Last decade has seen tremendous increase in the demand for economical and sustainable bioproducts replacing the petrochemical derived products. Consequently, interest in microbial surface active agents, also known as biosurfactants, has increased tremendously. Biosurfactants have properties that make them enviable not only for mankind but also for microorganisms.

The present study tried to explore the pristine rhizospheric diversity of wild plant to isolate biosurfactant producing bacteria. From rhizosphere of wild plant *Parthenium hysterophorus* two biosurfactant producing bacteria namely *Pseudomonas aeruginosa* strain A11 (Gene bank accession no. JN408078) and *Bacillus subtilis* strain A21 (Gene bank accession no. JN005770; Endo-rhizospheric isolate) were isolated along with the members of class Sphingobacteria Gammaproteobacteria, Alphaproteobacteria and Bacilli. Both the biosurfactant producing strain exhibited plant growth promoting traits. Thus, suggesting that biosurfactant producing bacteria may support plant growth in natural environment. Another interesting feature associated with strain A11 was its antibiotic resistance. Strain A11 exhibited resistance against several common antibiotics like Azithromycin, Colistin and Tobramycin. Thus, indicating that complex rhizospheric environment may have molded bacterium in to multi-antibiotic resistant strain.

Biosurfactant from strain A11 was characterized as rhamnolipid. It primarily consisted of dirhamnolipid with single monorhamnolipid congener. The dirhamnolipid fraction of rhamnolipid formed crystal making its separation handy. Under optimum condition strain A11 produced 4436.9mg/L and 8000mg/L of rhamnolipid in shake flask and in 5L fermenter, respectively while utilizing glycerol as carbon source. Rhamnolipid yield of strain A11 was more than several other reported strains which were isolated from hydrocarbon contaminated sites. Thus,

suggesting that region untouched by human activity can also be a promising site for isolating biosurfactant producing bacteria. Considering this, strain A11 can be a potential strain for commercial production of rhamnolipids as use of inexpensive glycerol as carbon source and high yield are conducive for large scale production.

In presence of some metals namely Cu^{2+}, Li^{1+}, Cr^{3+} and Cd^{2+} expression of rhamnolipid gene was increased as compared to the control. Thus, suggesting that rhamnolipids may provide some advantage to the bacterium under metal stress. This aspect can be explored further as it can disclose interesting aspects in relation to the natural roles played by biosurfactants.

Tn5-derived transposome mediated chromosomal integration of *RhlAB* was applied to construct the metabolic pathway of rhamnolipid biosynthesis in non-pathogenic *E. coli BL21*. Engineered strain *E. coli AB* was able to produce monorhamnolipid, unlike the native strain that produce mixture of rhamnolipids.

Purified rhamnolipid, monorhamnolipid and dirhamnolipid reduced the surface tension of water to 30mN/m, 42mN/m, and 36mN/m, respectively. Critical micelle concentration of rhamnolipid, monorhamnolipid and dirhamnolipid was 83mg/L, 150mg/L and 125mg/L, respectively. Emulsification ability and stability of dirhamnolipid was better than the monorhamnolipid.

Dirhamnolipid exhibited antimicrobial activity against several pathogenic bacteria like *Staphylococcus aureus* MTCC 3160, *Staphylococcus hominis* MTCC 4435, *Staphylococcus epidermidis* MTCC 435, *Salmonella typhi* MTCC 733, *Salmonella bovismorbificans* MTCC 1162, *Salmonella virchow* MTCC 1165, *Enterobacter oryzae*, *Pontibacter sp*, *Bacillus megaterium* , *Bacillus safensis* and *Bacillus subtilis*. Challenging above mentioned bacteria with dirhamnolipid caused

lysis of the cells, suggesting its potential application as an environment friendly disinfectant.

Surface conditioning with dirhamnolipid reduced the adherence of bacteria to polystyrene plates, brorosilicate glass and silicon rubber tubes. Treatment of already adhering bacteria with dirhamnolipid also significantly reduced the bacterial adherence. Thus, indicating that conditioning of surface with dirhamnolipid can be an effective strategy to reduce microbial adhesion and preventing colonization by pathogenic microorganisms.

Dirhamnolipid exhibited lethality against mycelium as well as ascospores of *Aspergillus fumigates* ATCC 204305, thus demonstrating its potential applicability in tropical ointments directed against cutaneous Aspergillosis. Fungal biofilm formation by *Fusarium solani* ATCC 36031 , *Paecilomyces marquandi* ATCC 10525, *Cladosporium cladosporoides* ATCC 16022, *Scopularioopsis acremonium* ATCC 58636, *Candida apicola* MTCC 1445, *Candida albicans* MTCC 1637, *Candida glabarata* 2367 and *Starmerella bombicola* MTCC 1910 was significantly reduced in the presence of dirhamnolipid, demonstrating its potential use as anti-fungal biofilm agent.

Application of rhamnolipids during biodegradation of triazine group of pesticides reduced rate of degradation in liquid broth. However, its application during degradation of herbicides contaminated soil increased the rate and extent of degradation significantly. Hence, suggesting that microbial surfactants must be judiciously used depending on the microorganism, condition and the compound to be degraded.

Bacillus subtilis strain A21 produced lipopeptides type biosurfactant which was characterized as surfactin isoforms. Glutamic acid, Leucine, Valine and Aspartic

acid constituted the amino acid portion of lipopeptides while carbon chain length of lipids varied from C-13 to C-15. Strain A21 produced 920.29 mg/L of surfactin utilizing sucrose as conventional carbon source. On unconventional carbon source, formulated *Ipomoea batatas* (sweet potato) extract, strain A21 produced 1863.93 mg/L of surfactin under optimized condition. Thus, indicating that *Ipomoea batatas* extract may be better fermentation media for production of surfactin as yield is more and its inexpensive nature.

Purified surfactin reduced the surface tension of water to 29mN/m with critical micelle concentration of 33mg/L. Emulsification ability and stability of surfactin suggested that it is not an efficient emulsifying agent. Due to presence of Aspartate and Glutamate, surfactin had negative residual charge, making it an anionic biosurfactant.

Soil washing by surfactin solution at 50CMC concentration removed cobalt, cadmium, nickel, copper and zinc ions form metal contaminated soil. Surfactin exhibited chelating specificity for divalent ions. Surfactin washed soil supported plant germination very similar to uncontaminated garden soil. However, no plant germination was observed in unwashed contaminated soil. This indicates that surfactin can be an effective surfactant for removal of metals from contaminated soil.

Metal chelating ability of surfactin maintained motility of *Bacillus licheniformis* strain CM100B under metal ion stress. Addition of surfactin to soft agar plates rescued (Cd^{2+}, $Pb2^{+}$) or enhanced (Co^{2+}, Cu^{2+}, Ni^{2+}, Mn^{2+}, Se^{4+}) the swarming ability of CM100B under metal ions stress. Surfactin may have formed complexion with metal ions and reduced or nullify the toxicity of metal ions for bacterium. In natural environments surfactin produced by *Bacillus subtilis* may be beneficial to other microorganisms. Also surfactin could be used in metal bioremediation process

in case of motile bacteria, as it will aid bacterium to cover wider area by enhancing or rescuing the motility bacteria under metal ion stress.

In conclusion, the results of this study indicate that the rhizosphere of wild plant can also be a source of biosurfactant producing microorganisms. Biosurfactants from rhizospheric bacteria are conducive for environmental and biomedical applications. Apart from serving mankind, biosurfactants from rhizospheric bacteria may also influence plant and bacterial physiology significantly. Further, looking into the natural roles of biosurfactant may disclose hidden and interesting secretes capable of adding new dimensions to biosurfactant research.