Selection of Fungi and Bacteria by Antagonism test for Construction of a Mixed Microbial Consortium for Bioremediation of Soil Contaminated with Bisphenol A

Gerardo López Garrido (gera_gera_4@hotmail.com) and Diana V. Cortés-Espinosa (dcortes@ipn.mx) (Instituto Politécnico Nacional, Tlaxcala, México)
Angel E. Absalón (angelabsalon@vaxbiotek.com) (Vaxbiotek AC Company, Puebla, México)

Background/Objectives. Bisphenol A (BPA) is an emerging pollutant found in water, soil and air; belonging to the group of endocrine disruptors, generally related to diseases in the male and female reproductive system, associated with eating disorders, diabetes, cardiovascular diseases and irritation of the digestive tract. The use of bioremediation processes can help us remove this contaminates from the environment by bioaugmentation/biostimulation techniques. The formation of microbial consortia can increase the removal of this pollutants from the environment when these are bioaugmented. The aim of this work was to select fungi and bacteria able to tolerate high concentration of BFA with the purpose of building a mixed microbial consortium for their application in bioremediation of soils contaminated with BFA.

Focus/Activities. Six strains of fungi (*Aspergillus niger*, *Aspergillus terreus, Aspergillus flavus*, *Aspergillus nomius,* H13R and H20R) and ten bacterial strains (*Bacillus* sp*., Bacillus pumilus, Bacillus luteolus, Cellulosimicrobium cellulans, Klebsiella* sp*., Klebsiella pneumoniae, Pseudomonas aeruginosa,* C2R and C5R) were previously selected for their high tolerance to BFA. The antagonisms test was carried out using Toyama's and Czapek medium plates with and without 350 ppm of BFA. For fungi-fungi antagonisms, $1x10^4$ spores from each strain were spotted onto an agar plate and incubated at 30°C until inhibition halos or antagonist effects between fungal colonies were observed. For bacteria-bacteria the challenged strain was massively planted at a concentration of $1x10^4$ CFU. Then with the help of a micropipette 10 µL of the antagonistic strains were superficially placed at a same concentration. They were incubated at 30°C and monitored until they observed inhibition halos. Fungi to bacteria antagonisms were evaluated by centrally spotting 10^4 spores of a fungal strain (antagonist) and incubating at 30°C. After observing sporulation (indicative of secondary metabolism), $1x10^4$ CFU of each bacterial strain were radially streaked aside fungal colonies and incubated until bacterial growth was observed or inhibited by the fungi. Bacteria to fungi antagonisms were evaluated by spotting $1x10^4$ CFU of each bacterial strain (antagonists) in the periphery of the plates and centrally inoculating $1x10^4$ spores of a fungal strain at the same time.

Results/Lessons Learned. In the fungus-fungal antagonist test, the fungal strains did not show antagonism, since growth inhibition was not observed between the challenged strains and the challenging strains in both media without BFA, although better growth was observed in the Czapek medium compared to the Toyama's means besides that the growth was delayed in the latter. In the medium plus the addition of 350 ppm BFA the growth was very low, and no contact could be observed between the strains. In the bacteria-bacteria antagonism test the strains showed a better growth in Czapek medium added with 350 ppm of BFA, no inhibition halos were observed, the strains of *B. pumilus, B. luteolus, C. cellulans,* C2R and C5R showed low growth in both media with and without BFA. Bacteria-fungi and fungi-bacteria test, the strain *Bacillus pumilus* and *Bacillus luteolus* showed antagonism to the growth of *Aspergillus flavus*, the rest bacteria and fungi strains no showed antagonism.