**Application of Biosurfactant Producing PGPR in Agriculture: A Mini Review**

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**Abstract:** This paper reviews the immeasurable use of biosurfactants in agricultural soil and agrochemical industries. Biosurfactants produced by bacteria, yeasts, and fungi, generally called as green surfactants. Biosurfactants are considered as eco-friendly and less toxic as compared to synthetic surfactant because they are biodegradable in soil and not persist for long duration in soil. Biosurfactants play a key role in motility, signaling, and biofilm formation governing plant–microbe interaction. In agriculture, biosurfactants can be used for biocontrol of phytopathogens as well as for increasing the bioavailability of nutrient for beneficial plant associated microbes. Besides, biosurfactants are the alternatives for enhanced biodegradation of hydrocarbon, heavy metals, and organic compounds. The use of green surfactant in agriculture field can replace the drastic effect of synthetic surfactant in soil. Thus there is a great require for disquisition of biosurfactants producing PGPR and their potential role in agronomy. Biosurfactant can be produced traditionally by exploiting potential microorganisms. On the other hand, metagenomics approaches can be made for unculturable microorganisms. Modern novel techniques, such as Gas chromatography mass spectrometry, nuclear magnetic resonance and fast atom bombardment mass spectrometry, MALDI-TOP mass spectrometry, etc. are being used for purification and identification of biosurfactants.

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**Keywords:** Biosurfactants, Phytopathogens, Agronomy, Biocontrol, Biodegradation Metagenomics.

**1. Introduction**

Biosurfactants are amphiphilic compounds which reduce surface and interfacial tensions by accumulating at the interface of immiscible fluids and increase the mobility, solubility and bioavailability. Biosurfactants are produced extra cellularly or as part of the cell membrane by bacteria, yeasts and fungi. *Pseudomonas aeruginosa* produces rhamnolipids, *Candida bombicola* produces sophorolipids and *Bacillus subtilis* produces a lipopeptide called surfactin (Mulligan, 2005). Biosurfactants play an indispensable natural role in the swarming motility of microorganisms to participate in cellular physiological processes like signaling, differentiation and biofilm formation. Furthermore, microbial behaviour may be altered by adding surfactant or biosurfactants in the medium. In addition, biosurfactants have been used as antimicrobial agents in disease control and degradation of chemical contaminants (Van Hamme *et al.*, 2006). Besides, biosurfactants also have a variety of potential application, such as enhanced transport of bacteria, enhanced oil recovery, biological control of phytopathogens, bioremediation of organic compound, and cosmetic additives (Badour *et al*., 2003). A promising alternative to agrochemicals is the use of biosurfactant-producing plant growth-promoting rhizobacteria (PGPR) with plant roots. Biosurfactant is used worldwide at large-scale as it is preferred to chemical surfactants for it being eco-friendly and easily biodegradable (Desai and Banat, 1997). The cost of biosurfactants is much inexpensive as compared to chemical surfactant. At present worldwide surfactant markets are around $9.4 billion per annum (Shaw, 1994). According to Greek (1991) demand of surfactants is expected to increase at a rate of 35% toward the end of the century. Thus, in this review mainly highlight an overview use of biosurfactant in agriculture.

**2. Plant Growth Promoting Activity of Biosurfactant Producing Rhizobacteria**

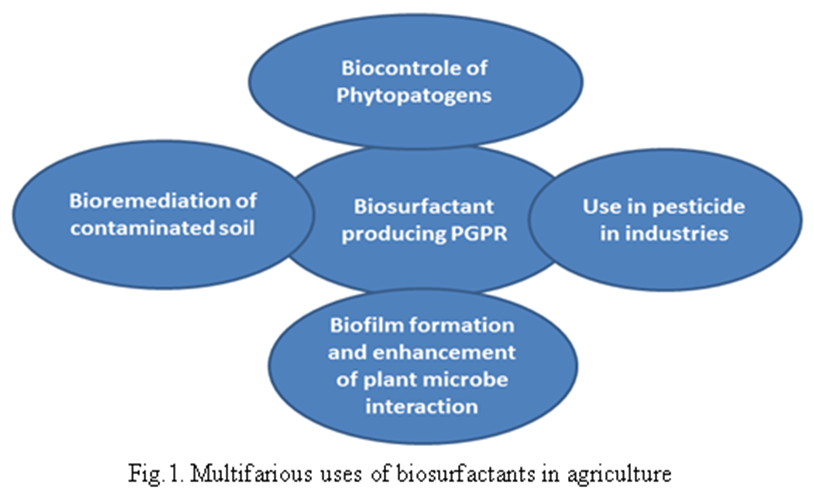
Plant growth promoting rhizobacteria (PGPR) are commonly used as bioinoculant for improving the growth and yield of agricultural crops. For development of an effective PGPR bioinoculant the bacterial species must bear diverse traits that can help in colonization of rhizosphere and survival under different stringent environment conditions. Biosurfactant producing rhizobacteria play a major role in plant growth promotion and enhancement of crop yield. Many species of biosurfactant producing bacteria reside in the rhizosphere region. A plant-growth promoting and biosurfactant-producing *Pseudomonas aeruginosa* A11 was also isolated from the rhizosphere of *Parthenium hysterophorus* (Singh and Cameotra, 2013). *Pseudomonas putida* strain 267 was also isolated from the rhizosphere of black pepper by Tran *et al*. (2008). The PGPR may have synergistic, neutral or antifungal properties. For example, biocompatibility between a biosurfactant producing *Bacillus subtilis* strain BS1 and an arbuscular mycorrhizal fungus *Glomus etunicatum* has been reported. Mycorrhizal colonization and BS1 bioinoculation increased the tolerance to strain of phenanthrene and increased plant biomass. BS1 strain secreted biosurfactant that enhanced the solubility of phenanthrene favoring the enrichment in rhizospheric soil and plant roots (Xiao *et al*., 2012). Different uses of biosurfactants in agriculture field are showed in Fig.1.

Similarly, *Bacillus amyloliquefaciens* strain KPS46 isolated from soybean secreted of compounds indole, lipopeptides and proteins that would have been involved in plant growth promotion (Buensanteai *et al*., 2008). Motility is an important event for colonization and microbial fitness in the plant environment, but the mechanisms employed by bacteria on plant surfaces not well known so far. Alsohim *et al*. (2014) isolated a viscosin biosurfactant producing Pseudomonas fluorescens SBW25 and studied the spreading motility and plant growth promoting activity. Alvarez *et al*. (2012) isolated plant associated rhizobacteria *Bacillus amyloliquefaciens* strains MEP218 and ARP23 and studies the production cyclic lipopeptides iturin or surfactin and fengycin.

**3. Biocontrol of Plant Pathogens by Biosurfactant Producing PGPR**

In agriculturecrop and post-harvested vegetables affecting by phytopathogenic fungi are a major drawback to food production and food storage. The farmers have become dependent on agrochemicals to face these problems. Intensive use of these compounds has led to the emergence of resistance among pathogens and severe unassertive environmental impacts. Hence, biocontrol of phytopathogens has emerged as a promising alternative to chemical pesticides for more rational and secure crop management (Arguelles-Arias *et al*., 2009). Cyclic lipopeptides (CLPs) biosurfactant producing strain of *Pseudomonas fluorescens* with antifungal propertieswas investigated in bulk soil and in the sugar beet rhizosphere (Nielsen and Sørensen, 2003). Biocontrol of root and foliar diseases of soybeans by using *Bacillus amyloliquefaciens* KPS46 has been reported by Thein and Prathuangwong *et al*. (2010).

Three families of *Bacillus* lipopeptides viz., surfactins, iturins and fengycins have demonstrated antagonistic activity against many phytopathogens including bacteria, fungi and oomycetes. These lipopeptides can also influence the ecological fitness of bacteria in terms of root colonization and beneficial interaction of *Bacillus* species with plants via stimulation of the host defense mechanisms. Different structural traits and physicochemical properties of these effective amphiphilic biomolecules explain their involvement in biocontrol of different plant pathogens (Ongena *et al*., 2007).



*Pseudomonas aeruginosa* LBI isolated from petroleum-contaminated soil produced rhamnolipids (RL) which showed good antibacterial activity (Benincasa *et al*., 2002). Biosurfactants have also been used for control of aflatoxin producing *Aspergillus parasiticum* NCIM 898 (Mule and Bhathena, 2012). *Phytophthora capsici* is a major pathogen of black pepper. Zoospores of *Phytophthora capsici* play an important role in host infection. A biosurfactant producing fluorescent *Pseudomonas* was isolated from the rhizosphere of black pepper and its genotypic diversity and biocontrol potential against *Phytophthora capsici* was determined (Tran *et al*., 2007). *Bacillus sonorensis* MBCU2 isolated from vermincompost-amended soil showed potential antagonistic activity against *Macrophomina phaseolina* *in vitro* (Pandya and Saraf, 2014). Lipopeptides are frequently produced by bacilli that exhibited antibacterial and antifungal activity against a wide spectrum of pathogenic bacteria and fungi exceptionally with surfactant activity (Kumar and Johri, 2012). Cell-free culture supernatant and purified biosurfactant were evaluated for biocontrol activity against *M. phaseolina* (Khare and Arora, 2011).

**4. Mode of action of Biosurfactant on Phytopathogens**

There areseveral mechanisms that explain the promotion of plant growth by bacteria residing in the rhizosphere. One of the major aspects of this stimulation is diseases suppression (Toure *et al*., 2004). Antimicrobial agents produced by PGPR play directly or indirectly a key role in biocontrol of phytopathogens (Leclere *et al*., 2005). The direct effect of biosurfactants on mycelia of *Pythium myriotylum* and *P. splendens* were hypothesized by Perneel *et al.* (2008)*,* whileDebode *et al.* (2007) found a major role of biosurfactants in the reduction of viability of *Verticillium* microsclerotia. Lipopeptides from the iturin and fengycin families showed formidable antifungal activity that suppressed the growth of a wide range of plant pathogens (Toure *et al*., 2004). Iturins produced by *B. subtilis* and other closely related bacilli comprise of iturins A–E, bacillomycins D, F, and L, and mycosubtilin (Stein, 2005). These molecules disrupt the yeast plasma membrane by forming small vesicles and also by aggregating membrane-spanning particles. They also release electrolytes and high molecular mass products and degrade phospholipids. Leclere *et al*. (2006) showed enhanced the spreading of *B. subtilis* 168 on B-medium after addition of a purified antifungal compound, mycosubtilin. In this process the role of mycosubtilin is based on decrease of the surface tension of the medium and increase in the wettability. This double activity could be considered as a synergistic effect towards phytopathogenic fungi in the field of biocontrol by increasing the ability of the bacteria to colonize target surfaces connected with the strong antifungal properties of mycosubtilin. The anti adhesive properties of the surfactin seem to have a remarkable potential for biocontrol of plant diseases (Ron and Rosenberg, 2001). Surfactin exhibits strong antibacterial and antifungal properties. This is probably due to its capability of permeabilizing cellular membranes (Heerklotz and Seelig, 2007).

**Table 1:** Recent reported bio-surfactant producing microbes from different cite.

|  |  |  |
| --- | --- | --- |
| **Microorganism** | **Source** | Reference/s |
| *Pseudomonas aeruginosa* | Oil Spilled soils | Padmapriya B *et al*.(2012) |
| *Pseudomonas aeruginosa* | Pyrene-contaminated soil | Cao *et al*. (2012) |
| *Stenotrophomonas maltophilia* | Asphalt-permeated soil | Belcher *et al*.(2012) |
| *Bacillus subtilis* | Contaminated Soil | Pemmaraju *et al*.(2012) |
| *Pseudomonas aeruginosa* | Oil-contaminated soil | Saikia *et al*.(2012) |
| *Agrobacterium tumefaciens, Bacillus cereus,* | Oil-contaminated soil | Saimmai *et al*.(2012) |
| *Pseudomonas aeruginosa strain and Kocuriaturfanesis strain* | Soil | Dubey *et al*.(2012) |
| *Bacillus amyloliquefaciens* | Oily sludge And petroleum-contaminated Soil | Liu *et al*.(2012) |
| *Acinetobacter calcoaceticus and Alcanivoraxdieselolei* | oil-contaminated sites | Hassanshahian *et al*.(2012) |
| *Klebsiella sp.* | Oil industry sludge | Jain *et al*.(2012) |
| *Pseudomonas sp.and Rhodococcus sp.* | crude oil contaminated site | Kumari *et al*.(2012) |
| *Pseudomonas aeruginosa* | Oil Field | Yan *et al*.(2012) |
| Pseudomonas aeruginosa | Rhizosphere | [Singh](http://link.springer.com/search?facet-creator=%22Anil+Kumar+Singh%22) and  [Cameotra](http://link.springer.com/search?facet-creator=%22Swaranjit+Singh+Cameotra%22) (2013) |
| *Pseudomonas sp.* | Rhizosphere soil | Liu *et al*.(2013) |
| *Paenibacillus macerans* | Oil-containing media | Liang *et al*.(2014) |
| *Serratia rubidaea* | Hydrocarbon contaminated soil | Nalini and Parthasarathi (2013) |
| *Bacillus subtilis* | Soil | Al-Wahaibi *et al*.(2014) |
| *Ochrobactrum anthropic* | Mangrove sediment | Noparat *et al*.(2014) |
| *Pseudomonas sp.* | Heavily petroleum hydrocarbon contaminated soil | Pacwa-Płociniczak *et al*. (2014) |
| *Lysinibacillus fusiformis* | River bank | Pradhan *et al*.(2014) |
| *Bacillus subtilis and Pseudomonas aeruginosa* | Oil-contaminated soil | Barin *et al*.(2014) |
| *Bacillus methylotrophicus* | Hydrocarbon contaminated aqueous medium | Chandankere *et al*.(2014) |
| *Streptomyces spp.* | Soils | Ferradji *et al*.(2014) |
| *B. subtilis subsp. spizizenii* | Uncontaminated soils | daSilva *et al*.(2015) |
| *Klebseilla sp.* | Hydrocarbon-contaminated soil | Ahmad *et al*.(2016) |

**5. Biodegradation of Organic Compound by Biosurfactant Producing PGPR**

Low solubility of certain hydrophobic soil contaminants limits the remediation process. However, the biosurfactant can improve the solubility and removal of hydrophobic compounds from contaminated soils. Farmers have manipulated the ecology of the soil by the addition or depletion of organic matter for centuries. The organic matter affects soil aeration, moisture holding capacity, drainage, structure, nutrient availability, and microbial ecology (Davey, 1996). The living components of soil require carbon as an energy source but there has been a ‘chemical drip, with minor organic energy input to the system for the last 65 years. This change of production has been harmful to soil health and water quality that has resulted in the increase in plant diseases and pests (Pimentel *et al*., 1991; Hoitink and Boehm, 1999). In agricultural soil increased contamination by different hydrocarbons is a serious environmental problem due to their persistence in nature for a long time. Biosurfactants promote biodegradation of hydrocarbons. The use of biosurfactants is an alternative over the chemical surfactant as the former is a better biodegradable and ecofriendly (Tambekar and Gadakh, 2013).

The significant biodegradation of polycyclic aromatic hydrocarbons (PAHs) was observed after 22 days in the presence of selected biosurfactants (Kosaric, 2001)*.* Oil spills may considerablydamage the soil quality. Scope of bioremediation strategies is limited due to low solubility and poor hydrocarbon accessibility which can be overcome by use of a class of biosurfactants called rhamnolipids (Kumar *et al.,* 2014). Composting is a biological process refers to biological degradation and transformation of organic materials under controlled environmental conditions. Numerous methods including additives, such as inoculation of microorganisms and the use of biosurfactants had been explored to find the effective ways for accelerated composting and improved compost quality (Zhang *et al.*, 2011). Long chain alkanes and polycyclic aromatic hydrocarbons have been denunciative the global environment due to its toxicity and low bioavailability. The remediation of soil contaminated PAHs is of major importance because most PAH compounds are known as carcinogens and mutagens (Wilson and Jones, 1993). Xia *et al*. (2014) isolated one novel strain of *Pseudomonas* from heavy oil contaminated soil which could degrade long-chain alkanes and polycyclic aromatic hydrocarbons (PAHs). The strain produced the surfactin, fengycin and lichenysin, which were mostly recognized as common metabolites produced by *Bacillus* sp.

Rhamnolipid is a biosurfactant produced by several species of *Pseudomonas* which moist the hydrophobic soils by lowering the cohesive and adhesive surface tension. *Pseudomonas* efficiently degrades alkanes, PAHs and produces the rhamnolipids. Biodegradation of organic compound by employing autochthonous and allochthonous microbial flora has been accepted as an important method for the treatment of oil sludge. Biosurfactants have also been used to recover oil from oil sludge and to enhance the biodegradation of oil sludge process (Helmy *et al*., 2008).

Application of rhamnolipid causes minimal adverse impact on the soil and groundwater as compared to that of chemical wetting agents. However, its application has more advantages when used to renovate irrigation in the agricultural soil, specifically under draught conditions. Furthermore, water surface tension decline linearly with the increase in concentration of rhamnolipid. The attractive forces between rhamnolipid molecules contribute to micelle formation and facilitate rhamnolipid transport (Renfro *et al*., 2014). Biosurfactants from a thermophilic strain *Acinetobacter calcoaceticus* BU03 effectively enhanced the solubility of PAHs. Inoculation of *A. calcoaceticus* BU03 or its biosurfactants significantly increased the emulsifying capacity of soil, and increased desorption of PAHs from soil to aqueous phase in which they can be degraded by *B. subtilis* B-UM. The application of biosurfactants produced by *A. calcoaceticus* was effective and enhanced the biodegradation of PAHs in thermophilic composting (Zhao and Wong, 2009).

**6. Hydrocarbon**

Hydrocarbon fuels are one of the most common oecumenical environmental pollutants which cannot be degraded easily due to their hydrophobic nature. This is particularly true with pollutants made up of many deferent compounds such as crude oil or petroleum. The uncontrolled industrial leakage, diesel oil and its constituents might act as a persistent soil and water pollutant. Petroleum hydrocarbons are becoming the major global problem for the environment. They are long time persistent in the environment, toxic and present significant health risks to human (Hentati *et al*., 2013). Autochthonous microorganisms used in bioremediation processes can reduce the risks of hydrocarbon-contaminated soils (Suja *et al*., 2014). Biodegradation of hydrocarbons in contaminated soil of wheat and mustard crops was investigated by biosurfactant producing bacterial consortium (Kumar *et al*., 2013). Biofilm- mediated bioremediation presents a conversant alternative to bioremediation with planktonic microorganisms. Three species of *Pseudomonas* biofilm-associated cells, namely *P. fluorescens, P. putida* and *P. aeruginosa* were found to degrade gasoline, xylene, benzene and cyclohexane. Changes in biofilm formation and siderophore production were monitored in the presence of different concentrations of benzene and xylene. All strains synthesized biosurfactant compounds and tolerated aromatic hydrocarbon more than the cyclic compounds. Hydrocarbon mixture or gasoline could be better biodegraded by bacterial consortium than alone inoculation alone (Meliani and Bensoltane, 2014).

Increase in public awareness of environmental pollution has resulted in the search and development of new technologies that help in decontamination of organic and inorganic contaminants. An alternative and eco-friendly method of remediation technology of environment contaminated with these pollutants is the use of biosurfactant-producing microorganisms and biosurfactants. Diversity of biosurfactants makes them an attractive group of compounds for potential use in a wide range of industrial and biotechnological applications (Pacwa-Płociniczak *et al*, 2011). The use of biosurfactants has been found to increase the degradation of crude oil or other hydrocarbons (Pruthi and Cameotra, 1997).

**Table 2: Some common biosurfactants and their origin.**

|  |  |  |
| --- | --- | --- |
| **Head group** | **Biosurfactant** | **Microorganism** |
| Fatty acids, neutral lipids, and | Fatty acid Neutral lipid | *Corynebacterium lepus* (Cooper *et al*. 1981), *N. erythropolis* (Kretschmer *et al.* 1982) |
| phospholipids | Phospholipid | *Thiobacillus thiooxidans* (Knickerbocker *et al*. 2000) |
| Lipopeptides | Surfactin | *Bacillus subtilis, Bacillus pumilus A* (Seydlova and Svobodova, 2008) |
|  | Viscosin | *Pseudomonas fluorescens, P. libanensis* (Laycock *et al*. 1991) |
|  | Serrawettin | *Serratia marcescens* (Matsuyama *et al*. 1992) |
| Glycolipids | Mannosylerythritol lipids | *Genus Pseudozyma* (yeast), *Candida antartica*, *Ustilago maydis* (Kitamoto *et al*. 2002) |
|  | Sophorolipids | *C. batistae, T. bombicola, C. lypolytica, C. bombicola, T.apicola, T.petrophilum, C. bogoriensis* (Van Bogaert *et al*. 2007) |
|  | Rhamnolipids | *Pseudomonas sp., P. aeruginosa* (Reiling *et al*. 1986) |
|  | Trehaloselipids | *Rhodococcus sp., Arthrobacter sp., R. erythropolis, N. erythropolis* (Lang and Philp1998) |
|  | Cellobiolipids | *Ustilagozeae, Ustilagomaydis* (Hewald *et al*. 2005) |
| Polymeric | Emulsan | *Acinetobacter calcoaceticus* (Rosenberg and Ron 1999) |
|  | Biodispersan | *A. calcoaceticus* (Rosenberg and Ron 1997) |
|  | Mannan–lipid–protein | *C. tropicalis* (Rosenberg and Ron 1999) |
|  | Alasan | *A. radioresistens* (Navonvenezia *et al.* 1995) |
| Siderophore | Flavolipids | *Flavobacterium* (Bodour *et al*. 2004) |

**Adopted from Biosurfactants: A General Overview (Gloria Soberon-Chavez and Raina M. Maier, 2011).**

**7. Biodegradation of Heavy Metal**

In recent years soil contamination caused by hazardous heavy metals has become a serious environmental problem. Biosurfactants can be used to detoxify heavy metals in contaminated soil to enhance the bioremediation. Most scientist and environmentalist have focused on the use of rhamnolipids. In the presence of a biosurfactant batch experiments were conducted to investigate arsenic (As) mobilization from mine tailings and to evaluate the feasibility of using biosurfactant in remediating As. Biosurfactant mediated sorption of mine tailings is essential for As mobilization. Arsenic mobilization was found to be articulately correlated with the mobilization of Fe and other metals. Biosurfactant enhance mobilization by helping to incorporate into the soluble complexes. Biosurfactants were used potentially to remove the bulk from mine tailings or contaminated soils under alkaline conditions (Wang and Mulligan, 2009).

Heavy metal tolerant and biofilm inhibiting *Bacillus* strains have been isolated and antimicrobial activities of biosurfactant produced by the strains have been studied. Biosurfactant produced by *Bacillus* sp.was a lipopeptide and exhibited strong surface activity (Sriram *et al*., 2011). A biosurfactant-producing PGPR *Bacillus* sp. J119 was isolated from heavy metal contaminated soil and investigated its effects on plant gr Sriram owth promoting features and heavy metal resistance. *Bacillus* sp. J119 was found cadmium uptake of maiz, rape, tomato and sudangrass in soil artificially contaminated with different levels of cadmium and promoted the plant growth (Sheng *et al*., 2008).

**8. Biosurfactants used in Biofilm Formation**

A bacterial cell can attach to surface and, after cell division and proliferation, forms aggregates which are generally referred to as biofilm. The bacterial cells secrete the protien and polysaccharides that form a hydrated gel- like slime that holds the biofilm together (Stewart and Franklin, 2008). Biofilm formation by *B. subtilis* is a complicated process that includes secretion of surfactin which is a lipopeptide antimicrobial agent (Bais *et al*., 2004). Biosurfactants produced by *Pseudomonas aeruginosa* play a major role in maintaining channels between multicellular structures in biofilms and in dispersal of cells from biofilms (Pamp and Tolker, 2007). Additional experiments revealed that lipopeptide production by plant beneficial strain *Bacillus amyloliquefacience* S499 is qualitatively and quantitatively imputed by the specific nutritional context of the rhizosphere but also biofilm related structure around root hairs (Nihorimbere *et al*., 2012).

**9. Screening of Unculturable Biosurfactant Producing Microorganisms**

The molecular approach such as functional metagenomics is concentrated for some unculturable biosurfactant producing microbes in the soil. This technique also gives the incredible knowledge of gene pool related to biosurfactant production which is still undiscovered. The data generated from such high throughput studies will accelerate application of biosurfactant in agriculture as well as the other fields. Metagenomics is the culture-independent genomic analysis of microbial communities. The study of metagenomics is mainly confined to those microorganisms which are non-culturable and thus cannot be sequenced. Their sequencing is based mainly on 16 S ribosomal RNA sequencinces which are conserved and generally differ from species to species. Construction of metagenomic libraries from different environment sample are showed in Fig. 2.

It is known that only 0.1 - 2% of all microorganisms observed in nature can be cultured *in vitro*. Therefore, the researchers are unable to study more than 99% microorganisms in some environments. Microorganisms sometimes have a unique and potentially very useful ability such as waste degradation or synthesis of compounds that could find use as drugs or antibiotics (Zeyaullah *et al*., 2009).

A powerful tool to calibrate the biosynthetic potential of an uncultivated bacterial population involves the formation of an eDNA library in a suitable host. For construction of eDNA library firstly isolation of high molecular weight eDNA, from a variety of different environments and enrichment of specific cell populations is common prior to eDNA isolation., for single organisms *Escherichia coli* is the most common host for genomic library construction. Vectors are generally used in construction of eDNA library. The choice of vector can also determine the flourishing or discomfiture of a particular project. Vectors used for library construction are cosmid and fosmid because they can accept 35–45 kb long fragment and can easily be transfected into *E. coli* to produce millions of clones. Larger insert libraries using bacterial artificial chromosomes (BACs) have also been successful but are less common due to the challenge of isolating DNA fragments of sufficient sizes from eDNA. When genomic library is constructed, it can be subordinate to functional or homology based screening protocols.

A common method for identification of discrete biocatalysts metabolites synthesized by small to medium-sized gene clustered is to screen metagenomics library clones for modified phenotypes or specific function of enzyme. This method is prevalent for the identification of industrially relevant enzymes such as cellulose, esterase, lipase and many more compiled (Satpute *et al*., 2010). For the study of microbial rhizosphere ecology molecular biological technique have revolutionized by enabling the identification for non-cultural bacteria. A variety of methods have been evaluated for the recovery of environment DNA that many further is used for cloning (Gabor *et al*., 2003). Hydrocarbon contaminated ecological niches are the most recommended sites for isolation of biosurfactant producing microbes. In recent time many advanced techniques have been developed for purification of microbial biosurfactant such as high pressure liquid chromatography, gas chromatography- mass spectroscopy, thin layer chromatography and phase separation technology (Sanchez *et al*., 2007; Heyd *et al*., 2008).

**10.** **Molecular Approach for profiling of Biosurfactant Producing PGPR**

In recent years many conventional methods used for screening of microbes for biosurfactant production are followed by the characterization of the biomolecule by infrared, gas chromatography mass spectrometry, nuclear magnetic resonance and fast atom bombardment mass spectrometry (Petrovic and Barcelo, 2004; Satpute *et al*., 2010). MALDI-TOP mass spectrometry is the new important technique reported for detection and separation of biosurfactant (Kurtzman *et al*., 2010). Along with the traditional methods, molecular techniques are being implemented to detect the presence for biosurfactant producing bacteria. PCR based technique targeting genes involved either in synthesis of biosurfactant (or regulation of biosurfactant production mainly for *Bacillus* spp. and *Pseudomonas* spp. ([Imanaka](http://www.sciencedirect.com/science/article/pii/0922338X9290055Y) *et al*., 1992; Ochsner *et al*., 1994).

**Conclusion**

Surfactants have multifarious applications in agriculture and the use of biosurfactants is more eco-friendly. Many phytopathogens cause the deleterious effect on crop and reduce the crop productivity. Therefore, the use of biosurfactants is an alternative that facilitates the biocontrol of many phytopathogens. Though many synthetic surfactants are available in the market, their use is supposed to be like pesticides and other chemicals. Hence the use of biosurfactant replaces the uses of harmful surfactant. Moreover, there is need to work on large scale production of biosurfactant so that net economic gain can be achieved from application of biosurfactants in agriculture as well as other sectors.

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**References**

1. Ahmad Z, Arshad M, Asghar, H N, Sheikh M A & Crowley D E. Isolation, Screening and Functional Characterization of Biosurfactant Producing Bacteria Isolated from Crude Oil Contaminated Sit. Int J Agri Biol 2016; 18.
2. Alsohim A S, Taylor T B, Barrett G A, Gallie J, Zhang X X, Altamirano‐Junqueira A E, Jackson R. The biosurfactant viscosin produced by *Pseudomonas fluorescens* SBW25 aids spreading motility and plant growth promotion, Environ microbial 2014; 16: 2267-2281.
3. Alvarez F, Castro M, Príncipe A, Borioli G, Fischer S, Mori G, Jofre E. The plant‐associated Bacillus amyloliquefaciens strains MEP218 and ARP23 capable of producing the cyclic lipopeptides iturin or surfactin and fengycin are effective in biocontrol of sclerotinia stem rot disease. Journal of applied microbiology 2012; 112(1):159-174.
4. Al-Wahaibi Y, oshi S, Al-Bahry S, Elshafie A, Al-Bemani A, Shibulal B. Biosurfactant production by *Bacillus subtilis* B30 and its application in enhancing oil recovery Col Surf B: Biointe 2014; 114: 324-333.
5. Arguelles-Arias A, Ongena M, Halimi B, Lara Y, Brans A, Joris B, Fickers P. *Bacillus amyloliquefaciens* GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens. Microbiol Cell Factories 2009; 8: 1.
6. Bais H P, Fall R & Vivanco J M. Biocontrol of *Bacillus subtilis* against infection of Arabidopsis roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. Plant physiol 2004; 134: 307-319.
7. Barin R, Talebi M, Biria D & Beheshti M. Fast bioremediation of petroleum-contaminated soils by a consortium of biosurfactant/bioemulsifier producing bacteria. Int J Environ Sci Technol 2014; 11: 1701-1710.
8. Belcher R W, Huynh K V, Hoang T V Crowley D E. Isolation of biosurfactant-producing bacteria from the Rancho La Brea Tar Pits, World J of Microbiol Biotechnol 2012; 28: 3261-3267.
9. Benincasa M, Contiero J Manresa M A, Moraes I O. Rhamnolipid production by *Pseudomonas aeruginosa* LBI growing on soapstock as the sole carbon source, *J Food En*, 54(2002) 283-288.
10. Bodour A A, Guerrero Barajas C, Jiorle BV, Malcomson ME, Paull AK, Somogyi A, Trinh LN, Bates RB, Maier RM, Structure and characterization of flavolipids, a novel class of biosurfactants produced by Flavobacterium sp strain MTN11, Appl Environ Microbiol 2003;70:114 -120.
11. Buensanteai N, Yuen G Y & Prathuangwong S. The biocontrol bacterium *Bacillus amyloliquefaciens* KPS46 produces auxin, surfactin and extracellular proteins for enhanced growth of soybean plant. Thai J Agric Sci 2008; 41: 101-116.
12. Cao L, Wang Q, Zhang J, Li C, Yan X, Lou X & Li S. Construction of a stable genetically engineered rhamnolipid-producing microorganism for remediation of pyrene-contaminated soil. World J Microbiol Biotechnol 2012; 28: 2783-2790.
13. Chandankere R, Yao J, Cai M, Masakorala K, Jain A K, Choi M M. Properties and characterization of biosurfactant in crude oil biodegradation by bacterium *Bacillus methylotrophicus* USTBa. Fuel 2014; 122:140-148.
14. Cooper D G, Zajic J E, Denis C. Surface active properties of a biosurfactant from *Corynebacterium lepus*, J Am Oil Chem Soc 1981; 58:77- 80.
15. daSilva F S P, Pylro V S, Fernandes P L, Barcelos G S, Kalks K H M, Schaefer C E G R, Tótola M R. Unexplored Brazilian oceanic island host high salt tolerant biosurfactant-producing bacterial strains, Extremophiles 2015; 19: 561-572.
16. Davey C B, Nursery soil management – organic amendments. In: LANDIS, T. D., DOUTH D. B. (Tech. Coordinators), National Proceedings, Forest and Conservation Nursery Associations. General Technical Report PNWGTR-389: USDA Forest Service PNWRS 1996; 6 -18.
17. Debode J, Maeyer K D, Perneel M, Pannecoucque J, Backer G D, Höfte M. Biosurfactants are involved in the biological control of *Verticillium microsclerotia* by *Pseudomonas* spp, J appl microbial 2007; 103: 1184-1196.
18. Desai J D & Banat I M. Microbial production of surfactants and their commercial potential, Microbiol Molecul Biol Rev 1997; 61: 47-64.
19. Deziel E, Lepine F, Milot S, Villemur R. rhlA is required for the production of a novel biosurfactant promoting swarming motility in *Pseudomonas aeruginosa*: 3-(3-hydroxyalkanoyloxy) alkanoic acids (HAAs), the precursors of rhamnolipids. Microbiol2003; 149: 2005-2013.
20. Dubey K V, Charde P N, Meshram S U, Shendre L P, Dubey V S, Juwarkar A A. Surface-active potential of biosurfactants produced in curd whey by *Pseudomonas aeruginosa* strain-PP2 and *Kocuria turfanesis* strain-J at extreme environmental conditions. Bioresource technol 2012; 126: 368-374.
21. Ferradji F Z, Mnif S, Badis A, Rebbani S, Fodil D, Eddouaouda K, Sayadi S .Naphthalene and crude oil degradation by biosurfactant producing Streptomyces spp. isolated from Mitidja plain soil (North of Algeria). Int Biodeterior Biodegr 2014; 86:300-308.
22. Gabor E M, de Vries E J, Janssen D B. Efficient recovery of environmental DNA for expression cloning by indirect extraction methods. FEMS Microbiol Ecol 2003; 44 :153-163.
23. Greek B F, Sales of detergents growing despite recession, Chem Eng News 1991; 69: 25–52.
24. Hassanshahian M, Emtiazi G, Cappello S. Isolation and characterization of crude-oil-degrading bacteria from the Persian Gulf and the Caspian Sea. Marine pollut bulletin 2012; 64:7-12.
25. Heerklotz H, Seelig J, Leakage and lysis of lipid membranes induced by the lipopeptide surfactin. Europ Biophys J 2007; 36: 305-314.
26. Helmy Q, Suryatmana P, Kardena E, Funamizu N W. Biosurfactants Production from *Azotobacter* sp. and its Application in Biodegradation o f Petroleum Hydrocarbon. J Appl Ind Biotechnol Trop Reg( 2008).
27. Hentati O, Lachhab R, Ayadi M, Ksibi M. Toxicity assessment for petroleum-contaminated soil using terrestrial invertebrates and plant bioassays. Environ moni assess 2013; 185: 2989-299.
28. Hewald S, Josephs K, B€olker M. Genetic analysis of biosurfactant production in Ustilago maydis. Appl Environ Microbiol 2005; 71:3033 3040.
29. Heyd M, Kohnert A, Tan T H, Nusser M, Kirschhöfer F, Brenner-Weiss G, Berensmeier S, Development and trends of biosurfactant analysis and purification using rhamnolipids as an example. Anal bioanal chemi 2008; 391:1579-1590.
30. Hoitink H A J, Boehm M J. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. Ann rev phytopathol 1999; 37: 427-446.
31. Imanaka T, Morikawa M, Ito M. Isolation of a new surfactin producer *Bacillus pumilus* A-1, and cloning and nucleotide sequence of the regulator gene, psf-1. J ferment bioeng 1992; 74: 255-261.
32. Jain R M, Mody K, Mishra A, Jha B. Physicochemical characterization of biosurfactant and its potential to remove oil from soil and cotton cloth. Carbohydrate polymers 2012; 89:1110-1116.
33. Khare E , Arora N K. Physiochemical and structural characterization of biosurfactant from *fluorescent Pseudomonas* with biocontrol activity against *Macrophomina phaseolina*, PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) FOR SUSTAINABLE AGRICULTURE 2011; 104 .
34. Kitamoto D, Isoda H, Nakahara T. Functions & potential applications of glycolipid biosurfactants from energy saving materials to gene delivery carriers*,* J Biosci Bioeng2002;94: 187 201.
35. Knickerbocker C, Nordstrom D K, Southam G. The role of “blebbing” in overcoming the hydrophobic barrier during biooxidation of elemental sulfur by *Thiobacillus thiooxidans*, Chem Geol 2002; 169: 425 433.
36. Kosaric N. Biosurfactants and their application for soil bioremediation. Food Technol Biotechnol 2001; 39:295-304.
37. Kretschmer A, Bock H, Wagner F. Chemical and physical characterization of interfacial active lipids from *Rhodococcus erythropolis* grown on normal alkanes. Appl Environ Microbiol1982; 44:864- 870.
38. Kumar A, Johri B N. Antimicrobial lipopeptides of *Bacillus*: natural weapons for biocontrol of plant pathogens In Microorganisms in Sustainable Agriculture and Biotechnology Springer Netherlands 2012; 91-111.
39. Kumar R, Bharagava R N, Kumar M, Singh S K, Govind K. Enhanced biodegradation of mobil oil hydrocarbons by biosurfactant producing bacterial consortium in wheat and mustard rhizosphere. J Pet Environ Biotechnol, 2013;4 .
40. Kumar R, Das A J, Juwarkar A A. Restoration of Petrol Contaminated Soil by PGPR Consortium Producing Rhamnolipids and Enhancement of Growth and Antioxidant activity of *Withania somnifera*. J Petro Environ Biotechnol 2014.
41. Kumari B, Singh S N, Singh D P. Characterization of two biosurfactant producing strains in crude oil degradation. Pro Biochemi 2012; 47: 2463-2471.
42. Kurtzman C P, Price N P, Ray K J, Kuo T M. Production of sophorolip biosurfactants by multiple species of the Starmerella (Candida) bombicola yeast clade. *FEMS* Microbiol Lett 2010; 31: 140–146.
43. Lang S, Philp J C. Surface active lipids in rhodococci. Antonie Van Leeuwenhoek Int J Gen Mol Microbiol, 1998; 74 : 59 70.
44. Laycock M V, Hildebrand P D, Thibault P, Walter J A, Wright J L C. Viscosin a potent peptidolipid biosurfactant and phytopathogenic mediator produced by a pectolytic strain of *Psedomonas fluorescens*. J Agric Food Chem1991;39: 483 -489.
45. Leclère V, Béchet M, Adam A, Guez J S, Wathelet B, Ongena M, Jacques P. Mycosubtilin overproduction by *Bacillus subtilis* BBG100 enhances the organism's antagonistic and biocontrol activities. Appl environ microbial 2005; 71: 4577-4584.
46. Leclère V, Marti R, Béchet M, Fickers P Jacques P. The lipopeptides mycosubtilin and surfactin enhance spreading of *Bacillus subtilis* strains by their surface-active properties. Arch Microbiol 2006; 186: 475-483.
47. Liang T W, Wu C C, Cheng W T, Chen Y C, Wang C L, Wang I L, Wang S L. Exopolysaccharides and antimicrobial biosurfactants produced by *Paenibacillus macerans* TKU029*.* Appl Biochem Biotechnol 2014;172: 933-950.
48. Liu W, Sun J, Ding L, Luo Y, Chen M & Tang C. Rhizobacteria (*Pseudomonas* sp. SB) assist phytoremediation of oily-sludge-contaminated soil by tall fescue (*Testuca arundinacea* L.). Plant soil 2013; 371:533-542.
49. Matsuyama T, Kaneda K, Nakagawa Y, Isa K, Hara Hotta H, Yano I. A novel extracellular cyclic lipopeptide which promotes flagellum dependent and independent spreading growth of *Serratia marcescens*. J Bacteriol, 1992: 174: 1769 -1776.
50. Meliani A, Bensoltane A. Enhancement of hydrocarbons degradation by use of pseudomonas biosurfactants and biofilms. J Petrol Environ Biotechnol 2014.
51. Mule A D, Bhathena Z P. Control of *Aspergillus parasiticum* NCIM 898 infection in potato tubers using biosurfactant. Asian J Exp Sci 2012; 26: 27-38.
52. Mulligan C N, Environmental applications for biosurfactants. Environ pollut 2005; 133: 183-198.
53. Nalini S, Parthasarathi R. Biosurfactant production by *Serratia rubidaea* SNAU02 isolated from hydrocarbon contaminated soil and its physico-chemical characterization. Biores Technol 2013; 147: 619-622.
54. Navonvenezia S, Zosim Z, Gottlieb A, Legmann R, Carmeli S, Ron EZ, Rosenberg E, Alasan. a new bioemulsifier from *Acinetobacter radioresistens*. Appl Environ Microbiol 1995; 61: 3240 -3244.
55. Nielsen T H, Sørensen J. Production of cyclic lipopeptides by *Pseudomonas fluorescens* strains in bulk soil and in the sugar beet rhizosphere. Appl Environ Microbiol 2003; 69: 861-868.
56. Nihorimbere V, Cawoy H, Seyer A, Brunelle A, Thonart P, Ongena M. Impact of rhizosphere factors on cyclic lipopeptide signature from the plant beneficial strain *Bacillus amyloliquefaciens* S499. FEMS Microbiol Ecol 2012; 79:176-191.
57. Noparat P, Maneerat S, Saimmai A. Utilization of palm oil decanter cake as a novel substrate for biosurfactant production from a new and promising strain of *Ochrobactrum anthropi*. World J Microbiol Biotechnol 2014; 30: 865-877.
58. Ochsner U A, Koch A K, Fiechter A, Reiser J. Isolation and characterization of a regulatory gene affecting rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. J Bacteriol 1994; 176: 2044-2054.
59. Ongena M, Jourdan E, Adam A, Paquot M, Brans A, Joris B, Thonart P. Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. Environ Microbial 2007; 9:1084-1090.
60. Pacwa-Płociniczak M, Płaza A, Piotrowska-Seget Z, Cameotra S S. Environmental applications of biosurfactants: recent advances. Int J Mole Sci 2011;12: 633-654.
61. Pacwa-Płociniczak M, Płaza G A, Poliwoda A, Piotrowska-Seget Z. Characterization of hydrocarbon-degrading and biosurfactant-producing *Pseudomonas* sp. P-1 strain as a potential tool for bioremediation of petroleum-contaminated soil. Environ Sci Pollut Res 2014, 21: 9385-9395.
62. Padmapriya B. Isolation and screening of biosurfactants produced by *Pseudomonas aeruginosa* from oil spilled soils. Int J Pharmaceut Biol Arch 2012; 3.
63. Pamp S J, Tolker N T. Multiple roles of biosurfactants in structural biofilm development by *Pseudomonas aeruginosa*,. *J Bacteriol* 2007; 189: 2531-2539.
64. Pandya U, Saraf M. Isolation and identification of allelo chemicals produced by *B. sonorensis* for suppression of charcoal rot of *Arachis hypogaea* L. J Basic Microbiol 2015; 55: 635-644.
65. Pemmaraju S C, Sharma D, Singh N, Panwar R, Cameotra S S , Pruthi V. Production of microbial surfactants from oily sludge-contaminated soil by *Bacillus subtilis* DSVP23. Appl Biochem Biotechnol 2012; 167: 1119-1131.
66. Perneel M, D'hondt L, De Maeyer K, Adiobo A, Rabaey K, Höfte M. Phenazines and biosurfactants interact in the biological control of soil‐borne diseases caused by *Pythium* spp, Environ Microbiol 2008 10: 778-788.
67. Petrovic M, Barcelo D. Analysis and fate of surfactants in sludge and sludge-amended soil. Tre A Chem 2004; 23: 10–11.
68. Pimentel D, McLaughlin L, Zepp A, Lakitan B, Kraus T, Kleinman P, Selig G. Environmental and economic effects of reducing pesticide use. Biol Sci 1991; 41: 402-409.
69. Pradhan A K, Pradhan N, Sukla L B, Panda P K, Mishra B K. Inhibition of pathogenic bacterial biofilm by biosurfactant produced by Lysini *bacillus fusiformis* S9. Biopro Biosy Eng 2014; 37: 139-149.
70. Pruthi V, Cameotra S S. Production and properties of a biosurfactant synthesized by *Arthrobacter protophormiae*—an antarctic strain. World J Microbiol Biotechnol 1997; 13: 137-139.
71. Reiling H E, Thaneiwyss U, Guerrasantos L H, Hirt R, Kappeli O, Fiechter A. Pilot plant production of rhamnolipid surfactant by *Pseudomonas aeruginosa*. Appl Environ Microbiol 1986; 51:985 989.
72. Renfro T D, Xie W, Yang G, Chen G. Rhamnolipid surface thermodynamic properties and transport in agricultural soil. Col Surf B: Biointerf 2014; 115: 317-322.
73. Ron E Z, Rosenberg E. Natural roles of biosurfactants. Environ Microbiol 2001; 3: 229-236.
74. Rosenberg E, Ron EZ. High and low molecular mass microbial surfactants. Appl MicroBiol Biotechnol 1999; 52: 154 162.
75. Rosenberg E, Ron E Z. Bioemulsans: microbial polymeric emulsifiers. Curr Opi *Biotecnol* 1997;8 :313 -316.
76. Saikia R R., Deka S, Deka M, Banat I M. Isolation of biosurfactant-producing *Pseudomonas aeruginosa* RS29 from oil-contaminated soil and evaluation of different nitrogen sources in biosurfactant production. Ann Microbiol 2012; 62:753-763.
77. Saimmai A, Kaewrueng J, Maneerat S. Used lubricating oil degradation and biosurfactant production by SC-9 consortia obtained from oil-contaminated soil. Ann Microbiol 2012; 62: 1757-1767.
78. Sanchez M, Aranda F J, Espuny M J, Marqués A, Teruel J A, Manresa A, Ortiz, A. Aggregation behavior of a dirhamnolipid biosurfactant secreted by *Pseudomonas aeruginosa*in aqueous media. J Coll Interf Sci 2007; 307: 246.253.
79. Satpute S K, Banpurkar A G, Dhakephalkar P K, Banat I M, Chopade B A. Methods for investigating biosurfactants and bioemulsifiers, Rev Crit Rev Biotechnol 2010; 30:127-144.
80. Seydlova G, Svobodova J. Review of surfactin chemical properties and the potential biomedical applications. Cent Eur J Med 2008; 3: 123 133.
81. Shaw A, Surfactants—94, Soap Cosmet Chem Specialities 1994; 70: 24–34.
82. Sheng X, He L, Wang Q, Ye H, Jiang C. Effects of inoculation of biosurfactant-producing *Bacillus* sp. J119 on plant growth and cadmium uptake in a cadmium-amended soil. J Haz Mater 2008; 155: 17-22.
83. Singh A K, Cameotra S. Rhamnolipids production by multi-metal-resistant and plant-growth-promoting rhizobacteria. Appl Biochem Biotechnol 2013; 170: 1038-1056.
84. Sriram M I, Kalishwaralal K, Deepak V, Gracerosepat R, Srisakthi K. Gurunathan S. Biofilm inhibition and antimicrobial action of lipopeptide biosurfactant produced by heavy metal tolerant strain *Bacillus cereus* NK1. Coll Surf B: Biointerf 2011; 85: 174-181.
85. Stein T, *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. Mol Microbiol 2005; 56: 845-857.
86. Stewart P S, Franklin M J. Physiological heterogeneity in biofilms. Nat Rev Microbiol 2008; 6: 199-210.
87. Suja F, Rahim F, Taha M R, Hambali N, Razali M R, Khalid A, Hamzah A. Effects of local microbial bioaugmentation and biostimulation on the bioremediation of total petroleum hydrocarbons (TPH) in crude oil contaminated soil based on laboratory and field observations. Int Biodeterior Biodegr 2014; 90 :115-122.
88. Tambekar D H, Gadakh P V. Biochemical and molecular detection of biosurfactant producing bacteria from soil. Int J Life Sci Biotechnol Pharm Res 2013; 2:204-211.
89. Thein A, Prathuangwong S. Novel strains of *Xanthomonas oryzae* pv. oryzae UV mutated induce systemic resistance in rice against bacterial leaf blight disease. Kasersart, J (Nat Sci) 2010; 44:1026-43.
90. Toure Y, Ongena M, Jacques P, Guiro A, Thonart P. Role of lipopeptides produced by *Bacillus subtilis* GA1 in the reduction of grey mould disease caused by *Botrytis cinerea* on apple. J Appl Microbiol 2004; 96: 1151-1160.
91. Tran H, Ficke A, Asiimwe T, Höfte M, Raaijmakers J M. Role of the cyclic lipopeptide massetolide A in biological control of Phytophthora infestans and in colonization of tomato plants by *Pseudomonas fluorescens*. New Phytol 2007; 175:731-742.
92. Tran H, Kruijt M, Raaijmakers J M. Diversity and activity of biosurfactant‐producing *Pseudomonas* in the rhizosphere of black pepper in Vietnam. J Appl Microbiol 2008; 104: 839-851.
93. Van Bogaert I N A, Saerens K, De Muynck C, Develter D, Soetaert W, Vandamme E J. Microbial production and application of sophorolipids. Appl Microbiol Biotechnol 2007;76: 23 34.
94. Van Hamme J D, Singh A. Ward O P, Physiological aspects: Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. Biotechnol Advances 2006 24: 604-620.
95. Wang S, Mulligan C N. Rhamnolipid biosurfactant-enhanced soil flushing for the removal of arsenic and heavy metals from mine tailings. Process Biochemi 2009: 44: 296-301.
96. Wilson S C, Jones K C. Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs). Rev Environ Pollut 1993; 81: 229-249.
97. Xia W, Du Z, Cui Q, Dong H, Wang F, He P, Tang Y. Biosurfactant produced by novel *Pseudomonas* sp. WJ6 with biodegradation of n-alkanes and polycyclic aromatic hydrocarbons. J Haz Mater 2014; 276:489-498.
98. Xiao X, Chen H, Si C, Wu L. Influence of biosurfactant-producing strain *Bacillus subtilis* BS1 on the mycoremediation of soils contaminated with phenanthrene. Int Biodeterior Biodegr 2012; 75:36-42.
99. Yan P, Lu M, Yang Q, Zhang H L, Zhang Z Z, Chen R. Oil recovery from refinery oily sludge using a rhamnolipid biosurfactant-producing *Pseudomonas*. Biores Technol2012;116: 24-28.
100. Zeyaullah M, Kamli M R, Islam B, Atif M, Benkhayal F A, Nehal M, Ali A, Metagenomics-An advanced approach for noncultivable micro-organisms. Biotechnol Mole Biol Rev 2009; 4:49-54.
101. Zhang F, Gu W, Xu P, Tang S, Xie K, Huang X, Huang Q. Effects of alkyl polyglycoside (APG) on composting of agricultural wastes. Waste Manage 2011; 31: 1333-1338.
102. Zhao Z, Wong J W. Biosurfactants from *Acinetobacter calcoaceticus* BU03 enhances the solubility and biodegradation of phenanthrene. Environ Technol 2009; 30: 291-299.

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