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# Biological Applications of Biosurfactants and Strategies to Potentiate Commercial Production

*Mohd Sajjad Ahmad Khan, Brijdeep Singh, and Swaranjit Singh Cameotra*

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13.1 INTRODUCTION

Biosurfactants are amphiphilic compounds produced by microorganisms such as bacteria, yeasts, and molds with pronounced surface and emulsifying activities (Singh et al. 2007). The presence of hydrophobic and hydrophilic groups confers these molecules the ability to accumulate between interfaces of dissimilar polarities like liquid–air, liquid–liquid, or liquid–solid interface, and thereby reducing surface and interfacial tension at the surface and interface regions, respectively (Karanth et al. 1999). Microbiologically produced surfactants are soluble in both organic (nonpolar) and aqueous (polar) solvents and categorized by their chemical composition and microbial origin. They include glycolipids, lipopeptides, polysaccharide–protein complexes, protein-like substances, lipopolysaccharides, phospholipids, fatty acids, and neutral lipids (Van Hamme et al. 2006).

Variation in the chemical nature of biosurfactants is responsible for its diverse properties and physiological functions such as increasing the surface area and bioavailability of hydrophobic water-insoluble substrates, heavy metal binding, bacterial pathogenesis, quorum sensing, and biofilm formation (Singh and Cameotra 2004). These compounds can be synthesized by the microorganisms growing on water-immiscible hydrocarbons as well as on water-soluble compounds (Mukherjee et al. 2006). But optimization of environmental conditions and nutrient sources or use of genetically altered microorganisms may lead to increased production of biosurfactants. Enhancing the yield of these biomolecules is highly esteemed for their exploitation in industries.

Primarily, biosurfactants attracted attention as hydrocarbon dissolution agents in the 1960s, and their applications have been greatly extended in the past five decades as an improved alternative to chemical surfactants (carboxylates, sulfonates, and sulfate acid esters), especially in agriculture, cosmetics, food, pharmaceutical, and oil industry (Banat et al. 2000). As far as biological applications of biosurfactants are considered, these molecules have an edge over their chemically synthesized counterparts such as

1. Biodegradability: Because of the low toxicity and simple chemical structure, these compounds do not persist in the environment and are degraded easily. This property prevents the problem of accumulation of biosurfactants such as shown by chemical surfactants. Therefore, these molecules are greatly environmental friendly and termed as green chemicals (Abdel-Mawgoud et al. 2010).

2. Biocompatibility and digestibility: Biological origin of these molecules imparts them an inherent characteristic of compatibility with organisms. This property allows their unabated usage in cosmetics, pharmaceuticals, and as functional food additives.

3. Availability of raw materials and economic production: Biosurfactants can be produced from relatively cheap raw materials and renewable feedstocks by microorganisms. These materials such as industrial wastes and by-products are available in abundance and provide carbon source ranging from hydrocarbons, carbohydrates to lipids and may be used separately or in combination with each other for microbial production.

4. Environmental control: The amphiphilic nature of biosurfactant is exploited in the processes for stabilization of industrial emulsions, control of oil-sputs, biodegradation, and detoxification of industrial effluents. Also, bioremediation of contaminated soil can be favored with the use of biosurfactants.

5. Specificity: The presence of specific functional groups imparts specificity in the action by the biosurfactant molecules. This property can be of paramount importance in detoxification of specific pollutants, de-emulsification of industrial emulsions, development of specific cosmetic, specialized pharmaceutical, and food applications.

6. Stability at extreme conditions: The most significant properties of these microbial products are effectiveness at the extremes of temperature, pH, and salinity. Along with these parameters and the unique structure markedly differing from the chemical surfactants can promote alternate usage for which the classical surfactants fail.
During the past few years, biosurfactant production by various microorganisms has been studied extensively and also the various aspects of biosurfactants such as their biomedical and therapeutic properties, natural roles, production using cheap alternate substrates, and commercial potential, which has been reviewed recently. Biosurfactants are diverse in their applications and are becoming beneficial molecules for industrial uses. When these are used in the food-processing, cosmetic, and pharmaceutical industries, biosurfactants have been included in formulations to serve as emulsifiers and solubilizers, as well as foaming, wetting, antiadhesive, antimicrobial, and antiviral agents (Rodrigues et al. 2006; Shete et al. 2006; Muthusamy et al. 2008). The principle aim of this chapter is to focus special emphasis on the exploitation of biosurfactants by various industries utilizing cheap substrates as carbon source. The reason for use of biosurfactants in vast applications is that biosurfactants possess the characteristic property of reducing the surface and interfacial tension using the same mechanisms as that for chemical surfactants. Unlike chemical surfactants, which are mostly derived from petroleum feedstock, these molecules can be produced by microbial fermentation processes using cheaper agro-based substrates and waste materials. The screening of over-producer microbial strains and optimization of cultural parameters are other strategies to increase biosurfactants yield and applications.

13.2 Classifications of Biosurfactants

Unlike chemically synthesized surfactants, which are usually classified according to the nature of their polar groups, biosurfactants are generally categorized mainly by their chemical composition and microbial origin. Biosurfactants are generally classified into two categories: (i) low-molecular-mass molecules, which efficiently lower surface and interfacial tension; and (ii) high-molecular-mass polymers, more effective as emulsion-stabilizing agents. The major classes of low-mass surfactants include glycolipids, lipopeptides, and phospholipids, whereas high-mass surfactants include polymeric and particulate surfactants. Most biosurfactants derived from diverse microbial sources are either anionic or neutral and the hydrophobic moiety is based on long-chain fatty acids or fatty acid derivatives, whereas the hydrophilic portion can be a carbohydrate, amino acid, phosphate, or cyclic peptide (Nitschke and Coast 2007) (Table 13.1).

13.3 Applications of Biosurfactants

Biosurfactants produced by microorganisms are becoming important biotechnology products for industrial and medical applications due to their specific modes of action, low toxicity, relative ease of preparation, and widespread applicability. They can be used as emulsifiers, de-emulsifiers, wetting and foaming agents, functional food ingredients, and as detergents in petroleum, petrochemicals, environmental management, agrochemicals, foods, and beverages, cosmetics and pharmaceuticals, and in mining and metallurgical industries. The addition of a surfactant of chemical or biological origin accelerates or sometimes inhibits the bioremediation of pollutants. Surfactants also play an important role in enhanced oil recovery by increasing the apparent solubility of petroleum components and effectively reducing the interfacial tensions of oil and water in situ. For medical applications, biosurfactants are useful as antimicrobial agents and immunomodulatory molecules. The properties of biosurfactants that make them useful in various applications are presented in Figure 13.1 and Table 13.2. Beneficial applications of biosurfactants in various industries are discussed in this chapter.

13.3.1 Role of Biosurfactants in Biodegradation Processes

Bioremediation typically involves augmentation of soil or other media, contaminated with pollutants with nutrients and sometimes microorganisms, to improve processes for biodegradation of the contaminants. Biodegradation rate of a contaminant in soil depends on its bioavailability
TABLE 13.1
Classification and Types of Biosurfactant Produced by Various Microorganisms

<table>
<thead>
<tr>
<th>Group</th>
<th>Class</th>
<th>Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolipids</td>
<td>Rhamnolipids</td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td></td>
<td>Trehalolipids</td>
<td><em>Mycobacterium tuberculosis, Rhodococcus erythropolis</em></td>
</tr>
<tr>
<td></td>
<td>Sophorolipids</td>
<td><em>Torulopsis bombicola, Torulopsis petrophilum</em></td>
</tr>
<tr>
<td>Fatty acids, phospholipids, and</td>
<td>Corynomycolic acid</td>
<td><em>Corynebacterium lepus</em></td>
</tr>
<tr>
<td>neutral lipids</td>
<td>Spiculisporic acid</td>
<td><em>Penicillium spiculisporum</em></td>
</tr>
<tr>
<td></td>
<td>Phosphatidylethanolamine</td>
<td><em>Acinetobacter sp., Rhodococcus erythropolis</em></td>
</tr>
<tr>
<td>Lipopeptides</td>
<td>Surfactin</td>
<td><em>Bacillus subtilis</em></td>
</tr>
<tr>
<td></td>
<td>Lichenysin</td>
<td><em>Bacillus licheniformis</em></td>
</tr>
<tr>
<td>Polymeric biosurfactants</td>
<td>Emulsan</td>
<td><em>Acinetobacter calcoaceticus RAG-1</em></td>
</tr>
<tr>
<td></td>
<td>Alasan</td>
<td><em>Acinetobacter radioresistens KA-53</em></td>
</tr>
<tr>
<td></td>
<td>Biodispersan</td>
<td><em>Acinetobacter calcoaceticus A2</em></td>
</tr>
<tr>
<td></td>
<td>Liposan</td>
<td><em>Candida lipolytica</em></td>
</tr>
<tr>
<td></td>
<td>Mannoprotein</td>
<td><em>Saccharomyces cerevisiae</em></td>
</tr>
</tbody>
</table>

FIGURE 13.1 Characteristics of biosurfactants useful in various applications.

to the metabolizing organisms, which is influenced by factors such as desorption, diffusion, and dissolution. A promising method that can improve bioremediation effectiveness of hydrocarbon-contaminated environments is the use of biosurfactants. Many of the most persistent contaminants exhibit low water solubility and hence, bioavailability of contaminants can often be improved by addition of emulsifiers. By reducing surface and interfacial tension among liquids, solids, and gases,
allowing them to disperse readily as emulsions, chemical or biological surfactants may have variable effects on contaminant biodegradation (Banat et al. 2000).

They can enhance hydrocarbon bioremediation by two mechanisms. The first includes the increase of substrate bioavailability for microorganisms, while the other involves interaction with the cell surface that increases the hydrophobicity of the surface allowing hydrophobic substrates to associate more easily with bacterial cells. Addition of biosurfactants can be expected to enhance hydrocarbon biodegradation by mobilization, solubilization, or emulsification (Urum and Pekdemir 2004; Nguyen et al. 2008; Nievas et al. 2008).

The capability of biosurfactants and biosurfactant-producing bacterial strains to enhance organic contaminants’ availability and biodegradation rates was reported by many authors (Rahman et al. 2003; Inakollu et al. 2004). Martínez-Checa et al. (2007) investigated the usefulness of the V2-7 bioemulsifier-producing strain F2-7 of *Halomonas eurihalina* in oil bioremediation process. First, they studied the capacity of strain F2-7 to grow and produce bioemulsifier in the presence of different hydrocarbon compounds. They observed that all analyzed hydrocarbons supported the growth of F2-7 strain and the production of V2-7 bioemulsifier. The ability of the analyzed strain to remove polycyclic aromatic hydrocarbons was investigated during the growth of this strain for 96 h in liquid medium supplemented with naphthalene, phenanthrene, fluoranthene, and pyrene. After the experiment, the obtained residual concentrations of fluoranthene (56.6%) and pyrene (44.5%) were higher than naphthalene (13.6%) and phenanthrene (15.6%). Efficiency of strain F2-7 in removing poly aromatic hydrocarbons (PAHs) confirmed its potential applicability in oil bioremediation technology.

### TABLE 13.2

**Industrial Applications of Chemical Surfactants and Biosurfactants**

<table>
<thead>
<tr>
<th>Industry</th>
<th>Application</th>
<th>Role of Surfactants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum</td>
<td>Enhanced oil recovery</td>
<td>Improving oil drainage into well bore; stimulating release of oil trapped by capillaries; wetting of solid surfaces; reduction of oil viscosity and oil pour point; lowering of interfacial tension; dissolving of oil</td>
</tr>
<tr>
<td></td>
<td>De-emulsification</td>
<td>De-emulsification of oil emulsions; oil solubilization; viscosity reduction, wetting agent</td>
</tr>
<tr>
<td>Environmental</td>
<td>Bioremediation</td>
<td>Emulsification of hydrocarbons; lowering of interfacial tension; metal sequestration</td>
</tr>
<tr>
<td></td>
<td>Soil remediation and flushing</td>
<td>Emulsification through adherence to hydrocarbons; dispersion; foaming agent; detergent; soil flushing</td>
</tr>
<tr>
<td>Food</td>
<td>Emulsification and de-emulsification</td>
<td>Emulsifier; solubilizer; demulsifier; suspension, wetting, foaming, defoaming, thickener, lubricating agent</td>
</tr>
<tr>
<td>Biological</td>
<td>Microbiological</td>
<td>Physiological behavior such as cell mobility, cell communication, nutrient accession, cell–cell competition, plant and animal pathogenesis</td>
</tr>
<tr>
<td></td>
<td>Pharmaceuticals and therapeutics</td>
<td>Antimicrobial, antifungal, antiviral agents; adhesive agents; immunomodulatory molecules; vaccines; gene therapy</td>
</tr>
<tr>
<td>Agricultural</td>
<td>Biocontrol</td>
<td>Facilitation of biocontrol mechanisms of microbes such as parasitism, antibiosis, competition, induced systemic resistance, and hypovirulence</td>
</tr>
<tr>
<td>Bioprocessing</td>
<td>Downstream processing</td>
<td>Biocatalysis in aqueous two-phase systems and microemulsions; biotransformations; recovery of intracellular products; enhanced production of extracellular enzymes and fermentation products</td>
</tr>
<tr>
<td>Cosmetic</td>
<td>Health and beauty products</td>
<td>Emulsifiers, foaming agents, solubilizers, wetting agents, cleansers, antimicrobial agent, mediators of enzyme action</td>
</tr>
</tbody>
</table>
Obayori et al. (2009) investigated the biodegradative properties of biosurfactant produced by *Pseudomonas* sp. LP1 strain on crude oil and diesel. The results obtained confirmed the ability of strain LP1 to metabolize the hydrocarbon components of crude and diesel oil. They reported 92.34% degradation of crude oil and 95.29% removal of diesel oil. Biodegradative properties of biosurfactant-producing *Brevibacterium* sp. PDM-3 strain were tested by Reddy et al. (2010). They reported that this strain could degrade 93.92% of the phenanthrene and also had the ability to degrade other polyaromatic hydrocarbons such as anthracene and fluorene.

Kang et al. (2010) used sophorolipid in studies on the biodegradation of aliphatic and aromatic hydrocarbons and Iranian light, crude oil under laboratory conditions. The addition of this biosurfactant to soil also increased biodegradation of tested hydrocarbons with the rate of degradation ranging from 85% to 97% of the total amount of hydrocarbons. Their results indicated that sophorolipid may have the potential for facilitating the bioremediation of sites contaminated with hydrocarbons having limited water solubility and increasing the bioavailability of microbial consortia for biodegradation.

### 13.3.2 Biosurfactants and Metals Remediation

The contamination of soil environments with heavy metals is very hazardous for human and other living organisms in the ecosystem. Owing to their extremely toxic nature, the presence of even low concentrations of heavy metals in the soil has been found to have serious consequences. Application of microorganisms was discovered many years ago to help in the reduction of metal contamination. Heavy metals are not biodegradable; they can only be transferred from one chemical state to another, which changes their mobility and toxicity. Microorganisms can influence metals in several ways. Some forms of metals can be transformed either by redox processes or by alkylation.

Metal wastes are produced by a variety of sources including mines, tanneries, and electroplating facilities and through the manufacture of paints, metal pipes, batteries, and ammunition. Metal contamination has been linked to birth defects, cancer, skin lesions, mental and physical retardation, learning disabilities, liver and kidney damage, and a host of other maladies. Microorganisms and their products, namely, biosurfactants are extensively used for the enhanced metal and metal co-contaminated site bioremediation (Singh and Cameotra 2004).

Biosurfactants can be applied to a small part of the contaminated soil: the soil is placed in a huge cement mixer, the biosurfactant–metal complex is flushed out, soil gets deposited back, and the biosurfactant–metal complex is treated to precipitate the biosurfactant, leaving behind the metal. The bond formed between the positively charged metal and the negatively charged surfactant is so strong that flushing water through soil removes the surfactant–metal complex from the soil matrix. This method can also be carried out for deeper subsurface contamination only with more pumping activities (Pacwa-Płociniczak et al. 2011).

According to Miller (1995), biosurfactants enhance the desorption of heavy metals from soils in two ways: (i) complexation of the free form of the metal residing in solution. This decreases the solution phase activity of the metal and, therefore, promotes desorption according to Le Chatelier’s principle. (ii) Direct contact of biosurfactant to sorbed metal at solid solution interface under conditions of reduced interfacial tension, which allows biosurfactants to accumulate at solid solution interface.

Considerable work has been done on rhamnolipid biosurfactant produced by various *Pseudomonas aeruginosa* strains capable of selectively complexing cationic metal species such as Cd, Pb, and Zn. Studies have shown that this surfactant complexes preferentially with toxic metals such as cadmium and lead than with normal soil metal cations such as calcium and magnesium, for which it has a much lower affinity (Herman et al. 1995; Torrens et al. 1998). The feasibility of using surfactin, rhamnolipid, and sophorolipid for the removal of heavy metals, Cu and Zn, from sediments has been evaluated by Mulligan et al. (1999, 2001). In one study by Jeong-Jin et al. (1998), biosurfactant-based ultrafiltration was used to remove the divalent metal ions, Cu²⁺, Zn²⁺, Cd²⁺, and Ni²⁺,
Biological Applications of Biosurfactants and Production Strategies

from aqueous solution containing either a single metal species or a mixture of metal ions. Todd et al. (2000) studied the effectiveness of rhamnolipid biosurfactants in the remediation of cadmium and naphthalene co-contaminated site. They observed reduced cadmium toxicity by *P. aeruginosa* rhamnolipid leading to an enhanced naphthalene biodegradation by a *Burkholderia* species.

Parthasarathi and Sivakumaar (2011) investigated the effects of biosurfactant produced by a mangrove isolate on a heavy metal-spiked soil remediation using two different methods of biosurfactant addition (pretreatment and direct application) at different concentrations (0.5–5%) for 10 days employing column and batch method of washings. The pre-addition of biosurfactant at 0.5% concentrations and further incubation for a month resulted in better chromium removal than the direct biosurfactant washing method. A maximum recovery of lead (99.77%), nickel (98.23%), copper (99.62%), and cadmium (99.71%) was achieved with a column washing method at 1% biosurfactant concentration. Release of 26% soluble fractions of nickel (pre-addition with biosurfactant) and 40% copper (direct application) was achieved by the column washing method at 1.0% concentration of biosurfactant. A total of 0.034 mg/10 g of lead, 0.157 mg/10 g of nickel, 0.022 mg/10 g of copper, 0.025 mg/10 g of cadmium, and 0.538 mg/10 g of chromium were found to remain in the spiked soil after column washing with 1.0% biosurfactant solution. However, pre-addition of 0.5% biosurfactant treatment helps in maximum removal of chromium metal leaving a residual concentration of 0.426 mg/10 g of soil, suggesting effective removal at very low concentration. The average extraction concentration of metals in batch washings was between 93% and 100%, irrespective of the concentration of biosurfactant studied.

### 13.3.3 Biosurfactants in the Cosmetic Industry

Multifunctional biosurfactants have several cosmetic applications because of their exceptional surface properties such as detergency, wetting, emulsifying, solubilizing, dispersing, and foaming effects. The most widely used biosurfactant glycolipids in cosmetics are sophorolipids, rhamnolipids, and mannosylerythritol lipids. Sophorolipids have good skin compatibility and excellent moisturizing properties, rhamnolipids are natural surfactants and emulsifiers that can replace petrochemical-based surfactants used in most of the cosmetic products. They also have been used in acne pads antidandruff, antiwrinkle, and antiaging products, deodorants, nail care products, and toothpastes in several different formulations, because of its high surface and emulsifying activities (Piljac and Piljac, 1999). Mannosylerythritol lipids are generally used in skin care formulations as the active ingredient to prevent skin roughness (Masaru et al. 2007).

### 13.3.4 Oil Recovery and Processing

Chemical surfactants and biosurfactants can increase the pseudo solubility of petroleum components in water (Pekdemir et al. 2005). Surfactants are effective in reducing the interfacial tensions of oil and water *in situ* and they can also reduce the viscosity of the oil and remove water from the oil prior to processing (Liu et al. 2004). Biosurfactants can be as effective as the synthetic chemical surfactants and for certain applications they have advantages such as high specificity. Most of the biosurfactants and many chemical surfactants employed for bioremediation purposes are biodegradable. Pollution of sea by crude oil, mostly caused by stranding of tankers, is one of the urgent and serious environmental issues across the world (Olivera et al. 2003). Chemical surfactants are used over few decades for the degradation of these hydrocarbons due to its high toxicity and low degradability.

#### 13.3.4.1 Microbial Enhanced Oil Recovery

Poor oil recovery in oil-producing wells may be due to either low permeability of some reservoirs or high viscosity of the crude oil resulting in poor mobility. The concept of microbial-enhanced oil recovery (MEOR) was first proposed nearly 80 years ago but received only limited attention until
the early 1980s (Stosur 1991). The ability of indigenous or injected microorganisms to synthesize useful fermentation products to improve oil recovery from the oil reservoirs is exploited in MEOR processes. Three strategies are recognized for biosurfactant application:

1. Biosurfactant production in batch or continuous culture and addition to the reservoir using the conventional way of MEOR
2. Production of biosurfactant by injected microbes at the cell–oil interface within the reservoir
3. Injection of selected nutrients into the reservoir to stimulate growth of indigenous biosurfactant-producing bacteria

The potential application of biosurfactants produced by the thermo- and halo-tolerant species of *Bacillus licheniformis* JF-2 and *Bacillus subtilis* has been explored for enhanced oil recoveries in laboratory columns and reservoirs with oil recoveries from 9.3% to 62% (Lin et al. 1994; Yakimov et al. 1997; Makkar and Cameotra 1998). Increases in oil recovery by about 30% have been reported from underground sandstone by using trehalolipids from *Nocardia rhodochrus* (Rapp et al. 1979). Oilfield emulsions, both oil in water and water in oil, are formed at various stages of exploration, production, and oil recovery and processing, represent a major problem for the petroleum industry (Manning and Thompson 1995). A process of de-emulsification is required to recover oil from these emulsions.

### 13.3.5 Biosurfactants in the Food Industry

Biosurfactants as biocompatible, biodegradable, and/or nontoxic compounds have the combination of particular characteristics that exhibit a variety of useful properties for the food industry, especially as emulsifiers, foaming, wetting, solubilizers (Banat et al. 2000), antiadhesive, and antimicrobial agents (Singh and Cameotra 2004).

#### 13.3.5.1 Food Emulsifier

Biosurfactants show several properties such as emulsion-based formulations that have great potential applications in many fields of food industry. An emulsion is a heterogeneous system, consisting of at least one immiscible liquid intimately dispersed in another in the form of droplets, having dispersed and continuous phase. The addition of emulsifiers is of special value for low-fat products (Rosenberg and Ron 1999) as it improves the texture and creaminess of dairy products. However, polymeric surfactants coat the droplets of oil and form very stable emulsions that never coalesce. This property is especially useful for making oil/water emulsions for cosmetics and food.

#### 13.3.5.2 Food Stabilizer

Biosurfactants act as controlling consistency in bakery and ice cream formulations. They are also utilized as fat stabilizer and antispattering agent during cooking of oil and fats (Kosaric 2001). In food processing, the addition of rhamnolipid surfactants improves the texture and shelf-life of starch-containing products, modifies rheological properties and stability of wheat dough (Van Haesendonck and Venzeveren 2004). Surfactants can also be used to control the agglomeration of fat globules, stabilize aerated systems, and texture of fat-based products (Kachholz and Schlingmann 1987). 1-Rhamnose, which already has an industrial application as precursor of high-quality flavor components like Furaneol (trademark of Firmenich SA, Geneva), is obtained by hydrolyzing rhamnolipid surfactants produced by *P. aeruginosa* (Linhardt et al. 1989).

### 13.3.6 Application in Medicine

One of the earliest noted antimicrobial activities of biosurfactants was that of iturin A, a potent antifungal lipopeptide produced by strains of *B. subtilis*. Iturin A has been proposed as an effective
antifungal agent for profound mycosis. Other members of the iturin group, including bacillomycin D and bacillomycin Lc, were also found to have antimicrobial activities (Eshita et al. 1995; Moyne et al. 2001; Singh and Cameotra 2004). Apart from antifungal and moderate antibacterial properties, surfactin (i) inhibits fibrin clot formation, (ii) induces formation of ion channels in lipid bilayer membranes, (iii) inhibits cyclic adenosine monophosphate (cAMP), (iv) inhibits platelet and spleen cytosolic phospholipase A2 (PLA2), and (v) exhibits antiviral and antitumor activities. Surfactin also has antmycoplasma properties and has been used in a fast and simple method for complete and permanent inactivation of mycoplasmas in mammalian monolayer and suspension cell cultures (Vollenbroich et al. 1997). Surfactin is active against several viruses, including semiliki forest virus, herpes simplex virus (HSV-1 and HSV-2), suid herpes virus, vesicular stomatitis virus, simian immunodeficiency virus, feline calicivirus, and murine encephalomyocarditis virus (Singh and Cameotra 2004).

13.3.6.1 Genetic Manipulation
It is stated by Gharaei-Fathabad (2011) that the establishment of an efficient and safe method for introducing exogenous nucleotides into mammalian cells is critical for basic sciences and clinical applications such as gene therapy. Lipofection using cationic liposomes is considered to be a safe way to deliver foreign gene to the target cells without side effects among various known methods of gene transfection (Fujita et al. 2009; Zhang et al. 2010). The use of liposomes made from biosurfactants has been used as an important strategy for gene transfection. In a study, Inoh et al. (2001) found that MEL-A dramatically increased the efficiency of gene transfection mediated by cationic liposomes with a cationic cholesterol derivative. Studies like these could lead to the development of effective and safe nonviral vector-mediated gene transfection and gene therapy procedures. Kitamoto et al. (2002) demonstrated that liposome based on biosurfactants shows increasing efficiency of gene transfection in comparison with commercially available cationic liposome. In the last decade, for the liposome-based gene transfection some techniques and methodologies have been developed. Members of the Candida antarctica strain produce two kinds of mannosylerythritol lipids (MEL-A and MEL-B) that exhibit antimicrobial activity, particularly against Gram-positive bacteria (Kitamoto et al. 1993). Ueno et al. in 2007 examined MEL-A-containing liposome for gene transfection by introducing biosurfactants in this field.

13.3.6.2 Immune Modulatory Action
Bacterial lipopeptides constitute potent nontoxic and nonpyrogenic immunological adjuvant when mixed with conventional antigens (Gharaei-Fathabad 2011). In rabbits and chickens (Rodrigues et al. 2006), a marked enhancement of the humoral immune response was obtained by coupling of poly-L-lysine (MLR-PLL) with the low-molecular mass antigens iturin-AL, herbicolin-A, and microcystin (MLR).

13.3.6.3 Toxic Activity against Microorganisms
Several biosurfactants have strong antimicrobial, antifungal, and antiviral activity; this versatile performance is conferred due to the diverse structures of biosurfactants (Zhao et al. 2010). The structure of biosurfactant is supposed to exert its toxicity on the cell membrane permeability as a detergent-like effect. Fernandes et al. (2007) investigated the antimicrobial activity of biosurfactants from Bacillus subtilis demonstrating that lipopeptides have a broad spectrum of antimicrobial activity against microorganisms with multidrug-resistant profile (Fernandes et al. 2007). Rodrigues et al. (2006) mentioned many biosurfactants produced by Candida antarctica, P. aeruginosa, B. subtilis, and B. licheniformis that have shown toxic activities against microorganisms.

13.3.6.4 Antiadhesive Agents
Adhesion of biosurfactants to solid surfaces might constitute a new and effective means of combating colonization by pathogenic microorganisms (Rivardo et al. 2009). Biosurfactants have been
Biosurfactants found to inhibit the adhesion of pathogenic organisms to the site of infection (Das et al. 2009). These surfactants can play the significant role of antiadhesive agent making them useful for treating many diseases as well as in use as therapeutic and probiotic agent. In addition, biosurfactant in pharmaceutical fields can be used as agents for stimulating stem fibroblast metabolism, while in premature infants the deficiency of pulmonary surfactant, a phospholipids protein complex also cause the failure of respiration. Moreover, isolation of genes for protein molecules of these surfactants and cloning in bacteria has made possible its fermentation production for medical application.

Biosurfactants play an essential natural role in the swarming motility of microorganisms and participate in cellular physiological processes of signaling and differentiation as well as in biofilm formation. Swarming motility and biofilm formation are the key actions in the colonization of a surface by bacteria, and increase the likelihood of nosocomial infections. Biosurfactants have been found to inhibit the adhesion of pathogenic organisms to solid surfaces or to infection sites. Surfactin decreases the amount of biofilm formed by *Salmonella typhimurium*, *Salmonella enterica*, *Escherichia coli*, and *Proteus mirabilis* in polyvinyl chloride wells, as well as in vinyl urethral catheters (Mireles et al. 2001). Precoating the catheters by running the surfactin solution through them before inoculation with media was just as effective as including surfactin in the growth medium. Given the importance of opportunistic infections with *Salmonella* species, including urinary tract infections of AIDS patients, these results have potential for practical applications. A biosurfactant of *Pseudomonas fluorescens* was found to inhibit the adhesion of *Listeria monocytogenes* LO28 to polytetrafluoroethylene and stainless-steel surfaces (Meylheuc et al. 2001). There are reports of inhibition of biofilm formed by uropathogens and yeast on silicone rubber by biosurfactants produced by *Lactobacillus acidophilus* (Velraeds et al. 1998).

**13.3.6.5 Antimicrobial Activity of Biosurfactants**

The antimicrobial activity of several biosurfactants has been reported in the literature for many different applications (Cameotra and Makkar 2004). For instance, the antimicrobial activity of two biosurfactants obtained from probiotic bacteria, *Lactococcus lactis* (Naruse et al. 1990) and *Streptococcus thermophilus* A, against a variety of bacterial and yeast strains isolated from explanted voice prostheses was evaluated (Rodrigues et al. 2004). Antibacterial and antiphytoviral effects of various rhamnolipids have been described in the literature (Bai et al. 1997; Benincasa et al. 2004). Seven different rhamnolipids were identified in cultures of *P. aeruginosa* AT10 from soybean oil refinery wastes and these showed excellent antifungal properties against various fungi (Abalos et al. 2001). Golubev et al. (2001) reported the production of an extracellular, low-molecular weight, protease-resistant thermostable glycolipid fungicide from the yeast *Pseudozyma fusiformata* (Ustilaginales). This fungicide was active against >80% of the 280 yeast and yeast-like species tested under acidic conditions (pH 4.0) at 20–30°C (Kulakovskaya et al. 2003). The purified glycolipids enhanced nonspecific permeability of the cytoplasmic membrane in sensitive cells, which resulted in ATP leakage.

Gomma (2013) investigated the antimicrobial effect of the lipopeptide biosurfactants produced by *B. licheniformis* strain M104 grown on whey. The biosurfactant was investigated for potential antimicrobial activity against different Gram-positive bacteria (*B. subtilis, B. thuringiensis, B. cereus, Staphylococcus aureus*, and *L. monocytogenes*), Gram-negative bacteria (*P. aeruginosa, E. coli, S. typhimurium, Proteous vulgaris*, and *Klebsiella pneumoniae*), and a yeast (*Candida albicans*).

**13.3.7 Application in Agriculture**

The dual hydrophobic/hydrophilic nature of biosurfactant can be widely exploited in areas related to agriculture for the enhancement of biodegradation of pollutants to improve the quality of agriculture soil, for indirect plant growth promotion as these biosurfactants have antimicrobial activity and to increase the plant microbe interaction beneficial for plant (Sachdev and Cameotra 2013). These
biosurfactants can replace the harsh surfactant presently used in pesticide industries as these natural surfactants are found to be utilized as carbon source by soil-inhabiting microbes (Lima et al. 2011) and this accounts for the biological removal of biosurfactants from the agricultural soil.

**13.3.7.1 Improvement of Soil Quality**

The productivity of agricultural land is affected by the presence of organic and inorganic pollutants that impart abiotic stress on the cultivated crop plant. To increase the quality of such soil contaminated by hydrocarbon and heavy metals, process of bioremediation is required. Microorganism-producing biosurfactants and/or biosurfactants can be effectively used for the removal of hydrocarbons as well as heavy metals (Sun et al. 2006). A very important phenomenon of desorption of hydrophobic pollutants tightly bound to soil particles is accelerated by biosurfactants. This is very crucial for bioremediation process. Biosurfactants can also enhance the degradation of certain chemical insecticides that are accumulated in the agricultural soil (Zhang et al. 2011). Biosurfactant from *Lactobacillus pentosus* has demonstrated reduction by 58.6–62.8% of octane hydrocarbon from soil (Moldes et al. 2011), thus exhibiting the biodegradation accelerator property of biosurfactant. It has been observed that a biosurfactant-producing species of *Burkholderia* isolated from oil-contaminated soil may be a potential candidate for bioremediation of variety of pesticide contamination (Wattanaphon et al. 2008).

**13.3.7.2 Plant–Pathogen Elimination**

Several biosurfactants from microbes have antimicrobial activity against plant pathogens and therefore they are considered to be a promising biocontrol molecule for achieving sustainable agriculture. An agricultural application of biosurfactants also facilitates biocontrol mechanism of plant growth-promoting microbes such as parasitism, antibiosis, competition, induced systemic resistance, and hypovirulence (Singh et al. 2007). Biological control involves the exploitation of selected microorganisms (termed antagonistic), using naturally occurring mechanisms, to suppress harmful organisms. The modes of action are parasitism, antibiosis, competition, induced systemic resistance, and hypovirulence. In many instances, surfactants enhance the effects of the microbial biocontrol agent. *Brevibacillus brevis* strain HOB1 produces surfactin isoform and this lipopeptide biosurfactant has demonstrated strong antibacterial and antifungal property which can be exploited for control of phytopathogens (Haddad 2008). Plant growth-promoting *Pseudomonas putida* produces biosurfactants that can cause lysis of zoospores of the oomycete pathogen *Phytophthora capsici*; causative agent of damping-off of cucumber (Kruijt et al. 2009). The lipopeptide biosurfactant produced by strains of *Bacillus* exhibits growth inhibition of phytopathogenic fungi like *Fusarium* spp., *Aspergillus* spp., and *Biopolaris sorokiniana*. Such biosurfactant can be used as biocontrol agent (Velho et al. 2011). Kim et al. (2011) have isolated biosurfactant from a strain of *Pseudomonas*, which has demonstrated insecticidal activity against green peach aphid (*Myzus persicae*). A possible plant pathogen *P. aeruginosa* was reported to be inhibited by biosurfactant produced by *Staphylococcus* sp., isolated from crude oil-contaminated soil (Eddouaouda et al. 2012). Biosurfactant-producing rhizospheric isolates of *Pseudomonas* and *Bacillus* have exhibited biocontrol of soft rot causing *Pectobacterium* and *Dickeya* spp. (Krzyzanowska et al. 2012).

**13.4 STRATEGIES TO ENHANCE PRODUCTION OF BIOSURFACTANTS FOR ITS INDUSTRIAL APPLICATIONS**

Improvement in production procedures and technologies has helped to some extent and can lead to further improvements. Researchers have emphasized the key parameters affecting the efficiency of biosurfactant production in terms of higher yields and lower production costs (Mukherjee et al. 2006). According to Syldatk and Hausmann (2010) the reasons for limited use of microbial surfactants in industry are the use of expensive substrates, limited product concentrations, low yields, and
formation of product mixtures rather than pure compounds. All these factors and other growth and upscale problems like the use of antifoaming agents add on to the high costs of the downstream processing. The main strategies to achieve this are through (i) the assessment of the substrate and product output with focus on appropriate organism, nutritional balance, and the use of cheap or waste substrates to lower the initial raw material costs involved in the process; (ii) the development of efficient bioprocesses, including optimization of the culture conditions and cost-effective separation processes to maximize recovery; and (iii) the development and use of overproducing mutant or recombinant strains for enhanced yields (Mukherjee et al. 2006; Makkar et al. 2011).

The use of the alternative substrates such as agro-based industrial wastes is one of the attractive strategies for economical biosurfactant production. Kosaric (1992) suggested the use of industrial and/or municipal wastewaters, rich in organic pollutants, to achieve a double benefit of reducing the pollutants while producing useful products. Another approach involves using raw substrates with negligible or no value. An approach for reducing the production costs is developing processes that use renewable low-cost raw materials or high-pollutant wastes. A wide variety of alternative raw materials are currently available as nutrients for industrial fermentations, namely, various agricultural and industrial by-products and waste materials (Makkar and Cameotra 2002; Savarino et al. 2007; Ferreira 2008; da Silva et al. 2009; Montoneri et al. 2009).

However, the production cost of synthetic surfactants is not affordable for their use in larger ecosystems. The literature evidenced that the marine microbes are scarcely explored for the production of biosurfactants. The sponge-associated marine bacteria are current focus of bioactive leads from the marine environment (Selvin et al., 2009).

13.4.1 Cheap Substrates: Economical and Promising Alternatives

Production economy is the major bottleneck in biosurfactant production, as is the case with most biotechnological processes. Often, the amount and type of a raw material can contribute considerably to the production cost; it is estimated that raw materials account for 10–30% of the total production costs in most biotechnological processes. Thus, to reduce this cost it is desirable to use low-cost raw materials (Makkar and Cameotra 2002). One possibility explored extensively is the use of cheap and agro-based raw materials as substrates for biosurfactant production. A variety of cheap raw materials, including plant-derived oils, oil wastes, starchy substances, lactic whey, and distillery wastes (DWs), have been reported to support biosurfactant production.

The availability of raw materials for scaled-up production processes and acceptable production economics has widened the scope of biosurfactants. Most of the biosurfactants are produced from agricultural residues and from industrial waste products. The main problem related to the use of alternative substrates as culture medium is to find a waste with the right balance of nutrients that permits cell growth and product accumulation (Makkar and Cameotra 1999). Thus they have additional advantages from the viewpoint of resource replacement and recycling.

Modern society produces high quantity of waste materials through activity related to industries, forestry, agriculture, and municipalities (Martins et al. 2006). These inexpensive agro-industrial waste substrates include olive oil mill effluent, plant oil extracts and waste, distillery and whey wastes (WWs), potato process effluent, and cassava wastewater. These waste materials are some examples of food industry by-products or wastes that can be used as feedstock for biosurfactant production. The use of such waste materials serves a dual role of generating a usable product and reducing waste disposal (Makkar et al. 2011).

13.4.1.1 Vegetable Oils and Oil Wastes

Several studies with plant-derived oils have shown that they can act as effective and cheap raw materials for biosurfactant production, for example, rapeseed oil, babassu oil, and corn oil. Similarly, vegetable oils such as sunflower and soybean oils were used for the production of rhamnolipid, sophorolipid, and mannosylerythritol lipid biosurfactants by various microorganisms. Apart from
various vegetable oils, oil wastes from vegetable oil refineries and the food industry were also reported as good substrates for biosurfactant production (Trummler et al. 2003; Pekin et al. 2005; Kim et al. 2006).

Several plant-derived oils, for example, jatropha oil, mesua oil, castor oils, ramtil oil, and jojoba oil, are not suitable for human consumption due to their unfavorable odor, color, and composition and are, therefore, available at much cheaper rates. Incorporation of these cheaper oils and oil wastes in the industrial production media might potentially reduce the overall costs of biosurfactant production, making them challenging targets for future R&D activities (Mukherjee et al. 2006).

13.4.1.1.1 Biosurfactant Production Using By-Products of Vegetable Industries
Vegetable oils are a lipidic carbon source and mostly comprise saturated or unsaturated fatty acids with 16–18 carbon atoms chain. Researchers have used variety of vegetable oils from canola, corn, sunflower, safflower, olive, rapeseed, grape seed, palm, coconut, fish, and soybean. The crude or unrefined oils extracted from oilseeds are generally rich in free fatty acids, mono-, di-, and triacylglycerides, phosphatides, pigments, sterols, tocopherols, glycerol, hydrocarbons, vitamins, protein fragments, trace metals, glycolipids, pesticides, resinous, and mucilaginous materials (Dumont and Narine 2007).

13.4.1.1.2 Biosurfactant Production Using a Single Substrate of Vegetable-Processing Industries
Mercade et al. (1993) were the first group to show the production of rhamnolipids by P. aeruginosa 47T2 when grown on olive oil mill effluent as the sole carbon source (a major waste problem in Spain). This study was important in demonstrating the possibility of using other lipophillic wastes for wider application. Kitamoto et al. (1993) studied the interfacial and antimicrobial properties of two kinds of mannosylerythritol lipids (MEL-A and B), biosurfactants, produced by C. antarctica T-34, when grown on soybean oil as substrate. As the biosurfactant produced in this study exhibited antimicrobial activity particularly against Gram-positive bacteria, the process could be more economical because of high-value application in pharmaceutical industry. Sim et al. (1997) have tested mixture of vegetable oils (canola oil, soybean, and glucose) for rhamnolipid production by P. aeruginosa UW-1 and reported 10–12-fold increase in rhamnolipid production on vegetable oils in comparison to glucose. Camargo-de-Morais et al. (2003) studied the production of a glycolipid with emulsifier properties during cultivation of Penicillium citrinum on mineral medium with 1% olive oil as carbon source. The growth-associated emulsifier production reached maximal activity at 60 h of cultivation with the production yield (Yp/s) of 0.54. An emulsifier that was stable in a wide range of pH and temperature was stimulated by high salt concentration implying a possible application in industrial waste or marine remediation. Chang et al. (2005) reported the production of biosurfactant by Pseudoxanthomonas kaohsiungensis sp. nov. strain J36T during cultivation on olive oil as the sole carbon and energy source.

Rufino et al. (2007) studied the cultivation of Candida lipolytica grown on groundnut oil for the production of a new biosurfactant. The preliminary investigation of chemical composition suggested that it was a lipopeptide in nature. The biosurfactant had a yield of 4.5 g/L and exhibited good surface activity, emulsification ability, and could withstand high salt concentration but was not thermo stable. They later also applied sequential factorial design to optimize biosurfactant production by C. lipolytica using soybean oil refinery residue as substrate (Rufino et al. 2008). In this study, they evaluated the impact of three cultivation factors, amounts of refinery residue, glutamic acid, and yeast extract. The biosurfactant product showed high surface activity and emulsifying ability and was very stable at wide range of pH (2–12), temperatures (0–120°C), and salinity (2–10% NaCl). They concluded that combination of an industrial waste and a cheap substrate is a promising approach to reduce production cost.

Coimbra et al. (2009) also showed the biosurfactant production by six Candida strains cultivated in insoluble (n-hexadecane) and soluble substrates (soybean oil, groundnut oil refinery residue, corn steep liquor, and glucose). These biosurfactants were able to remove 90% of the hydrophobic
contaminants from sand. Oliveira et al. (2009) used palm oil, a low-cost agricultural by-product, which is used in as raw material for soap and food industries, for biosurfactant production using *Pseudomonas alcaligenes* (a strain isolated from crude oil-contaminated soil). They achieved a biosurfactant concentration of 2.3 g/L and E24 more than 70% with the hexane, jet fuel, and crude oil.

Plaza et al. (2011) aimed at the development of economical methods for biosurfactant production by the use of unconventional substrates. The research investigated the potential of utilizing agro-industrial wastes to replace synthetic media for cultivation of *Bacillus* strains and biosurfactant production. In total, 21 of the waste products from dairy, sugar, fatty, fruit and vegetable-processing industries, breweries, and distillery were examined. Three bacterial strains were identified by 16S rRNA gene sequencing: *B. subtilis* (I′-1a), *Bacillus* sp. (T-1), and *Bacillus* sp. (T′-1). The best unconventional substrates for bacteria growing and biosurfactant production at 30°C under aerobic conditions were molasses, brewery effluents, and fruit and vegetable decocion from the processing factory.

13.4.1.1.3 Biosurfactant Production Using Mixed Substrates of Vegetable Industries

To make processes more economical some researchers followed an approach of mixed substrates as carried out by Casas and Garcia-Ochoa (1999), who utilized the capability of *Candida bombicola* to produce sophorolipid biosurfactant properties when grown in medium composed of two different carbon sources and a nitrogen source. One of the carbon sources was a readily available sugar to maximize biomass production and the second was sunflower oil and they were able to achieve 120 g/L sophorolipid in 8 days under the best operational conditions. Bednarski et al. (2004) reported the synthesis of biosurfactants by *C. antarctica* or *Candida apicola* in the cultivation medium supplemented with oil refinery waste (either with soap stock or post-refinery fatty acids). Enrichment of the medium with the oil refinery waste resulted in a 7.5–8.5-fold greater concentration of glycolipids in comparison to the medium without addition of oil refinery waste. Costa et al. (2006) evaluated the possible use of oil from Buriti (*Mauritia flexuosa*), Cupuaçu (*Theobroma grandiflora*), Passion Fruit (*Passiflora alata*), Andiroba (*Carapa guianensis*), Brazilian nut (*Bertholletia excelsa*), and Babassu (*Orbignya* spp.) for rhamnolipid production by *P. aeruginosa* LBI. They observed extensive surface tension reduction and good emulsification. The highest rhamnolipid concentrations were obtained from Brazilian nut (9.9 g/L) and passion fruit (9.2 g/L) oils. Another Brazilian group led by Prieto (Prieto et al. 2008) isolated *P. aeruginosa* LBM10 from a southern coastal zone in Brazil, which could produce a rhamnolipid-type biosurfactant growing on different cheap carbon sources, such as soybean oil, soybean oil soapstock, fish oil, and glycerol. A combination of sugarcane molasses and three different oils (soybean, sunflower, or olive oil) was used a low-cost media by Daverey and Pakshirajan (2009), for the production of sophorolipids from the yeast *C. bombicola*. They achieved a yield approx. 24 g/L in this mixed media in comparison to media with single constituents. This yield was comparable to the costly conventional synthetic medium containing yeast extract, urea, soybean oil, and glucose.

Fontes et al. (2012) studied the production of a biosurfactant synthesized by *Yarrowia lipolytica* using different renewable resources as carbon source was investigated. Crude glycerol, a biodiesel co-product, and clarified cashew apple juice (CCAJ), an agro-industrial residue, were applied as feedstocks for the microbial surfactant synthesis. The microorganism was able to grow and produce biosurfactant on CCAJ and crude glycerol, achieving maximum emulsification indexes of 68.0% and 70.2% and maximum variations in surface tension of 18.0 mN/m and 22.0 mN/m, respectively. Different organic solvents (acetone, ethyl acetate, and chloroform–methanol) were tested for biosurfactant extraction. Maximum biosurfactant recovery was obtained with chloroform–methanol (1:1), reaching 6.9 g/L for experiments using CCAJ and 7.9 g/L for media containing crude glycerol as carbon source. The results herein obtained indicate that CCAJ and the co-product of biodiesel production are appropriate raw materials for biosurfactant production by *Y. lipolytica*.

Luna et al. (2012) studied the use of two industrial wastes, corn steep liquor and groundnut oil refinery residue, as low-cost nutrients for the production of a biosurfactant by *Candida sphaerica* (UCP 0995). They used an optimized medium with distilled water supplemented with 9% groundnut...
oil refinery residue and 9% corn steep liquor as substrates to produce biosurfactants by *C. sphaerica*, at 28°C during 144 h under 200 rpm. The isolated biosurfactant was formed with a yield of 9 g/L. The biosurfactant showed high surface tension-reducing activity the 25 mN/m, a small CMC value (0.025%), thermal (5–120°C), and pH (2–12) stability with respect to surface tension reducing activity and to emulsification activity and tolerance under high salt concentrations (2–10%). The biosurfactant was characterized as glycolipid and recovered 95% of the motor oil adsorbed in a sand sample, showing great potential to be used in bioremediation processes, especially in the petroleum industry.

### 13.4.1.1.4 Biosurfactant Production from Vegetable Oil Industries’ Wastes

Benincasa et al. (2002) reported isolating a rhamnolipids producing *P. aeruginosa* strain LBI using soap stock as the sole carbon source. Soap stock is the waste from the sunflower oil process, the main co-product from the seed-oil-refining industry. Rhamnolipid concentration in the range of 15.9 g/L was achieved. Nitschke et al. (2005) evaluated the oil wastes as alternative low-cost substrates for the production of rhamnolipids by *P. aeruginosa* LBI strain. They used wastes obtained from soybean, cottonseed, babassu, palm, and corn oil refinery. The soybean soap stock waste was the best substrate, generating 11.7 g/L of rhamnolipids and a production yield of 75%. Another soybean-associated waste, which has been utilized for biosurfactant production, is soy molasses, a by-product of soybean oil processing. It contains high fermentable carbohydrate (30% w/v) and is about 60% of solids carbohydrate that makes it well suited for economical production of biosurfactants. Increased interest in consumption of healthy soy protein-based foods and drinks has established a sustained growing soy-based industry and as a result an abundance of waste by-products (Deak and Johnson 2006). It has been found that soy molasses act as carbon and nitrogen source for the fermentative production of sophorolipids by *C. bombicola* with yields of 55 g/L (Solaiman et al. 2007). In this study, they achieved a further cost reduction by substitution of expensive yeast extract and urea from the growth medium. In an effort to economize biosurfactant production, Thavasi et al. (2008) used a mixture of peanut oil cake and waste motor lubricant oil as a substrate for the biosurfactant production. Peanut oil cake, a rich source of protein and lipids, is a by-product during the peanut oil manufacturing process. The cost of peanut cake is negligible compared with other pure carbon sources and waste motor oil is a waste product generated by the geared motor vehicles’ after long use. They confirmed that *Bacillus megaterium*, *Azotobacter chroococcum*, and *Corynebacterium kutscheri* had the capability of using these substrates for biosurfactant production with better yields achieved with peanut oil cake. Recently, the authors have reported the biosurfactant production by *Lactobacillus delbrueckii* using peanut oil cake as the carbon source. The biosurfactant produced (5.35 mg/mL) was capable of promoting biodegradation to a large extent (Thavasi et al. 2011). These studies showed the suitability of peanut oil cake as a substrate for glycolipid biosynthesis (Makkar et al. 2011).

Govindammal and Parthasarathi (2013) studied the production of biosurfactant by *Pseudomonas fluorescence* MFS03 isolated from mangrove forest soil, Pitchavaram, Tamilnadu, India, using renewable substrates. The maximum biomass (11.73 mg/mL) and biosurfactant production (9.23 mg/mL) was observed with coconut oil cake at 120 and 132 h, respectively. Characterization of the biosurfactant revealed that it is a glycolipid with chemical composition of carbohydrate (48.5 μg 0.1/mL) and lipid (50.2 μg 0.1/mL). The biosurfactant shows higher emulsification activity (89%) with crude oil and coconut oil (84%) among the different hydrocarbon tested. Emulsification activity of the biosurfactant against different hydrocarbons showed its possible application in insecticide cleaning in vegetables. Monocrotophos with initial concentration of 100 ppm was washed out with 10 ppm concentration of the biosurfactant.

### 13.4.1.2 Lactic Whey and Distillery Wastes

Lactic whey from dairy industries has also been reported to be a cheap and viable substrate for biosurfactant production. The effluent from the dairy industry, known as dairy wastewater, supports
good microbial growth and is used as a cheap raw material for biosurfactant production (Dubey and Juwarkar 2004). Furthermore, the potential use of dairy wastewaters provides a stratagem for the economical production of biosurfactants and efficient dairy wastewater management.

In a study by Dubey et al. (2012), combinations of DW with other industrial wastes, namely, curd WW, fruit-processing waste (FPW), and sugar industry effluent (SIE) were evaluated to replace the use of water that was reported earlier for biosurfactant production from 1:3 diluted DW by using four new bacterial cultures BS-A, BS-J, BS-K, and BS-P, isolated from soil collected from a distillery unit. These isolates have the potential to produce biosurfactant from these individual wastes and in their combinations. Highest biomass and biosurfactant yields with higher reduction in the chemical oxygen demand (COD), total sugars, nitrogen, and phosphate levels were obtained in 1:1:1 proportion of DW + WW + FPW followed by DW + WW + SIE and individual wastes. The combinations of wastes improved the yields of biosurfactants by 18–41% and reduced COD of the combined wastes by 76–84.2%. Total sugars, nitrogen, and phosphate levels reduced in the range of 79–86%, 58–71%, and 45–59%, respectively. Among the four microbial isolates tested, BS-J and BS-P were the efficient biosurfactant producers and were identified as Kocuria turfanesis and P. aeruginosa based on the 16S rDNA sequence and phylogenetic analyses. Benefits derived by using combined DW with other wastes are improved production of biosurfactant as resource and saving precious water and the costly nutrients with concomitant reduction in pollution load of the wastes.

13.4.1.2.1 Biosurfactant Production from Dairy and Sugar Industry Wastes

The dairy industry has a considerable amount of by-products such as buttermilk, whey, and their derivatives. Whey is a liquid by-product of cheese production, rich in lactose (75% of dry matter), and containing other organic water-soluble components (12–14% protein). Daniel et al. (1998) reported the high yields of sophorolipids production with whey concentrate and rapeseed oil as substrate. However, in this study the organisms did not utilize lactose. Daverey and Pakshirajan (2010) also reported the production of sophorolipids by the yeast C. bombicola on medium containing mixed hydrophilic substrate (deproteinized whey and glucose), yeast extract, and oleic acid. They could achieve a yield up to 34 g/L under experimental conditions in optimized medium formulation.

Molasses is a co-product of sugar production, both from the sugar cane and sugar beet industry in India as runoff syrup from the final step of sugar crystallization after which further sugar crystallization becomes uneconomical. The main reasons for the widespread use of molasses as substrate are its low price compared with other sources of sugar and the presence of several other compounds and vitamins (Makkar et al. 2011). Molasses are mainly composed of sugars (sucrose 48–56%), non-sugar organic matter (9–12%), proteins, inorganic components, and vitamins. The total fermentable sugars are in the range of 50–55% by weight. Traditionally, molasses was used as an animal feed, production of pullulan, xanthan gum, citric acid, and in ethanol industries (Maneerat 2005). Molasses with its high sugar content is a good substrate for biosurfactant production as evidenced by many studies covering two decades. The possibility of using soy molasses as a relatively inexpensive and easily available resource to produce rhamnolipids was investigated by Rashedi et al. (2005). They reported that biosurfactant production by the bacterial strain on soy molasses was growth related. The specific production rate of rhamnolipid when using 2%, 4%, 6%, 8%, and 10% of molasses were 0.00065, 4.556, 8.94, 8.85, and 9.09, with rhamnolipids/biomass yield of 0.003, 0.009, 0.053, 0.041, and 0.213, respectively. Others such as Raza et al. (2007) reported the production of a microbial surfactant by growing P. aeruginosa EBN-8 mutant on clarified blackstrap molasses as a sole carbon and energy source. Maximum rhamnolipid (1.45 g/L) yields were observed, at 96 h of incubation on 2% total sugar-based molasses amended with sodium nitrate. In an effort to reduce the cost of surfactin production by B. subtilis BSS, Abdel-Mawgoud et al. (2008) optimized the environmental and nutritional production conditions for economizing of the production process. Optimized medium containing 16% molasses, 5 g/L NaNO₃, and the trace elements solution of ZnSO₄·7H₂O (0.16 g/L), FeCl₃·6H₂O (0.27 g/L), and MnSO₄·H₂O (0.017 g/L) gave surfactin yield of 1.12 g/L. In conclusion, both molasses and whey have been successfully utilized as substrate...
for biosurfactant production. In addition, rhamnolipid biosurfactant production using 18 strains of *Pseudomonas* sp. were investigated by Onbasli and Aslim (2009). The two strains with the highest yield of rhamnolipids production (*Pseudomonas luteola* B17 and *P. putida* B12) were further examined for rhamnolipid production on different sugar beet molasses concentrations. Maximum rhamnolipid production was achieved with 5% (w/v) of molasses and occurred after 12 h incubation. More studies, however, are required to overcome the problems associated with batch variability and ways to standardize the pretreatment requirement of these substrates for more productive output.

### 13.4.1.3 Starchy Substrates

Waste starchy materials are also potential alternative raw materials for the production of biosurfactants. Potato process effluents (wastes from potato-processing industries) were used to produce biosurfactant by *B. subtilis*. Cassava wastewater, another carbohydrate-rich residue, which is generated in large amounts during the preparation of cassava flour, is also an attractive substrate in biotechnological processes and has been used for surfactin production by *B. subtilis* (Noah et al. 2005; Nitschke and Pastore, 2006). These wastes are obtained at low cost from the respective processing industries and are as potent as low-cost substrates for industrial level biosurfactant production. Several other starchy waste substrates, such as rice water (effluent from rice-processing industry and domestic cooking), cornsteep liquor, and wastewater from the processing of cereals, pulses, and molasses, have tremendous potential to support microbial growth and biosurfactant production. Extensive research is needed to establish the suitability of these carbohydrate-rich substrates in industrial-level biosurfactant production process (Mukherjee et al. 2006).

#### 13.4.1.3.1 Biosurfactant from Starch-Rich Substrates

Starch is a major agricultural product of corn, tapioca, wheat, and potatoes, which are major crops. Other sources include sugar plants such as sugar beet, sugar cane, or sugar sorghum. Sugar and starch-processing industries also produce large amount of solid residues of starch-containing wastewater. The high fiber content of the solid residue makes them a good source for paper and packaging industries, whereas the carbohydrate-rich wastewater is a suitable substrate for production of microbial products. Biological wastes rich in starchy materials are suitable for biosurfactant production.

One such substrate is potato which is one of the important staple foods and a lucrative cash crop in many countries. Processing of potatoes results in starch-rich wastewater, potato peels, and unconsumable potatoes, which are rich substrates for the microbes. Fox and Bala (2000) attempted to produce biosurfactants utilizing potato-associated waste. They evaluated potato substrate as a carbon source for biosurfactant production using *B. subtilis* ATCC 21332. They compared growth, surface activity, and carbohydrate utilization of *B. subtilis* ATCC 21332 on an established potato medium, simulated liquid, and solid potato waste media and a commercially prepared potato starch in a mineral salts medium. The results obtained indicated the utilization of potato substrate and production of surfactant as indicated by high surface tension reduction. The efficiency of two *B. subtilis* strains for the production of biosurfactants in two fermentation systems using powdered potato peels as substrate was investigated (Das and Mukherjee 2007). Potato peels were immersed in very hot water followed by oven drying. The dried peels were grinded to a paste and stored at 4°C before further use. Both the fermentation process resulted in biosurfactant (lipopeptides) with good surface activity and yield. Wang et al. (2008) applied a *B. subtilis* strain B6-1, for the production of biosurfactant using soybean and sweet potato residues in solid-state fermentation.

### 13.4.1.4 Biosurfactant Production from Lignocellulosic Waste

Lignocellulotic materials are among the most abundant organic carbon available on earth (Kukhar 2009), and they are the major components of different waste streams from various industries, forestry, agriculture, and municipalities. Such waste materials are mostly burned releasing CO$_2$ that contributes to the greenhouse effect. Lignocellulose consists of mainly three types of polymers—cellulose, hemicellulose, and lignin—that are strongly intermeshed and chemically bonded by both
Biosurfactants

noncovalent forces and covalent cross-linkages. From an economical point of view, lignocellulosic-rich agricultural residues can be employed for producing useful biomolecules such as biosurfactants. There have been reports of some forms of lignocellulosic wastes for the production of biosurfactant. Portilla-Rivera et al. (2007) were the first to look into the capability of \textit{Lactobacillus} sp. to use hemicellulosic hydrolyzates from various agricultural residues for simultaneous production of biosurfactants and lactic acid. Such dual production strategy makes biosurfactant more economically viable in market and reduce the effects of waste burning on environment. In their efforts they achieved reduced surface tension and biosurfactant yield of 0.71 g/g of biomass, when hemicellulosic hydrolyzates from trimming wine shoots were used. This study is important considering the large amount of pruning wastes of vine stocks generated worldwide and the resulting constitutive monomeric sugar solutions, which are potential renewable sources for the other biomolecules like lactic acid. They concluded that hemicellulosic sugars from the agricultural residues are interesting substrates for the competitive cost production of biosurfactants.

13.4.2 Bioprocess Development: Optimum Production and Recovery

An efficient and economical bioprocess is the foundation for every profit-making biotechnology industry; hence, bioprocess development is the primary step toward commercialization of all biotechnological products, including biosurfactants. Any attempt to increase the yield of a biosurfactant demands optimal addition of media components and selection of the optimal culture conditions that will induce the maximum or the optimum productivity. Similarly, efficient downstream-processing techniques and methods are needed for maximum product recovery.

13.4.3 Process Optimization: The Best Combination of Essential Factors

Several elements, media components, and precursors are reported to affect the process of biosurfactant production and the final quantity and quality. Different elements such as nitrogen, iron, and manganese are reported to affect the yield of biosurfactants, for example, the limitation of nitrogen is reported to enhance biosurfactant production in \textit{P. aeruginosa} strain BS-2 (Dubey Juwarkar, 2004) and \textit{Ustilago maydis} (Hewald et al. 2005). Similarly, the addition of iron and manganese to the culture medium was reported to increase the production of biosurfactant by \textit{B. subtilis} (Wei et al. 2003). The ratios of different elements such as C:N, C:P, C:Fe, or C:Mg affected biosurfactant production and their optimization enhanced it (Amézcua-Vega et al. 2007).

Makkar and Cameotra (2002) studied the effects of various factors on growth and biosurfactant production by \textit{B. subtilis} MTCC 2423. They found that sucrose (2%) and potassium nitrate (0.3%) were the best carbon and nitrogen sources. The addition of various metal supplements (magnesium, calcium, iron, and trace elements) greatly affected growth and biosurfactant production. The effect of the metal cations, used together, is greater than when they are used individually. The biosurfactant production increased considerably (almost double) by addition of metal supplements. Very high concentrations of metal supplements, however, inhibited biosurfactant production. Amino acids such as aspartic acid, asparagine, glutamic acid, valine, and lysine increased the final yield of biosurfactant by about 60%. The organism could produce biosurfactant at 45°C and within the pH range of 4.5–10.5. The biosurfactant was thermostable and pH stable (from 4.0 to 12.0). The capability of the organism to produce biosurfactant under thermophilic, alkaliphilic, and halophilic conditions makes it a suitable candidate for field applications. Infrared, nuclear magnetic resonance, and mass spectroscopy studies showed the surfactant to be identical to surfactin.

Nutritional requirements for maximal production of biosurfactant by an oil field bacterium \textit{P. putida} were determined by Pruthi and Cameotra (2003). The optimal concentrations of nitrogen, phosphate, sulfur, magnesium, iron, potassium, sodium, calcium, and trace elements for maximal production of biosurfactants were ascertained, and they formulated a new “Pruthi and Cameotra” salt medium. Data from their study showed that maximal biomass (2.4 g/L) and biosurfactant
production (6.28 g/L) takes place after 72 h of growth on 2% hexadecane. The biosurfactant was produced optimally over pH and temperature ranges of 6.4–7.2°C and 30–40°C, respectively.

The biosurfactant production of a marine actinobacterium *Brevibacterium aureum* MSA13 was optimized by Kiran et al. (2010) using industrial and agro-industrial solid waste residues as substrates in solid-state culture (SSC). On the basis of the optimization experiments of their study, they reported that the biosurfactant production by MSA13 was increased to threefold over the original isolate under SSC conditions with pretreated molasses as substrate and olive oil, acrylamide, FeCl3, and inoculums size as critical control factors. The strain *B. aureum* MSA13 produced a new lipopeptide biosurfactant with a hydrophobic moiety of octadecanoic acid methyl ester and a peptide part predicted as a short sequence of four amino acids including pro-leu-gly-gly. The biosurfactant produced by the marine actinobacterium MSA13 can be used for the microbially enhanced oil recovery processes in the marine environments.

### 13.4.4 Downstream Processing: Fast, Efficient, and Cheap Product Recovery

Even if optimum production is obtained using optimal media and culture conditions, the production process is still incomplete without an efficient and economical means for the recovery of the products. Thus, one important factor determining the feasibility of a production process on a commercial scale is the availability of suitable and economic recovery and downstream procedures. For many biotechnological products, the downstream processing costs account for ~60% of the total production costs. Several conventional methods for the recovery of biosurfactants are: acid precipitation, solvent extraction, crystallization, and ammonium sulfate precipitation and centrifugation. A few unconventional and interesting recovery methods have also been reported in recent years. These procedures take advantage of some of the other properties of biosurfactants—such as their surface activity or their ability to form micelles and/or vesicles—and are particularly applicable for large-scale continuous recovery of extracellular biosurfactants from culture broth. A few examples of such biosurfactant recovery strategies include foam fractionation (Davis et al. 2001; Noah et al. 2005), ultrafiltration (Sen and Swaminathan, 2005), adsorption–desorption on polystyrene resins and ion exchange chromatography, and adsorption–desorption on wood-based activated carbon (Dubey et al. 2005).

### 13.4.5 Mutant and Recombinant Strains: The Hyper-Producer

The genetics of the producer organism is an important factor affecting the yield of all biotechnological products because the capacity to produce a metabolite is bestowed by the genes of the organism. The bioindustrial production process is often dependent on the use of hyper-producing microbial strains: even with cheap raw materials, optimized medium and culture conditions, and efficient recovery processes, a production process cannot be made commercially viable and profitable until the yield of the final product by the producer organisms is naturally high. Moreover, the industrial production process is dependent on the availability of recombinant and mutant hyper-producers if good yields are lacking from the natural producer strains. Even if high-yielding natural strains are available, the recombinant hyper-producers are always required, to economize further the production process and to obtain products with better commercially important properties.

Besides the natural biosurfactant producer strains, a few mutant and recombinant varieties with enhanced biosurfactant production characteristics are reported in the literature.

Sekhon et al. (2011) utilized olive oil as a carbon source, which has been explored by many researchers. However, studying the concomitant production of biosurfactant and esterase enzyme in the presence of olive oil in the *Bacillus* species and its recombinants is a relatively novel approach. In their study, *Bacillus* species isolated from endosulfan-sprayed cashew plantation soil was cultivated on a number of hydrophobic substrates. Olive oil was found to be the best inducer of biosurfactant activity. The protein associated with the release of the biosurfactant was found to be an esterase. There was a twofold increase in the biosurfactant and esterase activities after the successful cloning
of the biosurfactant genes from *B. subtilis* SK320 into *E. coli*. Multiple sequence alignment showed regions of similarity and conserved sequences between biosurfactant and esterase genes, further confirming the symbiotic correlation between the two. Biosurfactants produced by *B. subtilis* SK320 and recombinant strains BioS a, BioS b, BioS c were found to be effective emulsifiers, reducing the surface tension of water from 72 dynes/cm to as low as 30.7 dynes/cm. The attributes of enhanced biosurfactant and esterase production by hyper-producing recombinant strains have many utilities from industrial viewpoint. This study for the first time has shown a possible association between biosurfactant production and esterase activity in any *Bacillus* species. Biosurfactant–esterase complex has been found to have powerful emulsification properties, which shows promising bioremediation, hydrocarbon biodegradation, and pharmaceutical applications.

### 13.5 CONCLUSION

Surfactants, both chemical and biological, are amphiphilic compounds, which can reduce surface and interfacial tensions by accumulating at the interface of immiscible fluids and increase the solubility, mobility, bioavailability, and subsequent biodegradation of hydrophobic or insoluble organic compounds. Investigations on their impacts on microbial activity have generally been limited in scope to the most common and best characterized surfactants. Recently, a number of new biosurfactants have been described and accelerated advances in molecular and cellular biology are expected to expand our insights into the diversity of structures and applications of biosurfactants. Biosurfactants also exhibit natural physiological roles in increasing bioavailability of hydrophobic molecules and can complex with heavy metals, and some also possess antimicrobial activity. They have been exploited in this way, for example, as antimicrobial agents in disease control and to improve degradation of chemical contaminants. Considering the growing awareness on the climate change issues, the greener processes for the production of biosurfactants from industrial waste and bioremediation of petroleum hydrocarbons using biosurfactants will greatly reduce the uses of chemicals and xenobiotics in the environment.

The application of economical technologies and process based on utilization of waste conversion to products is also gaining ground. The commercial realization of the biosurfactants that are restricted by high production costs can be equipoise by optimized production conditions provided by utilization of the cheaper renewable substrates and application of novel and efficient multistep downstream processing methods. Recombinant and mutant hyper-producer microbial strains, able to grow on a wide range of cheap substrates, may produce biosurfactants in high yield and potentially bring the required breakthrough for their economic production. The true significance of these processes will be justified only when these studies will be scaled up to commercially viable processes.

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