

Exploiting the Synergism of a Tripartite Association of *Pinus patula*, Ectomycorrhizal Fungi and Rhizosphere Bacteria for the Biodegradation of Soil Phenanthrene and Fluoranthene

R.A. Adeleke, M.P. Maila, M.M. Bello-Akinosho, and M. Thantsha

Background/Objectives. The mutual association of mycorrhizae involving *Pinus patula*, ectomycorrhizal fungi and rhizosphere bacteria are well known for their ecosystem functions that they are currently explored for bioremediation purposes. Biodegradation is a cost-effective and ecosystem-friendly means of removing pollutants, such as polycyclic aromatic hydrocarbons (PAHs), from soil. The toxicity and adverse effects of PAHs to the environment and humans warrant their efficient removal. Biodegradation of PAHs by a mycorrhizal association of organisms is sustainable and promises to bring about enhanced degradation because the synergistic metabolic assemblages of the organisms will be utilized. The study therefore sought to sustainably biodegrade phenanthrene and fluoranthene contaminating soil by association of pine plant, ectomycorrhizal fungi and rhizosphere bacteria with ability to enhance soil fertility.

Approach/Activities. A greenhouse experiment was set up in which sterile agricultural soil was contaminated by 100 mg of each of phenanthrene and fluoranthene per kg of soil. The contaminated soil was potted and maintained at water holding capacity of 60 to 80% of the field capacity. Surface-sterilized pine seeds were germinated after which bacteria and ectomycorrhizal fungi (ECMF) were introduced into the pots in six different treatment combinations which included the control. Each treatment was replicated 18 times to facilitate destructive sampling at three different times. Biodegradation were efficiently monitored by the degradation genes, which included the alpha subunit of the polycyclic aromatic hydrocarbon ring hydroxylating dioxygenase (PAH-RHD) genes. Samples of each treatment were also analyzed for phenanthrene and fluoranthene by GC-MS. The diversity of bacteria associated with the biodegradation process was also evaluated by metagenomics sequencing on Illumina platform.

Results/Lessons Learned. The ring hydroxylating dioxygenase genes were amplified in all treatments containing PAHs. PAH-RHD genes were cloned and identified as being homologous to the alpha subunit of several bacterial hydroxylating dioxygenases at sequence similarity of 98% and above. The alpha subunit is the catalytic as well as substrate-determining domain of the gene. Biodegradation was demonstrated by the relative abundance of these genes, dissipation of PAHs in the various treatments and prevalence of unculturable bacteria known to be associated with hydrocarbon degradation. This was revealed by Illumina sequencing. Mycorrhizal associations thus promises sustainable biodegradation of typical PAHs which are important pollutants worldwide.