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MICROBIAL BIOFILMS: NOVEL BIOFERTILIZERS FOR RUBBER PLANTATIONS

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INTRODUCTION

Continuous cultivation of rubber lands as a mono cultural cropping system has reduced soil fertility and land productivity. Erosion of top soil over more than 100 years and removal of large amounts of mineral nutrients in similar proportions through timber during replanting have mainly contributed to poor soil fertility.

In general the soils of rubber growing areas are gravelly, highly porous and lack in plant nutrients and therefore unsuitable for arable farming according to standard land classification (Dissanayake *et al.,* 1999). In order to enhance soil chemical fertility, it is essential to apply fertilizers. With only half of the applied fertilizers getting into the crop (Bockman *et al.*, 1990), there is a marked economic loss and environmental pollution. With the global fuel crisis, prices of inorganic fertilizers have risen sharply and will continue to rise in the future. Cost of inorganic fertilizers, low efficiency in fertilizer use and continuous use of large doses of inorganic fertilizers and their consequent negative repercussions have drawn attention to develop new approaches in fertilizer use.

Biofertilizers are ready to use live formulates of beneficial microorganisms which on application to soil, mobilize the availability of nutrients by their biological activity in particular, and help to build up micro flora and in turn improve the soil health, in general. Nitrogen-fixing biofertilizers harvest atmospheric nitrogen and convert into ammoniacal form, which will be available to the plants or is released in to the soil with time. Phosphorus biofertilizers solubilize fixed forms of phosphorus already present in the soil and make it available for the use of plants. Composting biofertilizers are used for hastening the process of composting and enriching its nutrient value.

In conventional inoculant technology of biofertilizers, a major problem yet to be addressed is the poor survival of introduced microorganisms in the soils due to various environmental stress factors (Seneviratne *et al*., 2008a). As such, a high level of microbial effect may not be achieved by the conventional practice of plant inoculation with monocultures or mixed cultures of effective microbes.

However, certain microbes can attach to the surfaces and differentiate to form complex multi-cellular communities called biofilms and they have the potential to be more effective biofertilizers than single cultures (Jayasinghearachchi and Seneviratne, 2004). A biofilm consists of microbial cells (algal, fungal, bacterial and/or other microbial) and extracellular biopolymers they produce. The extra cellular polymeric substances provide structure and protection to the community. Such communities can be found in medical, industrial and natural environments. However, the density of biofilms in the nature is not adequate to give sufficient beneficial effects and therefore needs to be produced and multiplied in the laborototy. Biofilms can also be engineered *in-vitro* for various biotechnological applications such as agricultural and environmental applications, enzyme technology, drug discovery studies and green energy research (Seneviratne, 2003; Seneviratne *et al*., 2008b).

Biofilm development

Formation of biofilms begins with the attachment of free-floating microorganisms to a surface. These surfaces could either be living or non living. If the colonists are not immediately separated from the surface, they can strongly anchor themselves onto the surface, almost permanently. The first colonists facilitate the arrival of other cells by providing more divers adhesion sites and ultimately to form a three-dimensional structure of cells encased in exopolysaccharides. There are three major types of biofilms that occur in the soil. They are bacterial, fungal and fungal-bacterial biofilms (FBBs). In fungal-bacterial biofilms, fungi act as a living surface to which the bacteria adhere (Fig. 1a and b).

Biofilm formation is thought to begin when bacteria sense environmental condition where they live. Environmental conditions that require triggering biofilm formation vary among organisms. For example, *Pseudomonas aeruginosa* will form biofilm under almost any condition that allow growth. On the other hand, some strains of *Escherichia coli* will not form biofilm in minimal medium unless supplemented with amino acids. In contrast, some strains of *E. coli* are reported to make a biofilm only in low-nutrient medium (Dewanti and Wong, 1995). In addition to the nutritional content of the medium, temperature, osmolarity, pH, iron and oxygen contents are some other environmental factors that can influence biofilm formation (Watnick and Kolter, 1999).

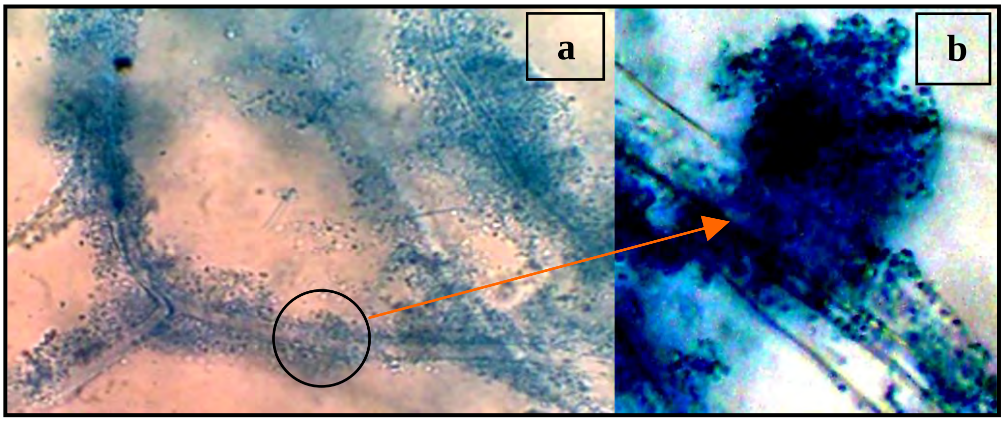

Fig.1. A microscopic view of bacterial cells attached to a fungal filament forming a fungal-bacterial biofilm (a) and a close-up of the biofilm (b).

Properties of biofilms

The biofilms are held together and protected by a matrix of excreted polymeric-compounds called exopolysaccharide. This matrix provides protection from variety of environmental stresses and facilitates communication among themselves through biochemical signals (Flemming, 1993). Biofilms have a unique pattern of gene expression which is different from their non-biofilm forming stages.

Advantages and disadvantages of biofilms

Biofilm communities are present in the rhizosphere (the root surface and the region immediately surrounding a root) and bacteria attached to roots then produce exopolysaccharides. This not only provides many advantages to bacterial cells, but also enhances soil aggregation by cementing particles together and leads to marked improvement of soil structure and helps plants avoid water stress (Amellal *et al*., 1998). Microorganisms living in rhizosphere biofilms enhance cycling of nutrients and help biocontrol of pest and diseases, resulting in improved agricultural productivity (Seneviratne, 2003).

Interactions between common soil fungi and Rhizobia have formed fungal-rhizobial biofilm and these interactions have fixed N_2 biologically. This was not appeared when the *Rhizobium* spp. was grown as a monoculture (Jayasinghearachchi and Seneviratne, 2004). The fungal-bacterial biofilms of beneficial endophytes have been observed to produce high acidity and plant-growth promoting hormones (Bandara *et al*., 2006). The higher acidity is generally important for pathogen suppression and enhanced weathering of the mineral substrates (Jayasinghearachchi and Seneviratne, 2006; Seneviratne and Indrasena, 2006).

The ability of bacteria to form films on living and non living surfaces could also create problems in plant and animal life. They sometimes can be harmful or pathogenic. Furthermore some pathogenic bacteria in biofilms can be more resistant to antibiotics and unfavorable environmental factors such as, extreme temperatures, pH values and osmolarity than when they are free living (Costerton *et al*., 1987).

Application in agriculture

The conventional practices of plant inoculation with effective microbes may not provide substantial benefits. It has now been invented biofilmed inocula developed *in vitro*, which give improved microbial action, compared to conventional biofertilizers (Seneviratne *et al*., 2008a). The knowledge gained through numerous recent studies has shown a promising trend in the application of biofilm-based biofertilizers known as biofilmed biofertilizers (BFBFs) in diverse crops.

The plant growth-promoting effects of BFBFs have been observed in rice (*Oryza sativa*), tea (*Camellia sinensis*), wheat (*Triticum aestivum*) and anthurium (*Anthurium andraeanum*) (Seneviratne *et al*., 2009). Apart from their growth promoting effect, biofilms help to increase soil carbon accumulation as well one of the most important fertility promoters in the soils (Jayasekara *et al*., 2008).

Recently, it has been found that chemical fertilizer application can be cut down by half when BFBFs are applied into tea plantations (Zavahir *et al*., 2009). However, this valuable technology has not still been tested in rubber growing soils, but experiments are underway to develop BFBFs using microorganisms isolated from rubber growing areas in Sri Lanka.

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