Enrichment, isolation and characterization of dichlorodiphenyltrichloroethane (DDT) microbial degraders from soils in Kenya

Julius Kinuthia Mwangi

A thesis submitted in partial fulfillment for the degree of Master of Science in Biotechnology in the Jomo Kenyatta University of Agriculture and Technology

2009

ABSTRACT

Dichlorodiphenyltrichloroethane (DDT) has been in use as an agricultural insecticide and as a disease vector control since 1939. Due to its persistence in the environment, its use has been surrounded by controversies regarding its safety to humans and the environment. There has been no reliable and replicable scientific evidence proving its harmful effects to humans though DDT was banned in many developed nations by 1970s. The DDT ban has seen to the reemergence of malaria and other insect borne diseases in the tropics. There being no better alternatives to DDT, its use to control malaria vector has been recommended by the World Health Organization. Responsible use of DDT should include research to determine possible ecotoxicalogical effects due to its accumulation and biomagnifications potential. In this study, the potential for biodegradation of DDT by soil microorganisms through enrichment and isolation of DDT biodegraders in uncontaminated tropical soils has been done. Microorganisms from both cultivated and uncultivated soils were found to grow in MM4 media with DDT (100 ppm) as the only carbon source. DDT degradation over a period of 29 days was higher in uncultivated soils (60.20%) than in cultivated soils (38.58%). Six isolates coded as isolate **101,** isolate **102,** isolate **103,** isolate **104,** isolate **105** and isolate **110** degraded DDT into l, l-dichloro-2, 2-bis (p-chlorophenyl) ethane (DDD). None of the isolates was capable of transforming DDT into l, l-dichloro-2, 2-bis (p-chlorophenyl) ethylene (DDE). The ability of the six isolates to degrade DDT from highest to lowest over a period of 31 days was **102** (58.08%), **101** (44.31%), **103** (39.72%), **104** (30.33%), **105** (28.97%) and **110** (28.48%). The degradative ability of the six isolates combined over the same period was higher (82.63 %) than that of any individual isolate (range of DDT degraded by the isolates was 28.48 %-58.08 %). The identity of the six isolates was determined through microscopic, biochemical and molecular techniques. Phylogenetic

analysis of the 16S rRNA gene sequences of the isolates showed them to belong to genera *Bacillus* for isolate **101** with a 16S rRNA gene sequence similarity of 99 % to *Bacillus cereus.* Isolates **102** and **110** were members of the genera *Stenotrophomonas* with 16S rRNA gene sequence similarity of 98 % to *Staphylococcus sciuri.* Isolates **103**, **104** and **105** were members of the genera *Stenotrophomonas* with 16S rRNA gene sequences similarity of 95 %, 97 % and 94 % respectively to *Stenotrophomonas maltophilia*. Isolates **103**, **104** and **105** could be new species. There are DDT biodegraders in the tropical soils as evidenced by the isolates.