Potential Application of Biofilms : A New Approach for Tea Gardens

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Introduction

Surface-attached microbial communities or biofilms are found in many environments, including the soil. They can also be engineered *in vitro* for various biotechnological applications (Seneviratne, 2003; Seneviratne *et al.*, 2007). This study describes the potential applications of developed biofilms as biofertilizers in tea cultivation. Biofertilizers improve the fertility of soils by interactive effects of living organisms. They could be nutritional, hormonal and/or biocontrol. The use of biofertilizers is already established in soybean and mung bean cultivation in Sri Lanka. The objective of this project was to find means of biofertilization and biocontrol for tea plants using the 'biofilm technology'.

Biofertilizers and biocontrol can be defined briefly as follows.

- Biofertilizers- A "microbial inoculant" usually refers to preparations of microorganism (s) that may be a partial substitute for chemical fertilization.
- Biocontrol (biological control)- is the control of pests, plant pathogens or weeds by disrupting their ecological status by biological means.

The 'rhizosphere' *i.e.* the zone of influence around the roots harbors a multitude of microorganisms that are affected by various stress factors. Among these are the dominant 'rhizobacteria' that prefer living in close vicinity of the root or on its surface and play a crucial role in soil health and plant growth. Both free-living and symbiotic bacteria are involved in this and help in plant matter degradation, nutrient mobilization and bio-control of plant diseases.

With the modern tools of science, the recent past has seen a growing interest in the study of microbial communities or biofilms associated with plant growth and development as well as the monitoring of microbes when they are in the soil and root environments. This is linked with environmental concerns for reduced chemical usage and an appreciation for the use of biological substances. This highlights the importance of the use of microbes as inocula. Thus in 2003, the potential use of developed biofilms in a multitude of biotechnological applications in agriculture, forestry and medicine, including plant growth promotion was suggested for the first time, based on the studies that had been conducted up to then at the Institute of Fundamental Studies (Seneviratne, 2003).

Microbes used as inocula

The method of purposefully introducing microorganism(s) into a system is known as 'inoculation'. The material containing such a microbial formulation is an 'inoculum' (plural inocula), which may contain bacteria, fungi, viruses or a combination of two or more of them. The immediate response to soil inoculation with an inoculum varies depending on the micro organism, plant species, soil type, inoculum density and environmental conditions. The heterogeneity of soil poses a key obstacle in inocula addition since the inoculated microbe sometimes cannot find an empty niche in the soil environment for survival. They must therefore interfere and quite often compete with the better-adapted native microflora.

The inoculation of plants with beneficial microbes can be traced as far back as 19th century (Nobbe and Hiltner, 1896). Many studies have been conducted on a variety of crops over the years where microbes have been added in disease control and to improve growth, Researchers world over, have studied the diversity of rhizobacteria in a variety of plants including cereals and legumes. Their function and resistance to diseases have been screened and field-tested in various agro-ecosystems. For example,

a) In a study conducted in India, a strain of the bacterium *Bacillus megatherium* was isolated from tea rhizosphere and tested for its ability to promote growth and cause disease reduction in tea plants. *In vivo* studies revealed the ability of this bacterium to promote growth of tea plants very significantly.
b) Brown root rot disease, caused by *Fomes lamaoensis* was markedly reduced by application of the bacterium *Bacillus megatherium* to the soil (Chakraborty *et al.*, 2006).
c) Inoculation of the bacterium *Bradyrhizobium elkanii* SEMIA 5019 in Soybean fields have shown yield increases over 5000 acres in Sri Lanka (Prof. S.A. Kulasooriya, personal communication).

The use of such microbial inocula in plant growth promotion and reduction of disease intensity have been shown due to a combination of several mechanisms. Although much is known about the survival of bacteria within the protective environment of an inoculum carrier, little is known about the stresses that bacteria must endure upon transfer to a competitive and often alien soil environment (Heijnen *et al.*, 1992;

Rodriguez-Navarro *et al.*, 1991; Van Elsas and Heijnen, 1990). Hence, inocula have to be designed to provide a dependable source of beneficial bacteria that survive in the soil and become available to the plant (Bashan, 1998).

This study, as stated above, endeavors to develop an inoculum in the 'biofilm' state.

What are Biofilms?

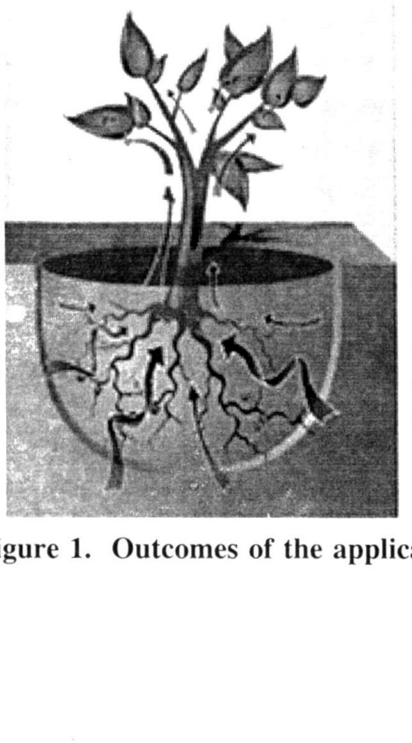
A 'Biofilm' is a complex aggregation of microorganisms which may be bacterial, fungal, algal and/or other microbial. They are often encased in a self-excreted and adhesive matrix called the extra-cellular polysaccharide (EPS) layer, which is protective to the microbial community. Biofilms may at times be attached to a biotic or abiotic surface and can be found in many natural, medical and industrial environments. The microbes undergo profound changes during their transition from planktonic (free swimming) organisms to the aggregated forms of biofilms.

Biofilms have proved to be beneficial with greater potential in concerned fields. Characteristics of biofilms (Figure 1) that make them excellent means of providing protection to plants include;

- Protecting cells from factors such as ultra-violet radiation, heat, acidity, salinity, *etc*.
- Protecting from inhibitory substances such as biocides and disinfectants when exposed.
- Resisting the access to the phages.
- Complementing biological control of pests and diseases.
- Increased agricultural productivity
- Increased nutrient recycling

Foliar

Root



Foliar and shoot application of biofilmed inocula

- Pathogen suppression by high acidity
- Growth hormone production for improved shoot growth

Soil application of biofilmed inocula

- Pathogen suppression by high acidity
- Mineral and organic nutrient release
- Growth hormone production and enhanced root and mycorrhizal growth

Figure 1. Outcomes of the application of biofilmed inocula to plants

Fungal-Bacterial Biofilms (FBB)

Many studies conducted both in the local and international arenas have shown that fungal-bacterial biofilms (FBB) are more effective than monocultured or bacterial-bacterial biofilms, or even a mixed culture of bacteria and fungi in their non-biofilm forming stages (Seneviratne *et al.*, 2007).

Application of FBBs extends to the area of polythene biodegradation, biosolubilization of rock phosphate, solubilization of mineral substrates, increased protein content of mushrooms, *etc*. The FBBs have also shown to provide better plant pathogen suppression by lowering the acidity and increasing plant growth promoting factors such as Indole Acetic Acid (IAA) (Bandara *et al.,* 2006). Bioactive compound production of a developed *Penicillium* spp.*Bradyrhizobium elkanii* SEMIA 5019 biofilm was observed to generate a higher number of detectable compounds with relatively high concentrations compared to component of monocultures (Zavahir and Seneviratne, 2007).

Presence of biofilms have shown to help bacteria survive in adverse conditions and previous studies have also shown that such biofilmed inoculants can improve N fixing symbiosis in legumes, contributing to plant's N nutrition probably as a result of former in the long term (Jayasinghearachchi and Seneviratne 2004), favoring the growth of even non-legumes.

Due to their efficiency and increased environmental stress tolerance, these FBBs (Figure 2) were chosen in this study.

Figure 2. A Microscopic view of a biofilm; a fungal hyphae with bacteria attached to its surface

The first objective when considering inoculation with microbes is to find the best microbe available (Bashan *et al.* 1993; Glick, 1995; Kloepper *et al.*, 1989). Next, a study of the specific inoculum formulation is undertaken. In practical terms, the chosen formulation determines the potential success of the inoculum (Fages, 1992). Once a feasible strain is chosen, it is optimized, tested and applied with the help of a carrier material.

The preliminary studies of developing such an inoculum was carried out using different fungal species isolated from tea rhizosphere. Bacteria used were *B. elkanii* SEMIA 5019 from soybean tested, a species from *Arachis pintoii* and other N fixing bacteria from refuse tea and vermicast.

Three months old tea plants were screened using the following treatments. Relative growth rate was measured at monthly intervals up to 4^{th} month. Each treatments included 10 tea plants.

A range of combinantions of treatments comprising of tea root fungus, refuse tea fungus, bacteria from rerfuse tea, *Arachis pintoii* and vermicast together with SEMIA 5019 were tested along with standard T 65 fertilized plants as a control. Yeast Manitol Broth (YMB) was used as the medium for inculation.

During the period of study all inoculated plants were maintained without any chemical fertilizer application. After 5 months, an enhanced root growth was seen in inoculum-treated plants compared to the control. This may possibly be due to the phosphate solubilizing ability of biofilms (Jayasinghearachchi and Seneviratne 2006) which in turn encouraged root growth.

Efficient strains were chosen from this preliminary study and new treatments were devised to continue this research. In the second stage, which is in progress at the TRI stations it is envisaged to obtain plants with increased shoot growth with minimal or no application of chemical fertilizers.

It is expected that this research would lead to technologies that are environmentally and socially acceptable and beneficial.

References

Bandara W M M S, Seneviratne G and Kulasooriya S A 2006 Interactions among endophytic bacteria and fungi: effects and potentials; J Biosci. 31, 645–650.

Bashan Y 1998 Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnology Adv. 16, 729-770.

Bashan Y, Holguin G and Lifshitz R 1993 Isolation and characterization of plant growth promoting rhizobacteria. *In* Methods in Plant Molecular Biology and Biotechnology. Eds. B. R. Glick and J. E. Thompson, 331-345, CRC Press, Boca Raton, Florida.

Chakraborty U, Chakraborty B and Basnet B 2006 Plant growth promotion and induction of resistance in *Camellia sinensis* by *Bacillus megatherium*. J Basic Microbiol. 86, 186–195.

Fages J 1992 An industrial view of *Azospirillum* inoculants: formulation and application technology. Symbiosis. 13, 15-26.

Glick B R 1995 The enhancement of plant growth by free-living bacteria. Can J Microbiol. 41, 109-117.

Heijnen C E, Hok-A-Hin C H and Van Veen J A 1992 Improvements to the use of bentonite clay as a protective agent, increasing survival levels of bacteria introduced into soil. Soil Biol Biochem. 24, 533-538.

Jayasinghearachchi H S and Seneviratne G 2004 A *bradyrhizobial-Penicillium* spp. biofilm with nitrogenase activity improves N_2 fixing symbiosis of soybean. Biol Fertil Soils. (2004) 40, 432-434.

Jayasinghearachchi H S and Seneviratne G 2006 Fungal solubilization of rock phosphate is enhanced by forming fungal-rhizobia biofilms. Soil Biol Biochem. 38, 405-408.

Kloepper J W, Lifshitz R and Zablotowicz R M 1989 Free-living bacteria inocula for enhancing crop productivity. Trends Biotechnol. 7, 39-44.

Nobbe F and Hiltner, L 1896. At the accommodation of various leguminous bacteria from developed nodules on different varieties. Annales Agronomiques 22, 494-495.

Rodriguez-Navarro D N, Temprano F and Orive R 1991 Survival of *Rhizobium sp.* (*Hedysarum coronarium* L.) on peat-based inoculants and inoculated seeds. Soil Biol Biochem. 23 No 4, 375-379.

Seneviratne G 2003 Development of eco-friendly, beneficial microbial biofilms. Curr. Sci. 85, 1395-1396.

Seneviratne G, Zavahir J S, Bandara W M M S and Weerasekera M L M A W 2007 Fungal-bacterial biofilms: their development for novel biotechnological applications. World J Microbiol Biotechnol (in press).

Van Elsas J D and Heijnen C E 1990 Methods for the introduction of bacteria into soil: a review. Biol Fertil Soils. 10, 127-133.

Zavahir J S and Seneviratne G 2007 Potential of developed microbial biofilms in generating novel bioactive compounds. Res J Microbiol. 2, 397-401.