

## 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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### 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 agriculture crop 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 properties was 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).

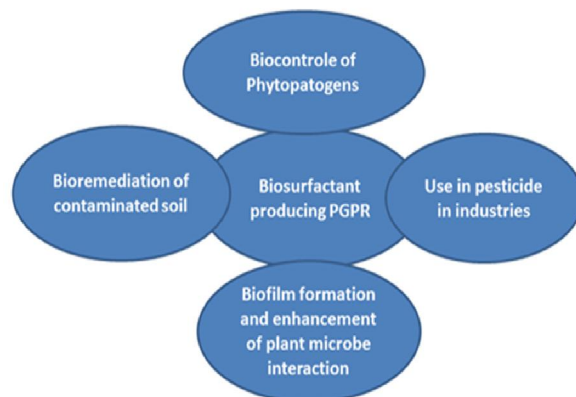


Fig.1. Multifarious uses of biosurfactants in agriculture

*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 Bhatena, 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 are several 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), while Debode *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
<i>Pseudomonas aeruginosa</i>	Oil Spilled soils	Padmapriya B <i>et al.</i> (2012)
<i>Pseudomonas aeruginosa</i>	Pyrene-contaminated soil	Cao <i>et al.</i> (2012)
<i>Stenotrophomonas maltophilia</i>	Asphalt-permeated soil	Belcher <i>et al.</i> (2012)
<i>Bacillus subtilis</i>	Contaminated Soil	Pemmaraju <i>et al.</i> (2012)
<i>Pseudomonas aeruginosa</i>	Oil-contaminated soil	Saikia <i>et al.</i> (2012)
<i>Agrobacterium tumefaciens</i> , <i>Bacillus cereus</i> ,	Oil-contaminated soil	Saimmai <i>et al.</i> (2012)
<i>Pseudomonas aeruginosa</i> strain and <i>Kocuriaturfanesis</i> strain	Soil	Dubey <i>et al.</i> (2012)
<i>Bacillus amyloliquefaciens</i>	Oily sludge And petroleum-contaminated Soil	Liu <i>et al.</i> (2012)
<i>Acinetobacter calcoaceticus</i> and <i>Alcanivoraxdieselolei</i>	oil-contaminated sites	Hassanshahian <i>et al.</i> (2012)
<i>Klebsiella</i> sp.	Oil industry sludge	Jain <i>et al.</i> (2012)
<i>Pseudomonas</i> sp.and <i>Rhodococcus</i> sp.	crude oil contaminated site	Kumari <i>et al.</i> (2012)
<i>Pseudomonas aeruginosa</i>	Oil Field	Yan <i>et al.</i> (2012)
<i>Pseudomonas aeruginosa</i>	Rhizosphere	Singh and Cameotra (2013)
<i>Pseudomonas</i> sp.	Rhizosphere soil	Liu <i>et al.</i> (2013)
<i>Paenibacillus macerans</i>	Oil-containing media	Liang <i>et al.</i> (2014)
<i>Serratia rubidaea</i>	Hydrocarbon contaminated soil	Nalini and Parthasarathi (2013)
<i>Bacillus subtilis</i>	Soil	Al-Wahaibi <i>et al.</i> (2014)
<i>Ochrobactrum anthropic</i>	Mangrove sediment	Noparat <i>et al.</i> (2014)
<i>Pseudomonas</i> sp.	Heavily petroleum hydrocarbon contaminated soil	Pacwa-Płociniczak <i>et al.</i> (2014)
<i>Lysinibacillus fusiformis</i>	River bank	Pradhan <i>et al.</i> (2014)
<i>Bacillus subtilis</i> and <i>Pseudomonas aeruginosa</i>	Oil-contaminated soil	Barin <i>et al.</i> (2014)
<i>Bacillus methylotrophicus</i>	Hydrocarbon contaminated aqueous medium	Chandankere <i>et al.</i> (2014)
<i>Streptomyces</i> spp.	Soils	Ferradji <i>et al.</i> (2014)
<i>B. subtilis</i> subsp. <i>spizizenii</i>	Uncontaminated soils	daSilva <i>et al.</i> (2015)
<i>Klebseilla</i> sp.	Hydrocarbon-contaminated soil	Ahmad <i>et al.</i> (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 considerably damage 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 phospholipids	Fatty acid Neutral lipid	<i>Corynebacterium lepus</i> (Cooper <i>et al.</i> 1981), <i>N. erythropolis</i> (Kretschmer <i>et al.</i> 1982)
Lipopeptides	Phospholipid	<i>Thiobacillus thiooxidans</i> (Knickerbocker <i>et al.</i> 2000)
	Surfactin	<i>Bacillus subtilis</i> , <i>Bacillus pumilus A</i> (Seydlova and Svobodova, 2008)
	Viscosin	<i>Pseudomonas fluorescens</i> , <i>P. libanensis</i> (Laycock <i>et al.</i> 1991)
	Serrawettin	<i>Serratia marcescens</i> (Matsuyama <i>et al.</i> 1992)
Glycolipids	Mannosylerythritol lipids	Genus <i>Pseudozyma</i> (yeast), <i>Candida antarctica</i> , <i>Ustilago maydis</i> (Kitamoto <i>et al.</i> 2002)
	Sophorolipids	<i>C. batistae</i> , <i>T. bombicola</i> , <i>C. lypolytica</i> , <i>C. bombicola</i> , <i>T. apicola</i> , <i>T. petrophilum</i> , <i>C. bogoriensis</i> (Van Bogaert <i>et al.</i> 2007)
	Rhamnolipids	<i>Pseudomonas sp.</i> , <i>P. aeruginosa</i> (Reiling <i>et al.</i> 1986)
	Trehalose lipids	<i>Rhodococcus sp.</i> , <i>Arthrobacter sp.</i> , <i>R. erythropolis</i> , <i>N. erythropolis</i> (Lang and Philp 1998)
	Cellobiolipids	<i>Ustilagozoeae</i> , <i>Ustilagomaydis</i> (Hewald <i>et al.</i> 2005)
Polymeric	Emulsan	<i>Acinetobacter calcoaceticus</i> (Rosenberg and Ron 1999)
	Biodispersan	<i>A. calcoaceticus</i> (Rosenberg and Ron 1997)
	Mannan-lipid-protein	<i>C. tropicalis</i> (Rosenberg and Ron 1999)
	Alasan	<i>A. radioresistens</i> (Navonvenezia <i>et al.</i> 1995)
Siderophore	Flavolipids	<i>Flavobacterium</i> (Bodour <i>et al.</i> 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 growth promoting features and heavy metal resistance. *Bacillus sp.* J119 was found cadmium uptake of maize, 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 protein and polysaccharides that form a hydrated gel-like slime that holds the biofilm together (Stewart and Franklin, 2008). Biofilm formation by *B.*

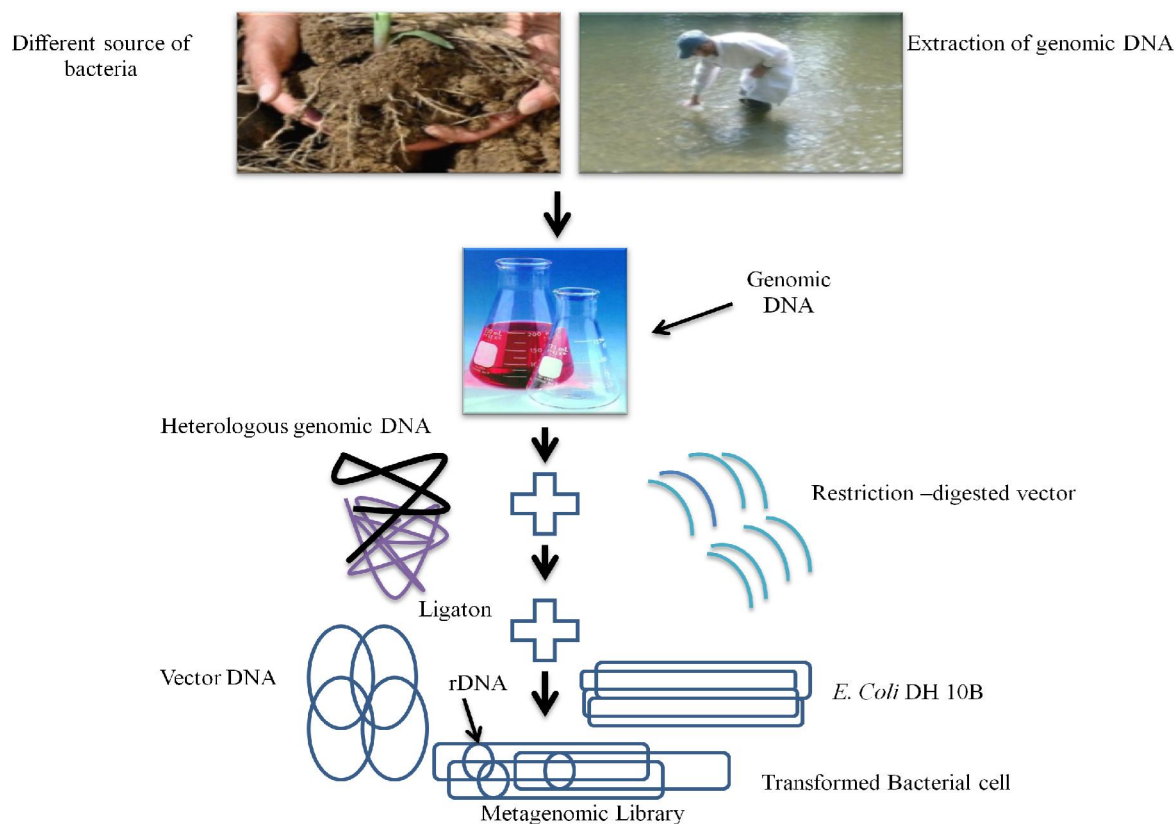
*subtilis* is a complicated process that includes secretion of surfactin which is a lipopeptide antimicrobial agent (Bais *et al.*, 2004). Biosurfactants produced by *Pseudomonas aeruginosa* play a major role in maintaining channels between multicellular structures in biofilms and in dispersal of cells 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 sequences 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 (Zeyaulah *et al.*, 2009).



**Fig.2.** Construction and screening of metagenomic libraries. Schematic representation of metagenomic libraries construction from different environment sample

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 *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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