African Journal of Microbiology Research Vol. 4 (3) pp. 185-196, 4 February, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2010 Academic Journals

Full Length Research Paper

Degradation of dichlorodiphenyltrichloroethane (DDT) by bacterial isolates from cultivated and uncultivated soil

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Accepted 3 December, 2009

The re-introduction of dichlorodiphenyltrichloroethane (DDT) to control mosquitos was recommended by the World Health Organization in 2007. In this study, the potential for biodegradation of DDT by soil microorganisms through enrichment and isolation of DDT biodegraders from soils without a history of prior exposure to DDT was done. Microorganisms from cultivated and uncultivated soils grew in minimal media with DDT (100 ppm) as the only carbon source. Six bacteria coded as isolates 101, 102, 103, 104, 105 and 110 degraded DDT to l, l-dichloro-2, 2-bis (p-chlorophenyl) ethane (DDD). None of the isolates degraded DDT into l, l-dichloro-2,2-bis (p-chlorophenyl) ethylene (DDE). Degradation by the mixed culture of the six isolates was higher (82.63%) than that of any individual isolates whose range was 28.48 - 58.08%. The identity of the isolates was determined through biochemical, morphological, physiological and molecular techniques. Isolate 101 was a member of the genus *Bacillus*; isolates 102 and 110 belonged to the genus *Staphylococcus* while isolates 103, 104 and 105 clustered with members of the genus *Stenotrophomonas*. This study showed that there are microorganisms in the soil that can degrade DDT and that the rate of degradation is dependent on the presence and numbers of microbes in the soil with the required degradative ability, environmental factors and access of the microbes to DDT.

Key words: DDT, biodegradation, bacterial isolates, phylogenetic analysis.

INTRODUCTION

DDT is still one of the first and most commonly used insecticides for indoor residual spraying because of its low cost, high effectiveness, persistence and relative safety to humans (Hecht et al., 2004). It is therefore a viable insecticide in indoor residual spraying owing to its effectiveness in well supervised spray operation and high excito-repellency factor. Although DDT is very effective in killing or repelling mosquitoes its use has been severely reduced and restricted to indoor residual spraying, due to its persistence in the environment and ability to bioconcentrate in the food chain (Cousins et al., 1998; Hickey, 1999).

One of the removal processes with significant impact on the fate of DDT in the environment is biodegradation (You et al., 1995). Biodegradation and bioremediation are matching processes to an extent that both of these are based on the conversion or metabolism of pesticides by microorganisms (Hong et al., 2007). A successful bioremediation technique requires an efficient microbial strain that can degrade largest pollutant to minimum level (Kumar and Philip, 2006).The rate of biodegradation in soil depends on four variables: (i) Availability of pesticide or metabolite to the microorganisms (ii) Physiological status of the microorganisms (iii) Survival and proliferation of pesticide degrading microorganisms at contaminated site and (iv) Sustainable population of the microorganisms (Dileep, 2008). Therefore, to attain an achievable bioremediation, it requires the creation of unique niche or microhabitats for desired microbes, so they can be successfully exploited.

So far, no micro-organisms have been isolated with the ability to degrade DDT as a sole carbon and energy

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source (Jacques et al., 2008), but organisms may degrade the organochlorine via co-metabolism under aerobic or anaerobic conditions. Most reports indicate that DDT is reductively dechlorinated to DDD under reducing conditions (Lai and Saxena, 1999). Extensive biodegradation of DDT and DDT metabolites in some bacteria has been demonstrated (Aislabie et al., 1998). The major bacterial pathway appears to involve an initial reductive dechlorination of the trichloromethyl group to form DDD. Further dechlorination to other intermediaries occurs resulting finally into non chlorinated compounds which are not harmful to the environment.

This study was based on the need to clean soil containing DDT in case it finds its way there and initiation of an assessment on impact of DDT on the tropical soil environment. The great versatility of microorganisms offers an inexpensive, simpler and more environmentally friendly strategy to reduce environmental pollution than non biological options (Jacques et al., 2008). The aim of this research was to isolate and characterize micro-organisms that could biodegrade DDT from the tropical soil. Knowledge of the genetics, physiology and biochemistry of these microbes could further enhance the microbial process to achieve bioremediation of DDT with precision and in a short time. The standard method for isolating microorganisms with the ability to degrade environmental pollutants is to enrich them from contaminated soils. This process has not been very successful for the isolation of microorganisms that can mineralize DDT. A novel approach for isolating DDT-degrading microorganisms is to screen alternative sources like uncontaminated soil and other materials.

MATERIALS AND METHODS

Chemicals

DDT and related metabolites were purchased from Sigma-Aldrich Chemical Company. All other chemicals, bacterial media and reagents were purchased from Oxoid limited- England, Scharlau Chemie- South Africa, Himedia laboratories and PVT limited- India. All the solvents and chemicals were high purity grade reagents.

Collection of soil samples

Six soil samples were collected from cultivated and uncultivated areas in Jomo Kenyatta University of Agriculture and technology, Juja campus-Kenya. DDT had not been used in the two areas previously hence they were uncontaminated with it. Collection of the soil samples was through digging and scooping 10 cm^3 of the soil using a sterile spoon. The soil samples were carried to the laboratory in sterile plastic containers.

The sampling area was largely covered by pyroclastic rocks (Muchena et al., 1998) that contain crystals of orthoclase anorthoclase and rarely crystals of aegirine. The tuffs did not show any great variation in thickness or composition.

The area had slopes ranging from 0-2 % and showed very little gravely sandy clay to clay texture and a friable moist consistence. The soil structure was generally weak and fertility was low in the non cultivated places. The pH range was from 5.6 in the topsoil to 8.4 in the subsoil. The soil had moderate to low organic matter from 1.0 - 1.7 %. The nitrogen (N) status was low ranging from 0.11 - 0.14%. Phosphorus value was below 10 ppm. Calcium, magnesium and potash were adequate. The soils had high cation exchange capacity. The cultivated parts had higher nitrogen, phosphorus and organic matter.

Isolation of DDT biodegrading bacteria by enrichment techniques

Soil samples (1 g) each were dissolved in 9 ml of 0.85% NaCl and diluted to 10^{-6} in ten-fold dilution steps. Dilutions of soil samples were inoculated into MM4 medium (Brune et al., 1995), that contained per litre, NaCl [1.7 g], KCl [6.5 g], $MgCl_2.6H_2O$ [0.50 g], $CaCl_2.2H_2O$ [0.10 g], NH_4Cl [5.6 g], $NaS0_4$ [1.0 g] and KH_2PO_4 [1.0 g]. The following were also added from sterile stock solutions: 1 M Na-phosphate buffer [40 ml, pH 7.0] and trace elements solutions, SL 11 [2 ml] and Se/W solution [2 ml] (George et al., 2005). Distilled water was used to top the above to one litre. Dilutions of 10^{-6} were enriched in MM4 medium with the following concentrations of DDT: 0, 20, 50 and 100 ppm. This was done to determine the highest concentration of DDT that could be used in subsequent tests. Then, an aliquot of 1.0 ml from dilution 10^{-6} from each of the six soil samples were inoculated in a 100 ml broth of MM4 medium containing DDT at (100 ppm). Cultures were incubated at 30°C in the dark until growth became constant and began to decline. Growth was determined by monitoring turbidity at optical density (OD_{600}) using a spectrophotometer. The incubation took 31 days. When the growth of enrichment cultures became constant, aliquots of 0.1 ml were streaked on MM4 medium solidified with 1.5% agar and containing 100 ppm DDT. The inoculated media were incubated at 30°C for six days. Distinct colonies were then picked, streaked in fresh media and incubated for another six days to obtain pure cultures.

Degradation of DDT by the isolates

Aliquots of 1.0 ml from actively growing pure culture of each isolate were inoculated separately in MM4 medium in which 100 ppm DDT was added and incubated for 31 days at 30°C. The initial concentration of DDT used was 5000 ug/ ml and dilutions in sterile media were made to a final concentration of 100 ppm DDT. Uninoculated medium with DDT and inoculated medium without DDT were used as controls. Turbidity, and concentrations of DDT and its metabolites DDD and DDE were measured after every two days. Isolates that grew in the medium and reduced the concentration of DDT in the medium were judged to be biodegrading DDT.

Analysis of DDT and its metabolites

The amount of DDT and its metabolites, DDD and DDE, were analyzed using High Performance Liquid Chromatography (HPLC, Shimadzu). This was done on a reverse phase C-18 Column 125 x 4 mm, 5 µM, equipped with UV-VIS detector (Ali and Aboul, 2002). Analysis was done at 25°C and the mobile phase was 99.9% methanol at a flow rate of 0.5 ml / min. Peak detection was at 240 nm. DDT was detected at a retention time of 3.78 min while DDD and DDE were detected at 3.2 and 4.3 min respectively. Peak areas of known concentrations of DDT, DDE and DDD were used to draw the standard curves. The curves were used to determine the

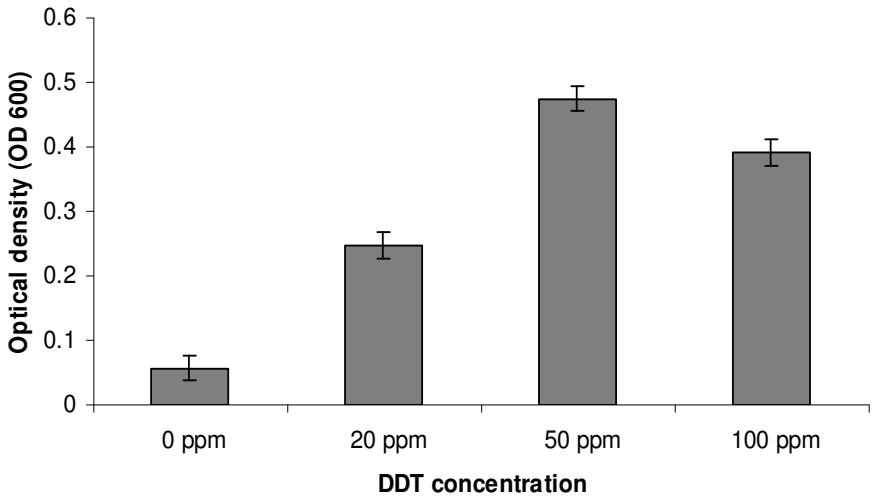

Figure 1. Growth of soil enrichment cultures at different concentrations of DDT.

concentration of DDT, DDD and DDE in the cultures and in the uninoculated control.

Characterization of isolates

Colony morphology was determined using a dissecting microscope. Biochemical tests that were also conducted included the triple sugar iron agar, citrate utilization, gelatin liquefaction, methyl red-Voges Proskauer, urease test, nitrate reduction, motility at 37°C, starch hydrolysis, egg yolk reaction, growth at 7 and 10% NaCl, phenylalanine test, tyrosine test, aerobic growth, anaerobic growth, H_2S production, casein hydrolysis and catalase test (Cappuccino and Sherman, 2001; Holt and Bergey, 1994). The ability of the isolates to utilize sodium acetate, benzoic acid, resorcinol, diazinon, isopropylamine salt of glyphosate, lactose, dextrose, sucrose, glucose, cellobiose and fructose was also assessed. The identity of the isolates was however confirmed through molecular characterization. Total bacterial DNA was extracted according to procedures described by Schmidt et al. (1991), purified and used as a template for amplification of 16S rRNA gene. PCR amplification was performed with a model Gene Amp 9800 thermal cycler (Applied Biosystems) using universal primers 27F 5'-GAG TTT G (AC) T CCT GGC TCA G- 3' forward primer and 1492R 5'-TAC GG (CT) TAC CTT ACG ACT T-3' reverse primer (Lane, 2001). The PCR Products were purified using QuickClean 5M Gel Extraction Kit and then sequenced. The forward and backward 16S rRNA gene sequences of the bacteria isolates were viewed and edited using Chromas software package (www.technelysium.com.au). They were then aligned using Bioedit sequence alignment editor software package (Hall, 1999) to provide full sequences of about 1500 nucleotide bases. The sequences were compared to sequences in the public databases with the BLAST search program on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/) to find closely related bacterial 16S rRNA gene sequences. The ARB database software package (Ludwig and Strunk, 1996) was also used to align and identify the closely related bacterial 16S rRNA gene sequences. The 16S rRNA gene sequences of the isolates and those of the closely related bacteria were then aligned and processed to produce Phylogenetic trees using MEGA software package (www.megasoftware.net).

RESULTS

Growth of enrichment cultures at different DDT concentrations

The maximum growths of enrichment cultures observed at DDT concentrations of 0, 20, 50 and 100 ppm, after a period of 15 days (Figure 1) were different from each other. There was minimal growth of 0.05 ($O.D_{600}$) at DDT concentration of 0 ppm over the same period. At DDT concentration of 20 ppm there was growth of up to 0.25 ($O.D_{600}$) after which growth declined. The highest growth observed of 0.48 ($O.D_{600}$) was at DDT concentration of 50 ppm. At DDT concentration of 100 ppm, growth was up to 0.39 ($O.D_{600}$), which was lower than the observed growth at 50 ppm.

Growth and rate of DDT degradation by the enrichment cultures

There were varying growths of the enrichment cultures from cultivated and uncultivated areas. This also translated into differences in DDT degradation. In both cases, DDT degradation was directly proportional to growth of the cultures (Figure 2). The enrichment culture from cultivated places had a long lag phase and reached its highest cell mass of 0.191 at OD_{600} on the 27^{th} day which was lower than that of enrichment culture from uncultivated places that reached its highest cell mass of 0.247 by the same period. In both cases, DDT degradation was quantifiable after three days with the enrichment culture from the uncultivated places degrading 60.20% in 29 days at a degradation rate of approximately 2.08 ppm DDT/day while the one from cultivated places degraded 38.58% of initial amount of

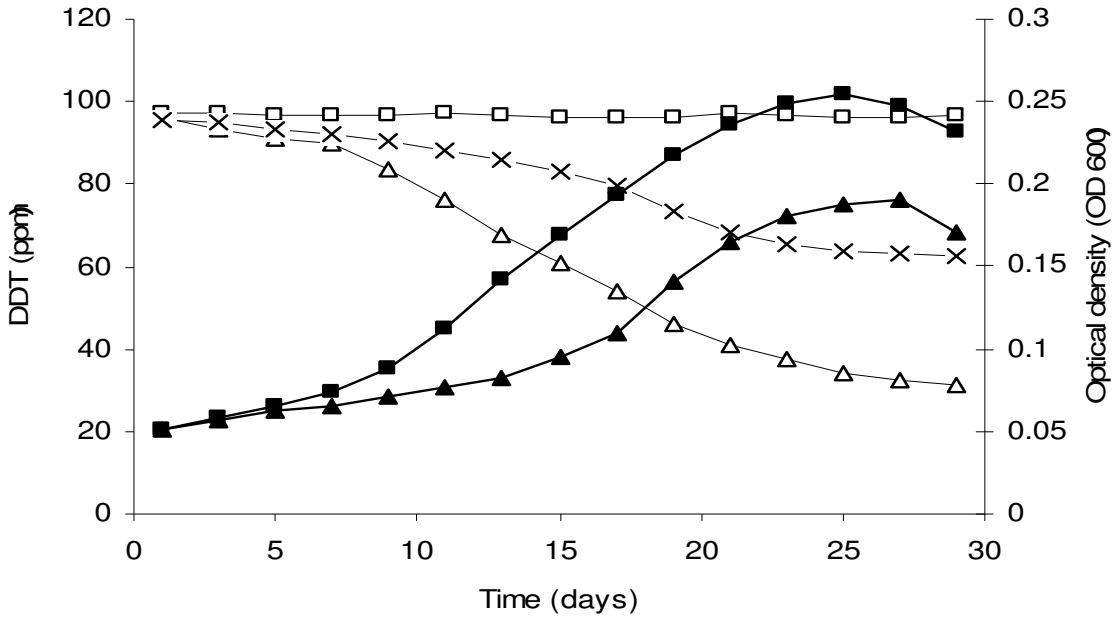

Figure 2. Growth and DDT degradation curves of soil enrichment cultures from cultivated and uncultivated places. Results are means where n=6 and SE <5% of means in all cases. The symbols represents: □- Control, ∆- DDT degradation curve and -■- growth curve for enrichment cultures from uncultivated areas; x – DDT degradation curve and ▲- growth curve for enrichment cultures from cultivated areas.

Table 1. Sources and amount of DDT degraded by isolates in pure and mixed culture.

Isolates	% DDT degraded	Source	
		Cultivated	Uncultivated
101	44.31		√
102	58.08		√
103	39.72	√	√
104	30.33	√	
105	28.97	√	
110	28.48	√	√
Six (mixed)	82.63	√	√

DDT over the same period at a degradation rate of approximately 1.33 ppm DDT/ day. Out of the six isolates that were capable of degrading DDT, the two (isolates 102 and 101) that degraded the highest amount of DDT (Table 1) were found in uncultivated places hence perhaps the higher degradation in enrichment culture from uncultivated places compared to the one from cultivated places.

Isolation of DDT degrading bacteria by enrichment technique

Through two independent enrichment steps, using MM4 media containing DDT as the sole source of carbon and energy, six isolates were obtained (Table 1). The isolates designated as 101, 102, 103, 104, 105 and 110, were found to individually biodegrade DDT into DDD. The biodegradation was indicated by the decrease in DDT concentration and the increase in DDD concentration. None of the isolates transformed DDT to DDE or further to CO_2 and water (Figures 3, 4 and 5).

Degradation of DDT by the isolates

Quantifiable degradation of DDT started after three days when the peak of DDD started to appear. From there on,

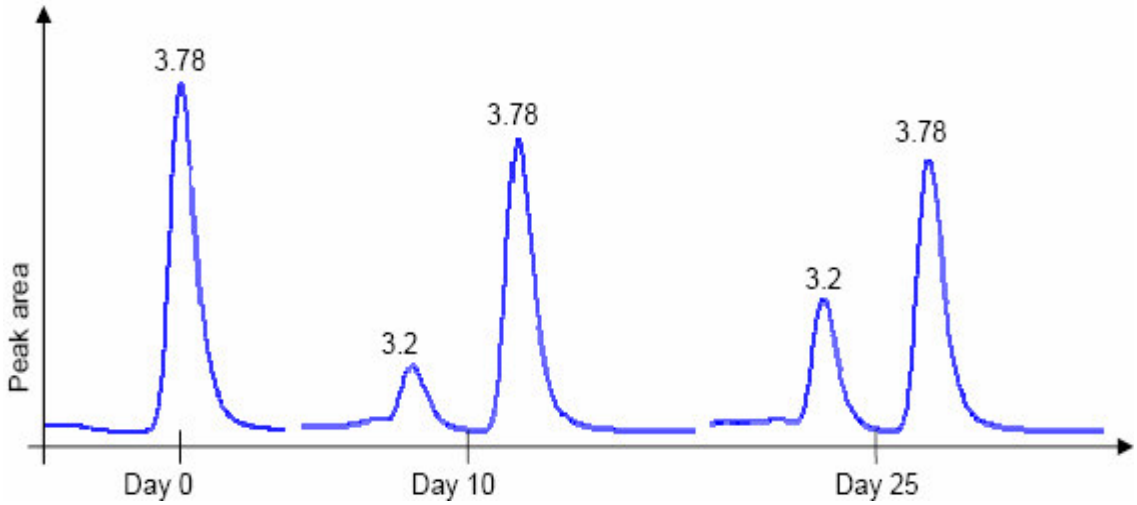

Figure 3. HPLC chromatograms of samples taken from uninoculated control and growth culture of isolate 110 after 10 and 25 days.

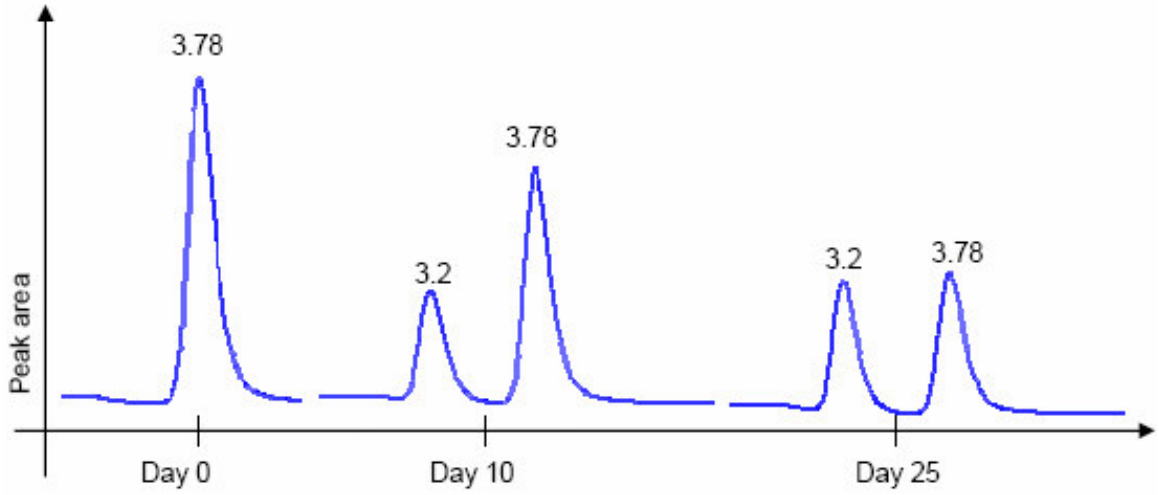

Figure 4. HPLC chromatograms of samples taken from uninoculated control and growth culture of isolate 102 after 10 days and 25 days.

the area and size of DDD peak continued to increase while that of DDT peak continued to decrease. Had the DDT been transformed to DDE, a resultant peak at a retention time of 4.3 min could have appeared hence its absence indicated that DDT was transformed to DDD only (Figures 3, 4 and 5). A decrease in DDT peak indicated more degradation as was an increase in DDD peak and this was well pronounced after 25 days. The chromatogram of isolate 110 which biodegraded the least amount of DDT (28.48%) in 31 days is shown in Figure 3. The chromatogram of isolate 102 which degraded the highest amount of DDT, for individual isolates (58.08%) in 31 days is also shown in Figure 4. For isolate 110, the DDT peak as at 25th day of incubation was larger than DDD peak showing less degradation compared to isolate 102 whose DDT and DDD peaks were almost equal in area and size. The DDD peak of the mixed isolates as at 25th day (Figure 5) was larger in size and area than the DDT peak indicating the highest transformation of DDT to DDD. In all the individual isolates and in the mixed isolates, DDD was the only product that resulted from the degradation of DDT. DDD was not degraded further. This was indicated by the increase in size of the DDD peak throughout the incubation period without any decrease at any time. Only the two peaks for DDT and DDD were observed.

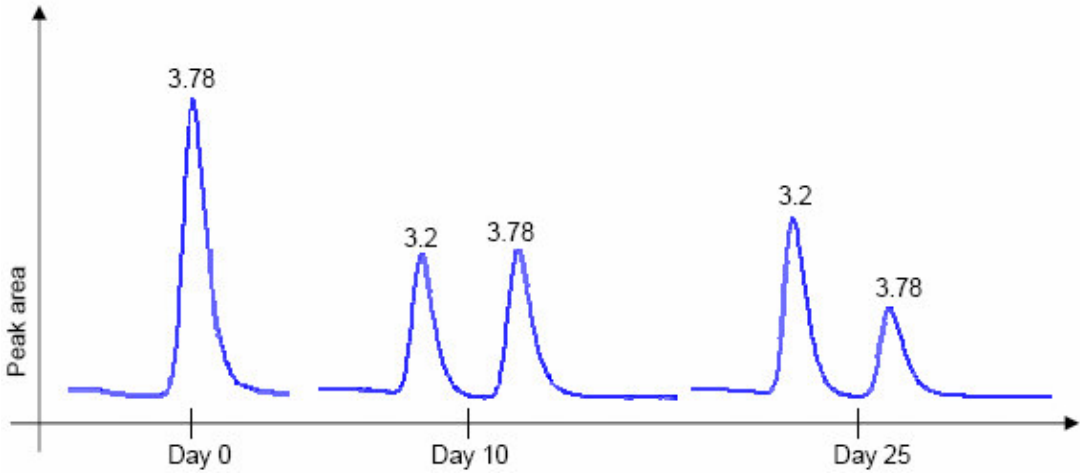

Figure 5. HPLC chromatograms of samples taken from uninoculated control and growth culture of six mixed isolates after 10 days and 25 days.

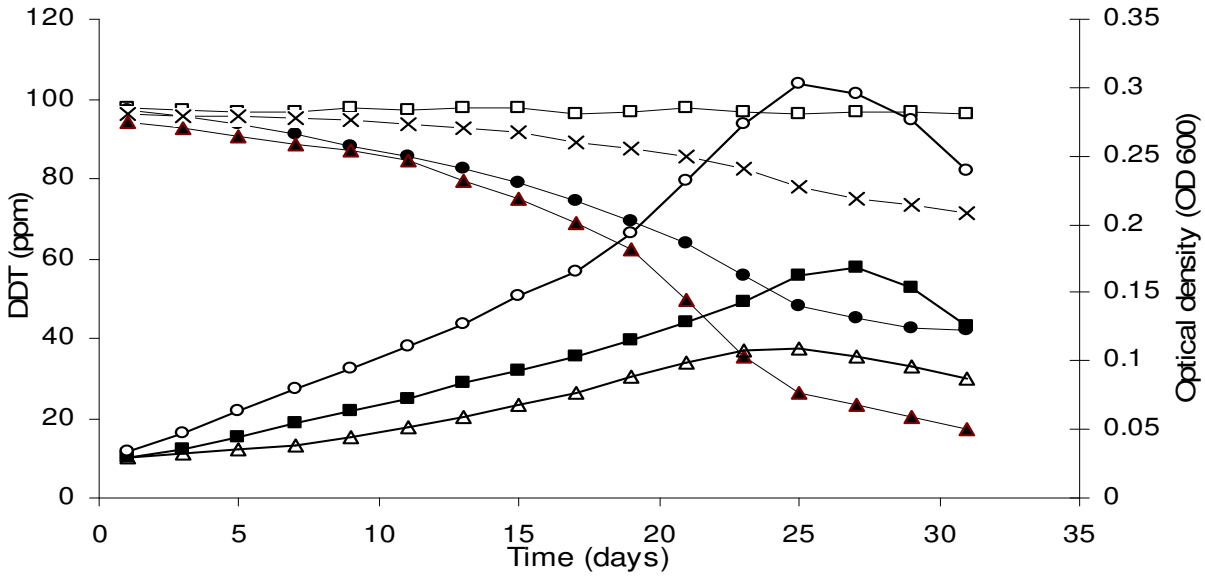

Figure 6. Bacterial growth and DDT degradation. Results are means where n=2 and SE <5 % of means in all cases. The symbols represents: □- Control, Δ- growth curve for isolate 110, -■- growth curve for isolate 102, -o- growth curve for the six mixed isolates , x -DDT degradation curve for isolate 110, • -DDT degradation curve for isolate 102, ▲- DDT degradation curve for the six mixed isolates.

Rate of growth and DDT degradation by the isolates

There was varying growth and different DDT degradation rates for each individual isolate and the mixed isolates. It was observed that an increase in turbidity corresponded to an increase in DDT degradation (Figure 6). Isolate101 was obtained from uncultivated places and degraded 44.31% of initial amount of DDT over a period of 31days with a degradation rate of 1.43 ppm/day. The isolate had a short lag phase and reached its highest cell mass of 0.157 (OD_{600}) on the 27th day. Of the six isolates, isolate 102 which was obtained from uncultivated areas (Figure6), had the highest cell mass evidenced by a higher OD value of 0.168 as at the 27th day and also had the highest degradative ability after degrading 58.08% of DDT in 31 days at a rate of 1.87 ppm/ day. Compared to isolates 102 and 101, isolate 103 which was from both cultivated and uncultivated places had a lower growth and degradative ability. At day 25, isolate 103 had grown to its highest cell mass of 0.125 and had degraded 39.72%

Table 2. Metabolic versatility of the isolates.

Metabolic versatility tests	Isolates					
	101	102	103	104	105	110
Lactose	-	-	-	+	+	-
Dextrose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Glucose	+	+	+	+	+	+
Mannose	+	+	+	+	+	+
Arabinose	-	+	-	-	-	+
Rhamnose	-	-	-	-	-	-
Maltose	+	+	+	+	+	+
Mannitose	-	-	±	±	+	-
Melibiose	-	-	-	-	-	-
Cellobiose	+	-	-	+	+	+
Fructose	+	+	+	+	+	+
Sodium acetate	+	+	-	-	-	+
Benzoic acid	+	+	+	+	+	+
Resorcinal	-	-	+	+	-	-
Diazinnon	-	-	-	-	-	-
Round up^{TM}	-	+	-	-	-	-

Symbols: + positive for substrate utilization, - negative for substrate utilization, ± variable reaction.

of DDT in 31 days at a rate of 1.28 ppm/ day. Unlike the other isolates, isolate 104 which was obtained from cultivated places did not have a sharp decline in growth after it had reached its highest cell mass of 0.117 on the 29th day of incubation. The rate of DDT degradation of the isolate was 0.97 ppm DDT/day having degraded 30.33% of DDT in 31 days. The amounts of DDT degraded in 31 days by isolate 105 (28.97%) at a rate of 0.93 ppm DDT/ day and isolate 110 (28.48%) at a rate of 0.91 ppm DDT/ day were very close to each other but ranked the lowest compared to those of other isolates.

The mixed culture of the six isolates had the highest cell mass at OD_{600} of 0.32 compared to individual isolates whose range was 0.12 - 0.17. DDT degradation was greatly enhanced when the six isolates were mixed (Figure 6). The mixed culture degraded 82.63% of the initial amount of DDT in 31 days at the highest rate of 2.67 ppm DDT/ day. The amount of DDT degraded by the mixed culture was higher than that of individual isolates.

Metabolic versatility of the isolates

The isolates differed in their ability to mineralize or transform a variety of aromatic and non aromatic compounds (Table 2). All the isolates could utilize glucose, fructose, dextrose, sucrose, mannose, maltose and benzoic acid. Only isolates 104 and 105 could utilize lactose, cellobiose and mannitose while only isolates 101, 104, 105 and 110 could utilize cellobiose. Isolate 103 utilized mannitose while only Isolate 102 and 110 utilized arabinose. Isolates 101, 102 and 110 could utilize sodium acetate. Resorcinol was utilized by isolates 103 and 104 while only isolate 102 could utilize isopropylamine salt of glyphosate (round up^{TM}). None of the isolates utilized Diazinnon, rhamnose, citrate and melibiose.

Identification of the isolates

The isolates were characterised using morphological, cellular and biochemical characterization (Table 3). The ability of the isolates to excrete extracellular enzymes was tested through hydrolysis of starch, casein and gelatin. The ability of the isolates to excrete intracellular enzymes was determined through tests on sugars fermentation, litmus milk reactions, hydrogen sulphide production, nitrate reduction, catalase reactions, urease, methyl red, voges-proskauer, citrate utilization and triple sugar- iron test.

The isolates further underwent molecular characterization. Phylogenetic analysis showed that isolate 101 was a member of the genus *Bacillus*. This was supported by the clustering pattern on the phylogenetic tree which indicated that the isolate clustered with *Bacillus*. This clustering pattern was supported by high bootstrap values of between 50 - 100% (Figure 7). This result was further confirmed after blasting the results which showed isolate 101 had I6S rRNA gene sequence similarity of 99% to *Bacillus cereus* DQ207729. Phylogenetic analysis

Table 3. Biochemical characteristics of the isolates.

Biochemical Tests		Isolates					
		101	**102**	**103**	**104**	**105**	**110**
Cell type	shape	rods	cocci	rods	rods	rods	cocci
Gram stain		+	+	-	-	-	+
Colony characteristics	color	cream	cream white	cream white	cream white	yellow	cream white
	shape	round	round	round	round	round	round
	elevation	raised	raised	raised	raised	raised	raised
	surface	smooth	smooth	smooth	smooth	smooth	smooth
TSI agar test	butt	+	+	+	+	+	+
	slant	+	+	-	+	-	-
Citrate utilization		-	-	-	-	-	-
Gelatin liquefaction		+	+	+	+	+	+
MR test		+	+	+	+	+	+
VP test		-	-	-	-	-	-
Urease test		+	+	+	+	-	+
Nitrate reduction		+	+	+	+	+	+
Motility at 37^{o}C		+	-	-	+	-	-
Starch hydrolysis		+	-	-	-	-	-
Egg yolk reaction		+	+	+	+	-	+
Growth at 7% NaCl		+	+	-	+	-	-
Growth at 10% NaCl		-	+	-	+	-	-
Phenylalanine test		-	-	-	-	-	-
Tyrosine test		+	+	-	+	-	+
Aerobic growth		+	+	+	+	+	+
Anaerobic growth		+	+	+	+	+	+
H_2S Production		-	-	-	-	-	-
Casein hydrolysis		+	+	+	+	+	+
Catalase test		+	+	+	+	+	+

Symbols: + positive reaction, - negative reaction, ± variable reaction.

of 16S rRNA gene sequences of isolate 102 and 110 showed that the two isolates clustered with the genus *Staphylococcus*. This clustering pattern was supported by high bootstrap values of between 50 - 100% (Figure 8). The isolates had I6S rRNA gene sequence similarity of 98% to *Staphylo-coccus sciuri* AB233332. Phylogenetic analysis of I6S rRNA gene of isolate 103, 104 and 105 showed that the three isolates clustered with the genus *Stenotropho-monas*. This clustering pattern was supported by high bootstrap values of between 50 – 100% (Figure 9). The isolates had sequence similarity of 95, 97 and 94% respectively to *Stenotrophomonas maltophilia* AB294553.

DISCUSSION

In this study, six DDT biodegrading bacteria were isolated from tropical soils that had no prior exposure to DDT. Previously, DDT metabolising microbes were isolated from areas where intensive DDT use had occurred (Lai and Saxena, 1999). The standard method for isolating microorganisms with the ability to degrade environmental pollutants is to enrich them from areas that were previously exposed to the pollutant. In this study, a different approach for isolating DDT-degrading microorganisms by screening alternative sources like soil that had no prior exposure to DDT proved successful. This could mean that tropical soils, unpolluted with DDT, contain some microorganisms that can degrade DDT. Since their identity has been established, a selective media and optimum growth conditions should be used to test their prevalence in the environment. Microbial transformation and volatilization are the major routes for DDT biodegradation in tropical soils whereas in temperate soils DDT may persist for long period of time (Diamond and Owen, 1996).

The amount of DDT degraded by the mixed culture of

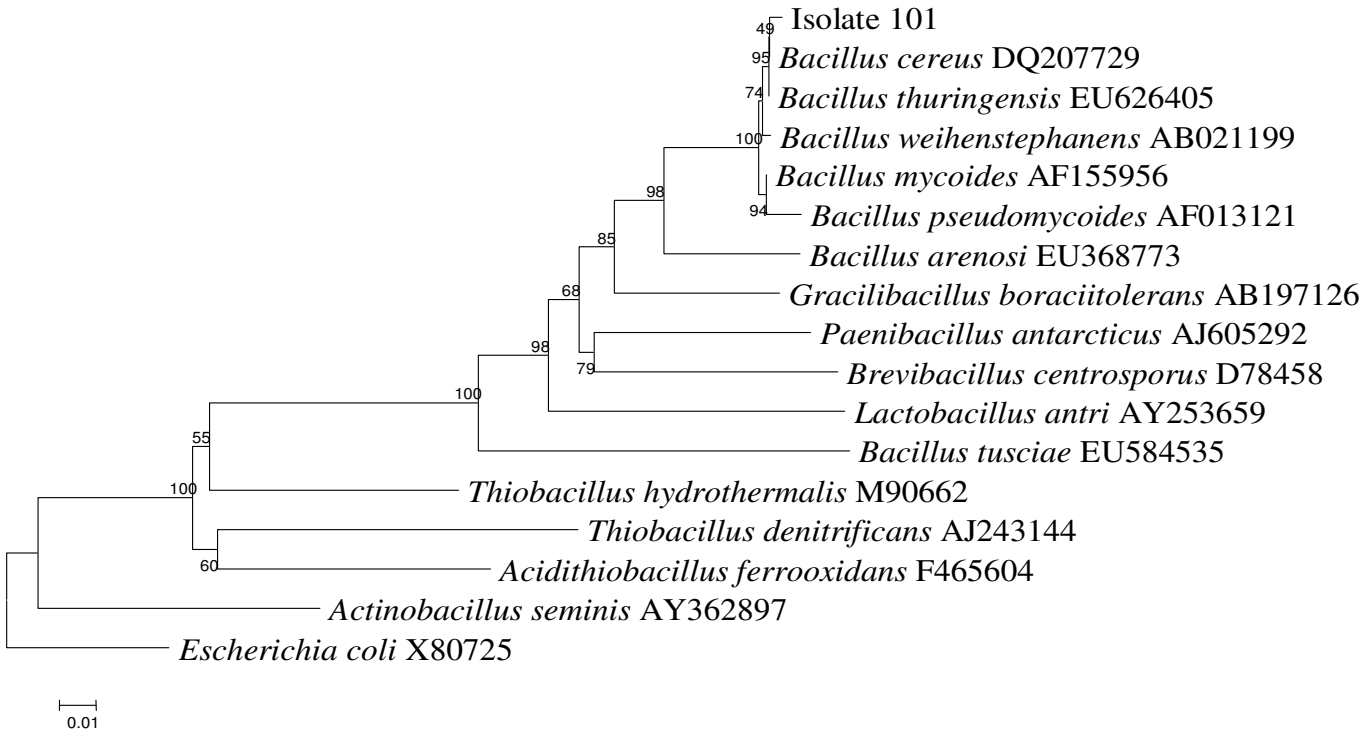

Figure 7. Phylogenetic tree showing position of isolate 101. The scale bar indicates approximately 1% sequence difference. Numbers at nodes indicate bootstrap values of each node out of 100 bootstrap resampling. The 16S rRNA gene sequence of *Escherichia coli X80725* was used as an outgroup.

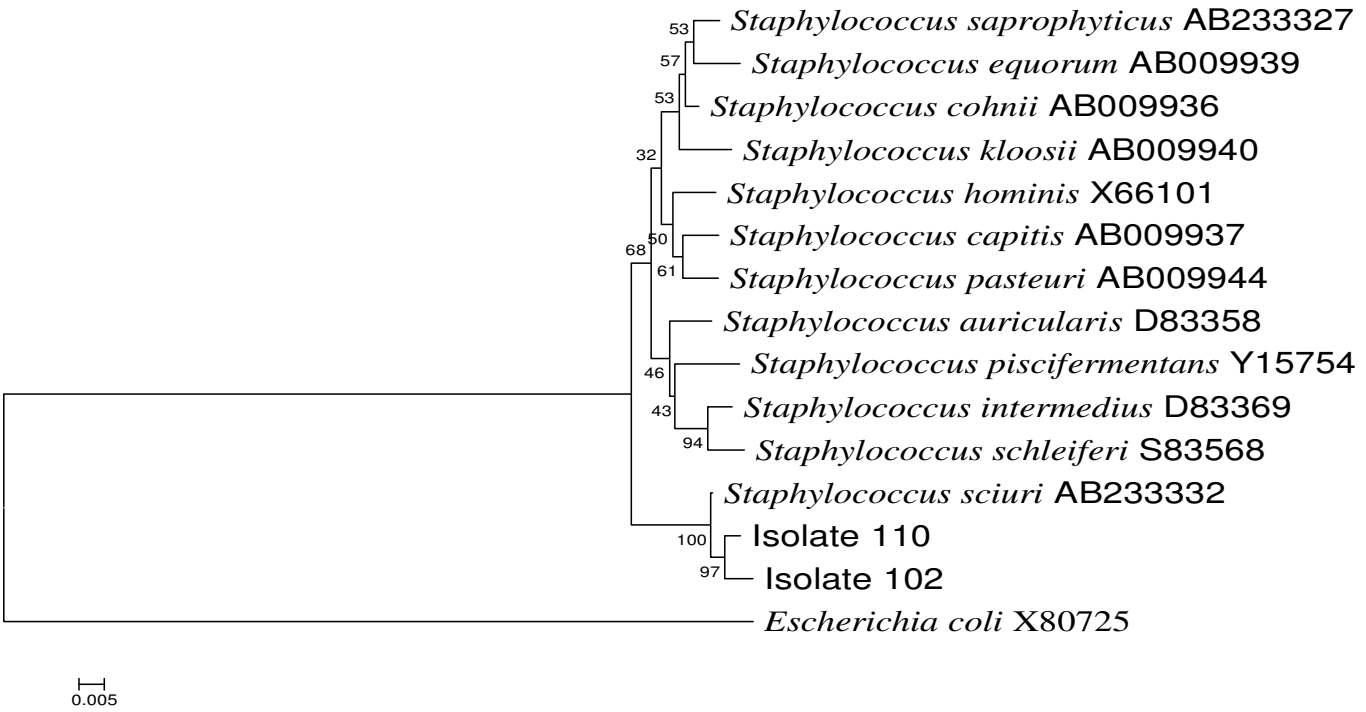

Figure 8. Phylogenetic tree showing position of isolates 102 and 110. The scale bar indicates approximately 0.5% sequence difference. Numbers at nodes indicate bootstrap values of each node out of 100 bootstrap resampling. The 16S rRNA gene sequence of *E. coli* X80725 was used as an out group.

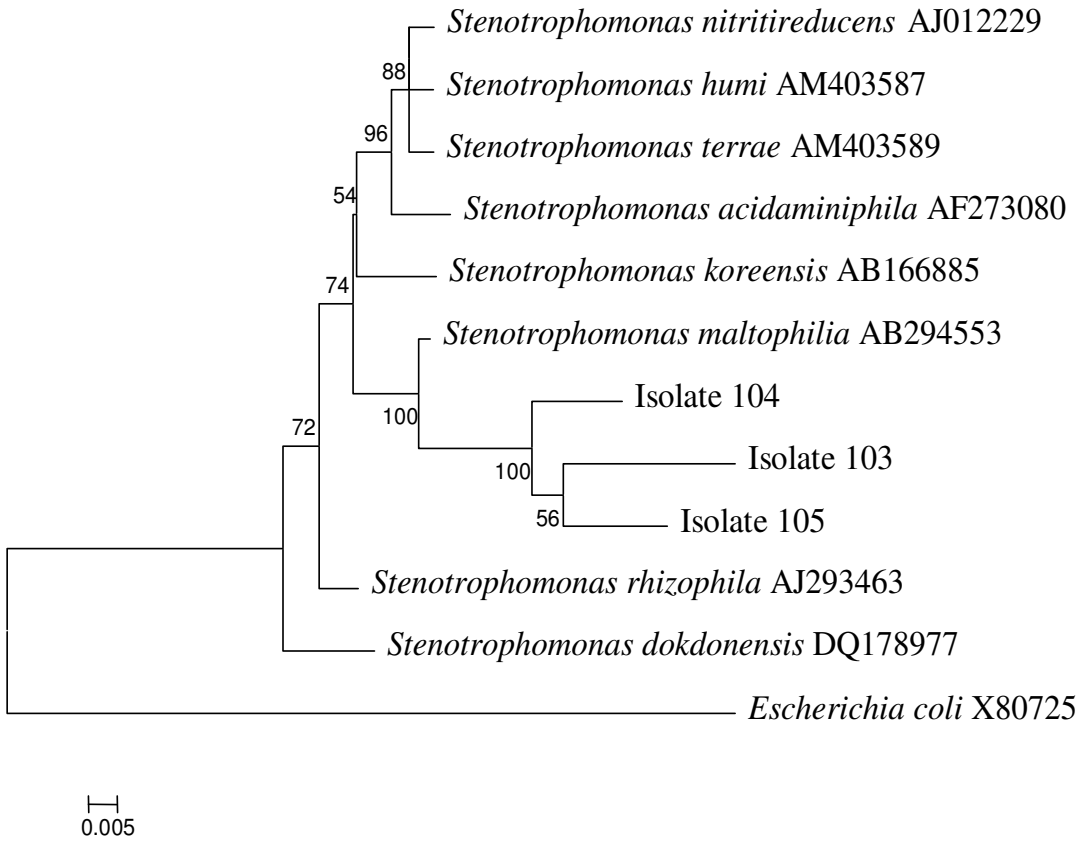

Figure 9. Phylogenetic tree showing position of isolate 103,104 and105. The scale bar indicates approximately 2% sequence difference. Numbers at nodes indicate bootstrap values of each node out of 100 bootstrap resampling. The 16S rRNA gene sequence of *E. coli* X80725 was used as an outgroup.

the six isolates was higher than that of any individual isolate. DDT degradation is greatly enhanced in a mixed culture perhaps due to their synergistic effect. Degradation of DDT was a slow process as it took the isolates about 31 days to degrade 28.48 - 58.08% of the initial amount of DDT. Juhasz and Naidu (2000) proposed that the tri Chlorine molecule is responsible for the resistance of DDT to degradation. Degradation of DDT involves two processes (Katayama et al., 1993), the uptake of DDT into the cell and the transformation of DDT in the cell. The rate of uptake into the cells is unlikely to be important as DDT is extremely hydrophobic; rather, the rate at which the chemical is transformed in the cell would be the rate-limiting step. Degradation in enrichment cultures from uncultivated areas was higher than that of the cultures from cultivated areas due to the presence of bacteria in the uncultivated areas with higher DDT degradative abilities. Again, soils with high organic matter content like the agricultural soils have significantly lower concentrations of bioavailable DDT as compared to other soils (Foght et al., 2001). The rate of degradation of DDT in soils is thus dependent on the presence and numbers of microbes in the soil with the required degradative ability and environmental factors which limit both growth and activity of the DDT metabolising microbes and access of the microbes to DDT.

DDT was degraded to DDD by the six isolates and none produced DDE. Formation of DDE is mostly through photochemical reactions in the presence of sunlight and through dehydrochlorination in bacteria (Pfaender and Alexander, 1972) and animals (Kurihara et al., 1988). Under reducing conditions, reductive dechlorination is the major mechanism for the microbial conversion of both the o, p'-DDT and p, p'-DDT to DDD (Fries et al., 1969). DDD has been identified as one of the major anaerobic transformation products of DDT (Aislabie et al., 1997). DDD formation by the isolates probably resulted from the reductive dechlorination of the aliphatic part of the DDT molecule. The reaction involves substitution of aliphatic chlorine for a hydrogen atom. The six isolates were capable of growing in both aerobic and anaerobic conditions and it is likely that degradation of DDT occurred during anaerobic growth. Although the experiments were incubated aerobically, lack of continuous shaking could

have led to anaerobic conditions. Gray et al. (1999) in a process with alternative aerobic and anaerobic stages reported a 95% reduction in DDT levels in the soils.

This study shows that as the DDT peaks were reducing, the DDD peaks were increasing and at no particular point did the DDD peak start to decrease even with the mixed cultures. This shows that under the conditions, DDD was probably the end product hence this was not a complete break down of DDT to CO_2 or to non chlorinated compounds like phenylacetic, phenylpropionic and salicylic acids. In various ecosystems, microorganisms cause only modest changes in the DDT molecule (Alexander 1985). Complete degradation of DDT is possible only through a cometabolic process (Pfaender and Alexander, 1976) and that only the first step in the process, the dechlorination of DDT to DDD can take place without an additional substrate, as was the case in this study. The major transformation products, DDD and DDE, are more toxic and recalcitrant than the parent compound. This is of concern as these compounds are metabolized slowly, if at all (Aislabie et al., 1997).

The identity of the six isolates as determined through characterization revealed that the isolates were members of the genera *Bacillus, Staphylococcus* and *Stenotrophomonas.* Previously, species of the genus *Bacillus* (Ramesh et al., 2004) and *Staphylococcus* (Dileep, 2008) have been reported to degrade DDT. Members of the genus *Stenotrophomonas* that degrade DDT have also been reported (Juhasz and Naidu, 2000). Other bacteria genera that have been implicated in DDT degradation are A*erobacter, Alcaligenes, Agrobacterium, Clostridium, Hydrogenomonas, Krebsiella, Streptomycs* and *Xanthomonas* (Ajay and Owen, 2004).

Conclusion and Recommendation

This study showed that there are microorganisms in the tropical soil previously not exposed to DDT that can partially degrade DDT. This study identified six DDT biodegrading bacteria. Isolate 101 had a sequence similarity of 99 % to *B. cereus*. Isolates 102 and 110 had a sequence similarity of 98 % to *Staphylococcus sciuri*. Isolates 103, 104 and 105 had a sequence similarity of 95, 97 and 94% respectively to *Stenotrophomonas maltophilia*. This suggests that the three isolates could be new species. The ability of the isolates to completely biodegrade DDT to CO_2 or to non chlorinated compounds through cometabolism should be assessed.

ACKNOWLEDGEMENT

This work was financially supported by Jomo Kenyatta University of Agriculture and Technology and Prof. Hamadi I. Boga. International Center of Insect Physiology and Ecology (ICIPE) through Dr. Dan Masiga also awarded me the Dissertation Research Internship Program (DRIP) fellowship. I am extremely indebted to them for the research funds without which it would not have been possible to do this work. KM was supported by the World Federation of Scientists during the course of the research.

REFERENCES

Aislabie J, Richards N, Boul H (1997). Microbial degradation of DDT and its residues: A review. N.Z. J. Agric. Res. 40: 269-282.

Aislabie J, Davison A, Franzmann P, Jardine D, Karuso P (1998). Co-metabolism of DDE by an aerobic bacterium. In 14th Australasian Biotechnology Conference, Adelaide, Australia 113 (E11).

Alexander M (1985). Microbial formation of environmental pollutants. Advan. Appl. Microbiol. 18: 1-73.

Ajay S, Owen P (2004). Applied bioremediation and phytoremediation. Springer-verlag Berlin Heidelberg pp. 36-37.

Ali I, Aboul E (2002). Determination of chiral ratio of o, p-DDT and o, p-DDD pesticides on polysaccharides chiral stationary phases by HPLC under reversed-phase mode. Environ. Toxicol. 17(4): 329-333.

Cappuccino J, Sherman N (2001). Microbiology: A laboratory manual (6th ed). Pearson education, Inc., San Francisco, CA.6: 1-101.

Cousins IT, McLachlan MS, Jones KC (1998). Lack of an aging effect on the soil air partitioning of polychlorinated biphenyls. Environ. Sci. Technol. 32: 2734–2740.

Diamond J, Owen R (1996). Long term residue of DDT compounds in forest soils in Maine. Environ. Poll. 92: 227-230.

Dileep K (2008). Biodegradation and bioremediation of pesticide in soil: concept, method and recent developments. Ind. Microbiol. 48: 35–40.

Foght J, April T, Biggar K, Aislabie J (2001). Bioremediation of DDT contaminated soils: A review. Bioremed. J. 225: 1058-1137.

Fries S, Charles G, Evans WC (1969). Anaerobic degradation of aromatic compounds. Ann. Rev. Microbiol. 42: 289-317.

George M, Don J, Noel R, Ames T (2005). Bergey's Manual of Systematic Bacteriology, 2nd ed. Springer, New York, ISBN 0-387-95040-0. pp. 359-361.

Gray N, Moser G, Moser L (1999). Compost decontamination of soil contaminated with chlorinated toxicants. US Patent- Google Patents. 5: 902-744.

Hall TA (1999). Bioedit: A user friendly biological sequence alignment editor and analysis program for windows. Nucleic Acids Symposium. 41: 95-98.

Hecht N, Guenzi W, Beard W (2004). Anaerobic conversion of DDT to DDD and aerobic stability of DDT in soil. Society of America proceedings, Soil Sci. 32: 522-527.

Hickey WJ (1999). Transformation and fate of polychlorinated biphenyls in soil and sediment. In Adriano DC, Bollag JM, Frankenburger WT JR, Sims RC. Bioremediation of contaminated soils. Agron. Monograph 37: 213–237.

Hong Q, Zhang Z, Hong Y, Li S (2007). A microcosum study on bioremediation of fenitrothion-contaminated soil using *Burkholderia sp*. FDS-1. Bioremed. Biodegrad. 59: 55–61.

Holt JG, Bergey H (1994). Bergey's Manual of Determinative Bacteriology, 9th ed. Williams and Wilkins, USA ISBN 978-0-683-00603-7 p. 182.

Jacques R, Okeke B, Bento F, Teixeira A, Peralba M, Camargo F (2008). Microbial consortium bioaugmentation of a polycyclic aromatic hydrocarbons contaminated soil. Bioresour. Technol. 99: 2637-2643.

Juhasz A, Naidu R (2000). Enrichment and isolation of non-specific aromatic degraders from unique uncontaminated (plant and faecal material) sources and contaminated soils. Appl. Microbiol. 89: 642-650.

Katayama S, Richards NK, Boul HL (1993). Microbial degradation of DDT and its residues: A review. N.Z. J. Agric. Res. 40: 269-282.

Kumar M, Philip L (2006). Bioremediation of endosulfan contaminated soil and water-optimization of operating conditions in laboratory scale reactors. J. Hazardous Mater. 136: 354–364.
Kurihara N, Ikemoto Y, Clark G (1998). Dehydrochlorination mechanisms of DDT Analogs catalyzed by housefly enzymes. Agric. Biol. Chem. 52: 1831-1833.
Lai R, Saxena D (1999). Accumulation, metabolism and effects of organochlorine insecticides on microorganisms. Microbiol. Rev. 46: 95-127.
Lane H (2001). Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) for studying soil microbial communities. Appl. Environ. Microbiol. 8: 353–373.
Ludwig W, Strunt O (1996). ARB: a software environment for sequence data. Retrieved on 9.9.2008 from http://www.mikrobiologie.tumuenchen.de/pub/ARB/documentation/arb.ps.
Muchena F, Wamicha W, Njoroge C (1998). Detailed soil survey of Jomo Kenyatta College of Agriculture and Technology, Juja (Kiambu District). Ministry of agriculture, National Agricultural laboratories 13: 12-33.
Pfaender F, M Alexander (1972). Effect of nutrient additions on the apparent co-metabolism of DDT. J. Agric. Food Chem. 21: 397-399.
Pfaender F, Alexander M (1976). Extensive microbial degradation of DDT in vitro and DDT metabolism by natural communities. J. Agric. Food Chem. 20: 842-846.
Ramesh C, Atur K, Ajay S, Owen P (2004). Bioremediation of pesticide contaminated soils, in: Ajay S and Owen P (2004). Applied bioremediation and phytoremediation. Springer-verlag Berlin Heidelberg pp. 35-37.
Schmidt M, DeLong F, Pace R (1991). Analysis of a marine picoplankton community by 16S rRNA gene cloning and sequencing. J. Bacteriol. 173: 4371-4378.
You G, Sayles H, Kupferle M, Bishop P (1995). Anaerobic bioremediation of DDT contaminated soil with non ionic surfactant. Appl. Environ. Microbiol. 3: 137-144.