oil recovery, removal of hazardous chemicals (such as 2,a- trichlorophenyloxyacetic acid, chlorophenols), wound healing, organ transplants, in the treatment of burn shock, arteriosclerosis, depression, dermatological diseases, papilloma viral infections, schizophrenia, and in cosmetics and detergents.
2. Sophorolipids	Yeast (*Turulopsis or Candida* sp.)	In oil recovery, cosmetics (especially for antidandruff, bacteriostatic agents, and deodorants), detergent as a macrophage activator, fibrinolytic agent, skin healing agent, desquamating agent, depigmenting agent, and in germicidal compositions (suitable for cleaning fruits, vegetables, skin and hair).
3.Trehalose lipids	Bacterial (*Rodicoccus, Mycobacterium, Arthrobacter, brevibacterium, Cornybacterium* sp.)	Bioremediation, emulsifying agent, enhanced oil recovery, removal of hazardous chemicals and metals, inducer for leukemic cell strain and surface activities, cell differentiation inducer, emulsifying dispersant for cosmetics or foods, gelling agent, raw material for liposome, additive to antiviral agent.
4.Cellobiose lipids	Bacterial (*Ustilgo* sp.)	In detergent.
Phospholipids	Bacterial and fungal (*Asperdillus, Acinetobacter, Arthobacter, Pseudomonas, Thiobacillus* sp.)	As a cytotoxic agent, treatment of HIV-1, hepatilis B and herpes viruses, as auxiliary agents in the production and application of spray mixtures containing plant protectants, as additive in anti-inflammatory, antiulcer and healing agents, etc.

Lipopeptides and lipoproteins:		
1. Surfactin	Bacterial (*Bacillus* sp.)	A powerful biosurfactant, mechanical dewatering of peat, treating or preventing hypercholesterolemia, good inhibitor of eukaryotic protein kinase activity.
2. Mycosubtilin	Bacterial (*Bacillus* sp.)	As an antibiotic.
3. Viscosin	Bacterial (*Pseudomonas* sp.)	As antibacterial, antiviral and antitrypan-osomal therapeutic agent, effective inhibitor of eukaryotic protein kinase activity.
4.Viscosinamide	Bacterial (*Pseudomonas* sp.)	Antifungal agent.
5. Germicidins	Bacterial (*Bacillus* sp.)	As antibiotics.
6. Polymyxins	Bacterial (*Bacillus* sp.)	As antibiotics.
7. Cerlipin (ornithine- and taurine- containg lipid)	Bacterial (*Gluconobactor* sp.)	Excellent biosurfactants.
8. Lysine containg lipids	Bacterial (*Acinetobacter* sp.)	Excellent biosurfactants.
9. Ornithine-containg Lipids	Bacterial (*Pseudomonas* and *Thiobacillus* sp.)	Excellent biosurfactants.
Polymeric biosurfactants:		
1. Biodispersan	Bacterial (*Acinetobacter* sp.)	As dispersing agent, mining and paper industries (prevents flocculation of minerials while stabilizing aqueous susupensions of minerals).
2. Emulsan	Bacterial (*Acinetobacter* sp.)	A very potent emulsifying agent used in cleaning oil-contaminated vessels, control of dental plaque and caries, treating chlamydial infection, patients having Gram-positive bacterial infections.
3. Liposan	Bacterial (*Candida* sp.)	Emulsifying agent.