Indian J. Applied & Pure Bio. Vol. 33(2), 131-134 (2018).

Biofilm: A future generation biofertilizer

Peer Mohmad Junaid* **and Fatima Khan****

*Department of Botany, Govt. Motilal Vigyan Mahavidyalaya, Bhopal-462 003 (India)
**Prof. of Botany, Govt. College Nasrullah Ganj-466 331 (India)
junaidpeerzada@gmail.com*; +919622524485
botanyfatima@gmail.com**; +919893573057

Abstract

All plants including leguminous plant roots have capability to secrete a wide range of compounds into the rhizosphere. Because of these exudates, the rhizosphere becomes nutrient rich for microbial growth. It is reported that 40% of carbon fixed by plants is converted into root exudates which contains water, enzymes, ions, free oxygen and compounds of carbon such as carbohydrates, organic acid, amino acids, proteins and mucilage. However, different types of soil has different abiotic factors such as temperature, light and soil moisture, therefore, root exudates vary with type of soil and nutrient availability. Due to the presence of root exudates around the roots, which create a niche and mediate positive and negative interaction among microorganisms. This symbiotic association between plants and microbes which are beneficial, is called as plant growth promoting rhizobacteria (PGPR), including nitrogen fixing bacteria. The efficiency of fungal bacterial biofilms (FBB^S) is however greater than their monocultures.

Some of the microbes show affinity and get attached to both biotic and abiotic plane surfaces and differentiate to form complex multicellular microbial communities called as biofilms. Microbial cells in association with extracellular biopolymer produced by the same cells to protect itself, is called as biofilm[9]. The extracellular matrix are mostly composed of exopolysaccharide (EPS) which is the basic material of biofilm formation, other components include proteins, DNA and products from bacterial lysis provide the matrix for biofilm formation. This allows attachment of the cells to a solid surface and to each other and later colonization of such surface.

Definition of biofilm :

Process of biofilm formation can also be defined as an assemblage of microbial cells that are irreversibly associated with a biotic or abiotic surface and confined in a matrix of primarily polysaccharide material allowing survival and growth in sessile environment. Scientists have realized that more than 99% of bacteria exist in nature as biofilms. They have also investigated that survival mechanisms for rhizobia subjected to stress by studying the formation of biofilms and describing many of the factors that mediate biofilm formation. The biofilm formed by non-spore forming bacteria protect its community from the fluctuation and often severe conditions of the rhizosphere, such as desiccation, extreme pH levels,[1] UV radiations, temperature, salt, nutrient availability, as well as tolerance against antibiotics, protection from protozoa predation and production of secondary metabolites and exoenzymes. Such transition from immobile to planktonic stage is mediated by numerous environmental signals as well as by accumulation of quorum sensing signals which mediate cell to cell communication in microbes. For the preparation of this manuscript, relevant literature[1-11] has been consulted.

Types of Biofilm :

There are almost three different types of biofilms that are formed in the soil:

- Fungal biofilms
- Bacterial biofilms
- Fungal bacterial biofilms (FBB^S)

The fungal biofilms are formed on non-living or abiotic surfaces in the soil. Same is the case with bacteria. The bacterial biofilms are formed on abiotic surfaces in the soil. With the first in-vitro development and observation of rhizobia forming biofilms, a series of studies was conducted to demonstrate its potential application for various purposes. It was observed that biofilms fixed nitrogen (N2) biologically as revealed by nitrogenase activity and N accumulation. Application of a developed biofilmed inoculant of the rhizobia can significantly increase N2 fixation in chickpea plant[9].

While, in case of the fungal bacterial biofilms (FBB^S), the fungi act as biotic surface to which the bacteria adheres to its fungal hyphae. The fungal and bacterial biofilms differ from the FBB^S on biotic surfaces and have lesser metabolic activity than FBB^S. The FBB^S have better growth and colonization abilities than their monocultures. The symbiotic association between bacteria and mycorrhizal fungi is one of the common example of FBB^S present in the soil. The adherence of biofilm around the roots of a plant provide multiple number of functions including growth of the crop plants, improvement in crop production, cycling of nutrients and control of pathogens.

Biofilm formation :

There are five stages of mature biofilm formation, which are:

- *Initial attachment:* Microorganisms adhere themselves with surface through reversible weak Vander wall forces.
- *Irreversible attachment by the production of EPS:* Once they adhere to surface through reversible weak Vander walls forces, microorganisms get permanently attached through cell adhesion structure like Pilli. This process ultimately results into production of EPS that serves to bind the cell to the surface and protect it from the surrounding environment.
- *Early development:* The EPS formed by such kind of bacterial cells is composed of polysaccharides, proteins, nucleic acids and phospholipids.
- *Maturation of the attached biofilm:* The maturation and speed of biofilm formation is affected by both environmental factors and the surface to which it is attached. The

rate of biofilm formation is increased on rough surfaces, porous and less hydrophobic materials. It also need suitable amount of nutrient availability, particularly the availability of phosphorus which increases the ability of cells to adhere.

- *Dispersion:* Finally, the existing cells of biofilm dispersed as aggregate cell colonies.

The rate biofilm formation and its cell growth is enhanced by high temperatures, EPS production and surface adherence.

Advantages of biofilm formation :

- As we know biofilms are found on the surface of plants or inside the plants. They can either be beneficial to the plants as in the case of nitrogen fixing rhizobium in roots of crop plants or either they may contribute in crop diseases.
- The biofilm help the bacterial cells to exchange the genetic material and several nutrient metabolites with each other in the microbial communities.
- The biofilm formed by hydrocarbanoclastic bacteria (HCB) can help in eliminating petroleum oil from polluted oceans and marine systems.
- They also help in removing pathogens, protozoa and suspended solids from the sewage treatment plants which include a secondary treatment stage in which waste water passes over biofilms grown on filters, which extract and digest organic compounds.

Biofilm and its importance in agriculture :

Biofilm inocula or liquid biofertilizer can be used as vigorous biofertilizer by introducing them into the soil.

- FRB^S were first time reported by Seneviratne and Jayasinghearachchi in 2003. A developed bio-filmed inoculant of the FRB significantly increased N2 fixation in soybean by ca. 30% compared to a conventional inoculant of rhizobium alone (monoculture inocula).
- The amount of plant growth substances (PGP) is enhanced by biofilmed inocula which in turn increase plant dry weight of rice by 25% as compared to monocultured inoculant[6].
- Biofilms also provide the microorganisms ability to survive under different environmental stresses. The biofilm formed by non-spore forming bacteria protect its community from the fluctuation and often severe conditions of the rhizosphere, such as desiccation, extreme pH levels,[1] UV radiation, temperature, salt, nutrient availability, as well as tolerance against antibiotics, protection from protozoa predation and production of secondary metabolites and exoenzymes.
- The application of biofilmed rhizobial inocula on *Pleurotus ostreatus* enhanced nitrogen fixation and also increased protein content of mushroom[9].
- The FRB^S increased nitrogen (N) and phosphorus (P) availabilities when inoculated directly to the soil. They also improved P bio solubilisation from rock phosphate.

Expected outcome of biofilmed inocula:

By the application of biofilmed inocula and FRBs fertility of soil has been increased in nutrient deficient soils. Selection of possible combinations of microbial communities for highest performance with concurrent biocontrolling and bio-fertilizing activities is a major upshot for upcoming biofilmed inocula as biofertilizer. We should undertake extensive research for enhancing biofilmed inocula in various food crops for their easy delivery under field conditions.

References :

1. Angus, A.A and A.M. Hirsch (2013). *Molecular microbial ecology of the rhizosphere, 2:* 703-712.
2. Dhilloin, G., D. Bhagat and P. Sharma (2012). *Biofertilizer newsletter, 20*(2):15-22.
3. Flemming, H.C. and J. Wingender (2010). *Nat. Rev. Microbiol., 8*(9): 623-633.
4. Fujishige, N.A., Kapadia, P.L. De Hoff and A.M. Hirsch (2006). *FEMS microbiol. Ecol., 56:* 195-206.
5. Nissipaul, M., S. Triveni, R. Subhashreddy, and B. Suman (2017). *Int. J. Curr. Microbiol. App. Sci., 6*(6): 1846-1852.
6. Rafique, M., K. Hayat, T. Mukhtar, Amna1, A.A. Khan, M.S. Afridi, T. Hussain, T. Sultan, M.F.H. Munis, M Imran and H.J. Chaudhary (2015). Bacterial biofilm formation and its role against agricultural pathogens. In: *The battle against microbial pathogens,* (Ed. Mende Vilas) 337-382.
7. Rogel, M.A., E.O. Orrillo and E.M. Romer (2011). *Syst. and appl. Microbiol. 34:* 96-104.
8. Seneviratne, G. and H.S. Jayasinghearachchi (2003). *J. Biosci., 28:* 243–247.
9. Seneviratne, G. and I.K. Indrasena (2006) *J. Biosci., 31:* 639–643.
10. Seneviratne, G., M.L. Kecskes and I.R. Kennedy (2008). *Australian centre for Int. Agri. Res.,* 126-130.
11. Seneviratne, G., M.L.M.A.W. Weerasekara, K.A.C.N. Seneviratne, J.S. Zavahir, M.L Kecske´s and I.R. Kennedy (2010). *Plant Growth and Health Promoting Bacteria,* 81-95.