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Biofilmed Biofertilizer: Promising Technology for Tomorrow

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Abstract: In the context of modern agricultural sciences , the technology of biofilmed biofertilizer is comparatively new in the south asian countries though basic research on the biological properties of biofilm is rather old. The unique structure of biofilm enables it to survive in adverse conditions including higher temperature, low moisture and acidic conditions. It has some unique features of development that enables it to include adhering members from adjoining areas to exist together as a socio-biological community. Recent advances in the subject has indicated several communities including fungal members deriving benefit in addition to bacteria from the biofilm community. Within the biofilm, the microbial rates of metabolism is also found to be higher and that helps the members better in their survival. Considering all these basic parameters of biofilms, its use as biofertilizer has been discussed with the examples of recent applications.

Key words: Biofilmed biofertilizer, FBB, FRB.

Introduction

Biofilms can be described as microbial cells that are self-embedded by a solid extracellular matrix consisting of exopolysaccharides (EPS)and that proliferate on a surface , interface or substratum remaining firmly attached to it. Structurally biofilms are defined as matrix-enclosed bacterial population adherent to each other and / or to surfaces or interfaces [4]. This definition also encompasses microbial aggregates or floccules found elsewhere in pores or porous surface.

Biofilms can flourish on a wide range of biotic and abiotic surfaces. Microbes have a lot of difference from their planktonic life to the complex surface attached biofilm covered existence. They are comparatively more resistant to the changes in the surrounding be it temperature, pH or salinity. Thus, physiologically biofilm microbes are superior to their non-biofilm counterparts when applied to the natural soil environment as bio-fertilizer or biocontrol agents. Some of the biotechnological aspects have been reported [14].

Whenever the plant root surfaces are ambient for colonization by rhizospheric microbial strains (fungal/ bacterial), possible biofilm forming microbes can be an ideal choice to be encouraged as biofertilizer. The biofilm community have the unique characteristic of genetic exchange among themselves and thereby up-regulating some gene expressions favourable to them.

Distribution of biofilm

Bacteria are considered to be ubiquitous due to their varied phenotypic existence. Biofilms are one such phenotypic peculiarity that makes them survive adverse conditions. Biofilms are found in industry, medical , agricultural environments. They can also be engineered as per the need and applications. Genes and regulatory elements function as per the needs of the biofilm community.

The distinct benefit derived from the biofilms developed under laboratory conditions have shown considerable promises. Hence, efforts are also put to exploit these biofilm systems as nutrient

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augmenting biofertilizers.

Stages of Biofilm formation

A biofilm formation has five distinct stages [5], as given below

a) Initial attachment stage
b) Irreversible attachment by the production of EPS
c) Early development stage
d) Maturation of Biofilm assembly
e) Dispersion stage

Weak reversible Van der Waals forces are the cause of microbial biofilm attachment to surface. Further cell adhesion structures (eg. pili) help them attain permanent anchoring on the surface. The adhesion and establishment of biofilm bacteria are rapid, usually remain unaltered by physical or chemical nature of the surface concerned or by the fluid flows like streams / currents of river.

EPS is a complex compostion with probable constituents like polysaccharides, proteins, phospholipids and nucleic acids.

Visualization of these microbial biofilms and subsequent imaging can be done through techniques like confocal scanning laser microscopy (CSLM). CSLM permits the non-destructive imaging & analysis of living, highly hydrated microbial biofilms without any harsh chemical treatment or embedding treatments [4].

Biofilms in the soil

Microbial biofilms that are attached to the soil can broadly be classified into three categories.

a) Bacterial (including Actinomycetes) biofilms

When the biofilm members are composed of bacteria, and the sticky binding material is predominantly exopolysachharides, the biofilm is known as bacterial biofilm. The most common biofilm formation is reported by *Pseudomonas* spp - *Ps. aeruginosa*.

b) Fungal biofilms

When the biofilm is composed of highly intertwined fungal hyphae and the binding material is exopolysachharide-like substances (but not EPS), one can classify them as fungal biofilms.

c) Fungal-bacterial biofilms

They are described in the following paragraphs. Both fungal and bacterial biofilms are formed on the soil surface. They are highly compact within the biofilm and respond to soil nutrient dynamics collectively. However , as the density of such biofilms is not adequate, they may not effect significant changes in the soil. Fungal-bacterial biofilms (FBBs) are more interactive as the fungi act as the biotic surface on which the bacteria are attached. The proliferation of bacteria on the fungal surface gives the biofilm bacteria enhanced metabolic advantage than that of bacterial monoculture. Fungal partners also show higher degree of metabolic diversity in terms of sustaining biofilm activities. This metabolic advantage can also be extended over natural multi-species fungal biofilm or multi-species bacterial biofilm over soil surface [13]. Normal fungal-bacterial biofilm associations in soil have already been reported [1] which promotes mycorrhizal symbiosis [6].

Biofilms attached to the plant roots are also found to be participating extensively in the nutrient recycling around the roots and to repel the advances of pathogenic organisms.

Some of the rhizobial members are common to form biofilm association with non-mycorrhizal soil fungi , they are known as fungal-rhizobial biofilms (FRBs). FRBs are more successful in terms of N_2 fixation as they fix the nitrogen biologically as reported by nitrogenase activity and N accumulation data [7].

Advantages of biofilms

1) Efficient uptake of dissolved organic matter through the participation of exoenzymes, signal molecules and ion channels.

2) Genetic and metabolic exchange among the participating microbial members within the biofilm community.

3) Attaining of quick removal of organic matter, pathogens/parasites and suspended soilds from waste water.

4) Capable of removing petroleum and petroproducts in contaminated marine systems.

5) FBBs can also help the plant growth through the production of hormones like indole acetic acid (IAA) [3].

Biofilmed biofertilizers: Applications

The *in-vitro* development of FRBs has ben reported by Seneviratne and Jaysinghearachchi (2003). The incorporation of soybean nodulating *Bradyrhizobium elkanii* SEMIA 5019 in the FRB significantly raised the N_2 fixation in soybean(by approximately 30 %) over rhizobium only inoculants. But neither only the bradyrhizobial strain nor the fungal partner alone can fix N_2 [8].

Co-inoculation of of PGPR (plant growth-promoting rhizobacteria) and AMF (arbuscular mycorrhizal fungi) in rainfed wheat was found to produce the highest protein contents of grains compared to their monocultures [12]. But biofilm formation exclusively by mycorrhizal fungi has not yet been reported, though non-mycorrhizal fungi like *Candida* spp [15], *S.cerevisiae* has been already reported [11].

Bashan [2] has reviewed the role of microbial inocula in agriculture, mixed inoculation using AMF and diazotrophic bacteria has been reported. Solubilisation of rock phosphate can be achieved through the biofilm developed from *Penicillium* spp, *Pleurotus ostreatus* and *Xanthoparmella mexicana* when compared to fungus only cultures [14].

Successful control of phytopathogens have also been achieved through biofilm inocula of *Pleurotus ostreatus- Pseudominas fluorescens* [9]. *P. fluorescens* plays the main biofilm forming role in the association. *Pleurotus ostreatus* participating in FRBs increased N_2 fixation. Also it has enhanced protein content in mushroom [13].

Biofimed inocula was observed to support their rhizobia to survive high salinity (400 mM NaCl) and tannin concentrations (0.4 mM tannic acid) by 10^5 times and 12 times respectively [14]. These may help develop biofilmed biofertilizer technology for salinity affected soils.

It was also studied that the formation of microcolonies by biofilm members and production of toxins may allow bacterial biofilms (eg. *Pseudomonas aeruginosa*) to resist protozoan grazing and persist in the environment [10]. Thus biofilm microbes are tough enough to survive within competitive environment in the soil.

Conclusions

As per the available studies and analysis, biofilmed biofertilizers are superior in terms of efficiency within the soil and of the survival in agricultural lands. They are also helpful for reducing/ removing the pathogenic attack and invasion. FBBs/ FRBs are more effective than their respective mono or mixed cultures. For depleted nutrient status , application of FRBs can be a judicious yet sustainable choice for the agricultural practices. Still, more investigative efforts are needed to choose effective microbial strains or combinations needed for a particular FBB or FRB in terms of the practical nutritional gains achieved by the crop plants for long term applications. It has also been inadequate for research persons to prescribe blanket recommendations for a particular crop plant species without details of the *in vitro* studies followed by suitable field trials. Crop based recommendations can be given once the field trials are significantly successful.

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