Abstract No: 276 ***Plant Science and Forestry***

RICE ROOT COLONIZATION BY AN AZORHIZOBIAL-FUNGAL BIOFILM

Thilini Perera[1], Shamala Tirimanne[1]* and Gamini Seneviratne[2]

[1]Department of Plant Sciences, Faculty of Science, University of Colombo, Sri Lanka
[2]Institute of Fundamental Studies, Hantana, Kandy, Sri Lanka
**shamala.tirimanne@gmail.com*

Rice is the most consumed cereal crop in Sri Lanka and cultivation of rice demands a very high input of nitrogen fertilizers. Use of nitrogen fertilizer over a long period is known to cause a number of environmental and socio-economic problems, and Biological Nitrogen Fixation (BNF) of rice would be the excellent alternative. A free living bacterium, *Azorhizobium caulinodans* ORS 571 is unique over other nitrogen fixers due to its ability to fix nitrogen non-symbiotically and the ability to tolerate up to 3 % v/v oxygen. Both of these characters are of extreme importance in BNF of a non-legume such as rice, under aerobic conditions. Biofilmed-biofertilizers are a novel approach with very high potential in practical application and benefits. This study aims at studying the potential of developing a biofilm with the bacterium *A. caulinodans* and an *Aspergillus* sp. isolated from the rice rhizosphere, and its ability of colonizing rice roots and enhancing biological nitrogen fixation. The bacterium was labeled with a green fluorescent protein marker (GFP) for reliable and accurate detection *in vivo*.

GFP-labeling was carried out by inserting the gfp gene containing plasmid pBBR5-hem-gfp5-S65T in to *Azorhizobium caulinodans* ORS 571, with the help of a helper plasmid (pRK2013) by tri-parental mating. A broth culture of *A. caulinodans* was inoculated on to an *Aspergillus* sp. culture and appropriate conditions were given for the development of the biofilm. The resulting biofilm was inoculated on to rice seedlings of variety BG 359 and the plants were allowed to grow for 15 days. After 15 days the roots were analyzed for bacterial colonization, and acetylene reduction assay was conducted as an indicator of BNF.

A successful *A. caulinodans-Aspergillus* biofilm was observed as a result of inoculation. Maximum colonization by the GFP-labeled *Azorhizobium caulinodans* on the mycelia of *Aspergillus* spp. and healthy biofilm formation was observed on the third day after inoculation. The biofilm was stable for two more days and then deteriorated. Three day old biofilm inoculation to rice and the observations on the fifteenth day revealed that the biofilm colonizes the rice roots and are visible as green shiny clusters on and around the root hairs, under the epifluorescent microscope. Acetylene Reduction Assay carried out with fifteen day old rice plant roots revealed that there is an enhanced nitrogenase activity in the rice roots inoculated with the biofilm compared to the control containing *A. caulinodans* only.

From this preliminary study, it can be concluded that the GFP-labeled *A. caulinodans* is capable of forming a biofilm with the *Aspergillus* spp. isolated from the rice rhizosphere and this biofilm can successfully colonize the rice root surface and contribute to enhanced nitrogen fixation.

Financial assistance given by the National Research Council, Sri Lanka (NRC-11-21) is acknowledged.