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Isolation and Characterization of Xenobiotic Pesticide Degrading Bacterial Species in Flower Farms Around Lake Naivasha, Kenya

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Abstract
Certain microorganisms especially bacteria and fungi are able to use xenobiotic organic compounds as their carbon and nitrogen source for metabolism. Flower farms around lake Naivasha basin uses several agrochemicals especially pesticides to control pests and improve flower production. The aim of this study was to isolate and characterize morphologically and biochemically the main bacterial species that are able to grow and tolerate the pesticide contaminated farm soils. Soil samples were collected from randomly selected five greenhouses from each five flower farms namely Crescent, Elsamere, Karuturi, Malewa and Sewage farms around Lake Naivasha basin. The collected samples were processed for bacterial isolation using the nutrient agar, mac' Conkey agar, blood agar, Luria-Bertani and Minimum Salt Media nutrient media. The conventional methods of swabbing and streaking were used. Pure colonies of isolates organisms were identified and characterized using standard microbiological technique. Morphological, cultural and biochemical characterization of bacterial species isolated from the flower farm soil samples identified mainly Pseudomonas auriginosa, Escherichia coli, Rhodococcus erythropolis and Bacillus subtilis species. Bacterial growth in pesticide consortia was quantified by monitoring colony growth of the species in liquid culture over time. The viable cell counts were determined turbidimetrically at $O.D_{696}$nm. All the isolated bacterial species were able to grow in flower farm soil contaminated with organochloride and organophosphate pesticide residues. B. subtilis recorded the highest growth at 1.77±0.07 $O.D_{696}$nm in pesticide mixture consortia. There was lower growth in organochloride pesticide consortia as compared to organophosphate pesticide consortia.

Keywords: Lake Naivasha; Flower farms; Organochlorides; Organophosphates; Xenobiotic pesticides; Bacterial tolerance

Introduction
The large scale use of xenobiotic pesticides in agriculture has led to widespread contamination of the environment. Many such compounds have been designated as priority pollutants by the United States Environmental Protection Agency because of their recalcitrance, toxicity, carcinogenicity and potential teratogenicity [1].

The presence of halogen substituents often renders simple chemical structures recalcitrant [2]. Organisms possessing oxygenases, reductases or dehalogenases, which are capable of catalyzing the cleavage of the carbon-halogen bonds, can mineralize haloalkanes and thereby utilize them as sources of carbon and energy [3].

Consequently, there has been considerable interest in the possibility of using such organisms and their enzymes for the detoxification of xenobiotic chemicals. Although most organisms have detoxifying abilities (that is, mineralization, transformation and or immobilization of pollutants), microorganisms, particularly bacteria, play a crucial role in biogeochemical cycles for sustainable development of the biosphere [4]. Horizontal gene transfer, high growth rates and metabolic versatility make them to evolve quickly and to adapt themselves to changing conditions of environment, even at extreme environmental conditions that does not permit the proliferation of other living organisms [5]. A large number of microbial communities have been characterized with their responses to pollutants, in order to identify the potential bacterial degrader that can adapt to use these chemicals as their novel growth and energy substrate [6]. Microbial degradation of petroleum and other hydrocarbons is incredibly an intricate course of action that is mainly restricted principally by the availability of phosphorus and nitrogen [7, 8]. Apart from these conditions, pH, moisture, oxygen and temperature are prime factors influencing chemical biodegradation [9]. Modern molecular techniques has to be identified for isolation of plasmid DNA, construction of DNA probes in recent perspective to explore the efficient genes implicated in catabolism of xenobiotics and to study the genetic diversity of environmentally significant microorganisms [10].

Materials and Methods
Sampling Sites
Lake Naivasha is a freshwater lake in Kenya, outside the town of Naivasha in Nakuru County, which lies North West of Nairobi. The lake geographical coordinates are 0˚46'6.70"S 36˚ 21'2. 32"E. It is

part of the Great Rift Valley. The most significant activity in Lake Naivasha, albeit for large scale farmers, is the extensive irrigated greenhouse floriculture and horticulture industry. Livestock ranching and private game sanctuaries and conservation areas also exist in the catchment [11]. To meet the market demands for quality flowers, fruits and vegetables in Europe, the horticultural farmers use large volumes of pesticides [11, 12].

Sample Collection

Soil samples were collected from randomly selected five greenhouses from each five flower farms namely Crescent flower farm, Elsamere flower farm, Karuturi flower farm, Malewa flower farm and Sewage flower farm around Lake Naivasha basin. Systemic random sampling method was used to collect the samples. Four sampling points for each greenhouse within the farms were randomly selected i.e. two points within the greenhouses and two water drainage points around the greenhouses. A soil core was dug using hoe and scooped using a spade down to the depth of 5-10 cm (for assessment of adsorption depth) from the four different locations from each greenhouses and approximately 200 g of the scooped core taken. The cores from each greenhouse were thoroughly mixed to give a composite sample of 100g.

Chemicals and Growth Media

Analytical-grade aldrin, dieldrin, endosulfan, dimethoate, Malathion and methyl parathion were purchased through local suppliers from Sigma-Aldrich Inc. All other chemicals and solvents were of highest analytical-reagent grade. Liquid minimal salts medium (MSM) consisted of 5.97g $Na_2HPO_4 .12H_2O$, 2.27g KH_2PO_4, 1g NH_4NO_3, 0.5g $MgSO_4 .7H_2O$, 0.02g $MnSO_4 .4H_2O$, 0.01g $CaCl_2.2H_2O$, 0.025g $FeSO_4$ dissolved in 1Liters of distilled deionized water (DDW). Unless otherwise stated, MSM was adjusted to pH 7.0 and standard pesticides added at 1000 mgL^{-1} in powdered form. Flower farm soil- amended MSM contained 15g agar L^{-1}, the solution added to the cooled medium after autoclaving. Luria-Bertani (LB) medium was used for general bacterial growth.

Isolation and Characterization of Bacterial Species

5 mg of the flower farm soil samples was dissolved in 10 ml of DDW and the mixture then inoculated with nutrient growth media. For isolation of *Pseudomonas auriginosa*, 20 ml of glycerol and 10 g of Tryptone was mixed with 100mls of MSM media and dissolved in 1000 ml distilled water. A loopful of the resulting bacterial suspension was streaked onto asparagine plates containing 1.5% agar. About 1 g of phosphorus was also added to promote pyocyanin production which is unique to *P. auriginosa* and was noted as a blue-green water soluble pigment that imparts a greenish color into the media. *E. coli* and *B. subtilis* were isolated by inoculating soil samples with LB growth media mixed with 10 g Tryptone, 5 g yeast extract and 10 g NaCl suspended in 1 Lt of DDW, while *R. erythropolis* was isolated by inoculating soil samples with 100mls of MSM nutrient media. 1-chlorobutane was added as the sole carbon source. After 7 days, 5mL of culture was inoculated to 100 mL fresh MSM and LB media depending on the species being isolated and incubated under the same conditions for another 7 days. After 5 sequential rounds of enrichment (i.e. 35 days exposure to nutrient media), 100 mL of culture was plated onto nutrient agar and incubated at 28ºC for 5 days with vigorous shaking at 200 rpm to provide aeration for the bacteria. Small amount of Dapsome fungicide was added to inhibit the growth of fungi.

Identification Tests of Isolated Bacterial Species

Identification of pesticide-tolerant bacteria isolated from flower farm soil samples was based on morphological, physiological and biochemical characterization according to Bergey's Manual of Systematic Bacteriology with some modifications [13]. After incubation, plates were studied for the colonies of microbes grown on the media. Microorganisms grown on mac' Conkey agar are capable of metabolizing lactose which produces acid by-products that lower the pH of the media which causes the neutral red indicator to turn red, and if sufficient acid is produced, a zone of precipitated bile develops around the colony [14]. Catalase activity was determined by the presence of bubbles in a 3% H_2O_2 solution [13, 14]. Oxidase activity was analyzed by oxidation of 1%w/v *p*-aminodimethylaniline oxalate. Motility was determined with an optical microscope using the hanging-drop technique. Starch hydrolysis was analyzed as described by Cowan and Steel Manual for the identification of medical bacteria [14].

Bacterial Growth in Pesticide Consortia

The objective of this study was to assess the tolerance of bacterial isolates against organochlorides aldrin, dieldrin, endosulfan, and organophosphates dimethoate, Malathion and parathion individually and as a mixture *in vitro*. Bacterial growth in pesticide consortia was quantified by monitoring colony growth of the species in liquid culture over time. The experimental setup was done in two ways. The first approach was to add pesticide standard equivalent to the analyzed concentration of the residues in the soil samples (spiking) and incubate the bacterial isolates in MM media to each pesticide soil sample residues plus the standard and the growth of the isolates measured after every 2 days, 10 days, 15 days and 21 days and the growth compared with isolates in MM media only. The second approach was to incubate the bacterial isolates in MM media to soil samples containing mixtures of pesticide residues and the growth of each bacterial isolates measured after 2 days, 10 days, 15 days and 21 days and compared with isolates in MM media only.

Cells were collected by centrifugation (6000 g for 5 min.), washed twice and re-suspended to an $OD_{696nm} = 0.8$ (Lambda Bio Spectrophotometer, Perkin Elmer, USA) in sterile water. The cell suspension (approx. 1×10^8 cells mL^{-1}) was used to inoculate (2% w/v) 100 mL flasks of MSM and LB with soil samples and incubated at 28ºC on a rotary shaker (150 rpm). Uninoculated MSM/LB (without soil samples) served as the negative control and each treatment was replicated 3 times. The viable cell counts were determined turbidimetrically to give O.D. values at 696nm in Lambda Bio Spectrophotometer, Perkin Elmer, USA). Standardization was done using 0.5McFarland solution (1.0 $OD_{696nm} = 1.5\times 10^8$ CFU/ml).

Statistical Analysis

Bacterial growth in pesticide and flower farm soil consortia was assessed by quantifying and comparing the growth between treatments, each consisting of three (3) replicates. All data were analyzed by analyses of variance (ANOVA), using SPSS 16.0 statistical software (SPSS Inc., USA). Pairwise comparisons of means were used to compute Fisher's least significant difference values (LSD, P= 0.05)

Results and Discussions

Morphological and Biochemical Identification of Bacterial Species

Four bacterial strains capable of utilizing organochlorides and organophosphates as carbon source for growth were isolated from

the agricultural flower farm soil sample around Lake Naivasha. They were identified according to their morphological, physiological and biochemical characterization based on Bergey's Manual of Systematic Bacteriology with some modifications [13]. Bacterial characterization, based on the morphological, biochemical and molecular tests indicated that the isolated species were *Pseudomonas auriginosa*, *Escherichia coli*, *Rhodococcus erythropolis and Bacillus subtilis*. *P. auriginosa and E*. coli are gram-negative bacterial species while *R. erythropolis and B. subtilis* are gram positive. Further all the bacterial species isolates were catalase positive. *E. coli* is oxidase negative while the rest are oxidase positive. All the isolated species are aerobic, motile and rod shaped. The flower farm soil samples collected from major flower farm green within the farms around Lake Naivasha basin were processed to identify the microorganisms present. The results for morphological and biochemical characteristics such as staining; motility test and cultural characters, colony morphology, Biochemical characters for samples are shown in the Table 1 below.

Table 1: Morphological and biochemical characteristics of bacterial species isolated from flower farm soil samples

SS	Shape	MT	Cap	Sp	CT	IN	OX	GS	Bacterial species Identified	BBL Crystal (% Confidence)
BS1	Rods	+	-	-	+	+	+	-	*P. auriginosa*	N/A
BS2	Rods	+	-	-	+	+	-	-	*E. coli*	N/A
BS3	Rods	+	-	+	+	+	+	+	*R. erythropolis*	94.8
BS4	Rods	+	+	+	+	-	+	+	*B. subtilis*	95.2

SS = Sample species; MT = Motility; Cap = Capsulation; Sp = Spore formation; CT = Citrate test; IN = Indole test; OX = Oxidase test; GS = Gram staining. N/A = Test not applicable. + = Positive reaction; - = Negative reaction

Bacterial Growth in Pesticide Residues Consortia

The bacteria predominant in farm soil are largely derived from soil organic and inorganic contents, as well as oxygen contents. Basically the bacteria are responsible for the degradation of organic and inorganic compounds. They derive their nutritional requirement from the compounds presented to them in the farm soil [15]. They are able to synthesize their enzymes, metabolic intermediates, structural proteins, lipids and nucleic acids from carbon compound in the soil together with other elements. They derive energy from metabolism of either organic compounds (chemoorganotrophic metabolism), or inorganic compounds (chemolithotrophic metabolism), such as reduced sulfur or nitrogen compounds [16]. Very extensive colonization of the pesticide consortiums were observed as this organic substrate represents a source of nutrients for the bacterial inoculant, but also for the indigenous soil micro flora. Under the conditions used in the present study all the four test bacterial isolates i.e. *P. auriginosa, E. coli, R. erythropolis and B. subtilis* were viable in natural soil, overcoming the competition with the native soil micro flora. All the tested bacterial species showed remarkable growth rates in both pesticide consortia inoculated with MSM nutrient media and those inoculated in MSM media only. All the bacterial species were observed in the plates during inoculation period. The bacterial species were periodically observed for growth after 2days, 10 days, 15 days and 21days. Standardization was done by 0.5McFarland standard solution at Optical Density (OD) 696nm spectrophotometrically (1.0 OD_{696nm} = 1.5×10^8CFU/ml).

This result has clearly indicated that the flower farm soils around Lake Naivasha has some pesticide contamination, confirming the outcomes of some studies, and hence the presence of such bacterial species which are known to have bioremediation potential of several xenobiotic chemicals in the environment [11,12,17]. The farm soils analyzed in this study has clearly shown that they are loaded with indicator organisms which are the indication of the presence of xenobiotic pesticides commonly used in major flower farms. Table 2 below shows the growth rate of bacterial species isolated from flower farm soil in specific pesticide consortia and in mixture of all the pesticide consortia. Bacterial species incubated with nutrient media only (without pesticide consortia) acted as negative control.

Table 2: Growth rate (average of 3 replicates ± mean standard deviation) expressed in OD(696nm) for P. auriginosa*, E. coli, R. erythropolis and B. subtilis* species subjected to aldrin, dieldrin, endosulfan, dimethoate, malathion and parathion pesticides individually and as mixture of pesticides and the Control (isolates not incubated with any pesticide) augmented with MM media at 15ºC

	Days	Control	OC1	OC2	OC3	OP1	OP2	OP3	Mixture
P. auriginosa	0	0.19±0.00^{a}	0.19±0.00^{a}	0.19±0.00^{a}	0.19±0.00^{a}	0.19±0.00^{a}	0.19±0.00^{a}	0.19±0.00^{a}	0.19 ±0.00^{a}
	2	0.34±0.02^{d}	0.32±0.03^{c}	0.28±0.03^{cd}	0.43±0.01^{b}	0.54±0.01^{a}	0.45±0.01^{b}	0.56±0.01^{a}	0.41±0.04^{b}
	10	0.45±0.03^{e}	0.78±0.01^{bc}	0.62±0.02^{d}	0.68±0.02^{cd}	0.77±0.01^{bc}	0.95±0.01^{a}	0.84±0.01^{ab}	0.77±0.11^{bc}
	15	0.26±0.01^{d}	0.86±0.01^{c}	0.88±0.02^{c}	1.21±0.04^{b}	0.89±0.01^{c}	1.65±0.01^{a}	1.25±0.01^{b}	0.95±0.05^{c}
	21	0.52±0.05^{d}	0.96±0.01^{c}	1.45±0.00^{ab}	1.45±0.02^{ab}	1.43±0.01^{ab}	1.65±0.10^{a}	1.42±0.00^{ab}	1.27±0.04^{b}
E coli	0	0.13±0.00^{a}	0.13±0.00^{a}	0.13±0.00^{a}	0.13±0.00^{a}	0.13±0.00^{a}	0.13±0.00^{a}	0.13±0.00^{a}	0.13±0.00^{a}
	2	0.48±0.03^{a}	0.25±0.01^{c}	0.36±0.01^{bc}	0.42±0.02^{ab}	0.44±0.01^{ab}	0.34±0.01^{bc}	0.25±0.01^{c}	0.45±0.01^{ab}
	10	0.70±0.04^{b}	0.47±0.01^{cd}	0.67±0.01^{abc}	0.75±0.01^{ab}	0.86±0.04^{a}	0.65±0.00^{bcd}	0.45±0.02^{d}	0.76±0.06^{ab}
	15	0.94±0.07^{cd}	0.88±0.02^{bcd}	0.86±0.03^{cd}	1.25±0.00^{ab}	1.48±0.00^{a}	0.96±0.03^{bcd}	0.65±0.02^{d}	1.05±0.10^{bc}
	21	1.44±0.09^{a}	1.25±0.02^{a}	1.34±0.01^{a}	1.45±0.01^{a}	1.66±0.02^{a}	1.65±0.01^{a}	1.24±0.02^{a}	1.31±0.04^{a}

R. erythropolis	0	0.21±0.00^{a}	0.21±0.00^{a}	0.21±0.00^{a}	0.21±0.00^{a}	0.21±0.00^{a}	0.21±0.00^{a}	0.21±0.00^{a}	0.21±0.00^{a}
	2	0.40±0.02^{a}	0.48±0.10^{a}	0.40±0.03^{a}	0.47±0.07^{a}	0.48±0.03^{a}	0.39±0.05^{a}	0.48±0.04^{a}	0.52±0.03^{a}
	10	0.70±0.02^{b}	0.69±0.12^{b}	0.75±0.06^{ab}	0.65±0.00^{b}	0.77±0.06^{ab}	0.80±0.06^{ab}	0.87±0.01^{a}	0.76±0.06^{ab}
	15	0.95±0.03^{b}	0.95±0.04^{ab}	0.94±0.06^{ab}	0.88±0.03^{b}	0.99±0.06^{ab}	1.06±0.08^{ab}	1.12±0.11^{a}	0.88±0.06^{b}
	21	1.33±0.04^{a}	1.39±0.09^{a}	1.41±0.04^{a}	1.31±0.06^{a}	1.44±0.06^{a}	1.45±0.06^{a}	1.39±0.10^{a}	1.44±0.11^{a}
	0	0.22±0.00^{a}	0.22±0.00^{a}	0.22±0.00^{a}	0.22±0.00^{a}	0.22±0.00^{a}	0.22±0.00^{a}	0.22±0.00^{a}	0.22±0.00^{a}
	2	0.37±0.02^{b}	0.37±0.04^{ab}	0.36±0.03^{ab}	0.43±0.12^{ab}	0.48±0.04^{ab}	0.48±0.07^{ab}	0.50±0.06^{a}	0.48±0.04^{ab}
B. subtilis	10	0.68±0.03^{b}	0.73±0.04^{ab}	0.77±0.00^{ab}	0.62±0.20^{b}	0.91±0.04^{a}	0.71±0.04^{ab}	0.80±0.03^{ab}	0.80±0.03^{ab}
	15	0.94±0.04^{b}	0.88±0.00^{b}	0.96±0.04^{b}	0.77±0.27^{b}	1.42±0.02^{a}	1.00±0.02^{b}	0.91±0.04^{b}	0.94±0.06^{b}
	21	1.50±0.04^{a}	1.41±0.04^{ab}	1.45±0.06^{ab}	1.01±0.39^{c}	1.17±0.06^{a}	1.51±0.03^{ab}	1.480.09^{ab}	1.77±0.07^{bc}

Means on the rows with different superscripts letters are significantly different for each bacteria. OC1 = Aldrin; OC2 = Dieldrin; OC3 = Endosulfan; OP1 = Dimethoate; OP2 = Malathion; OP3 = Parathion.

The growth rate for all the bacterial species was highest in pesticide mixture consortia as compared to individual pesticide consortia and media not inoculated with pesticide residues that served as control. The highest growth rate was observed from *B. subtilis* in pesticide mixture consortium at 2.65×10^{8} cfu/ml from the initial 3.3×10^{7} cfu/ml for the same bacterial species inoculated in MM media only. *P. auriginosa* showed the highest mean growth rate in Malathion consortium at 2.45×10^{8} cfu/ml from the initial 2.85×10^{7} cfu/ml during the same period. *E. coli* had the highest growth rate of 2.49×10^{8} cfu/ml in dimethoate consortium from the initial 1.95×10^{7} cfu/ml while *R. erythropolis* had the highest growth rate of 2.17×10^{8} cfu/ml in malathion consortium from the initial 3.15×10^{8} cfu/ml. B. subtilis had the highest growth rate of 2.26×10^{8} cfu/ml malathion consortium from the initial 1.33×10^{7} cfu/ml. There was generally lower growth rate in organochloride pesticides as compared to organophosphate pesticides.

Figures 1(a) to 1(f) below illustrate the graphical growth of the four isolated bacterial species in aldrin, dieldrin, endosulfan, dimethoate, Malathion and methyl parathion pesticide residue consortia. All the bacterial species that were inoculated with the pesticides consortium showed higher rate of growth in pesticide mixtures than in specific consortium. The growth rate was also higher in the three organophosphate pesticide consortia than in organochloride consortia aldrin, dieldrin and endosulfan probably due to complexity in bioremediation of organochloride xenobiotics as a result of the presence of stronger C-Cl which most bioremediating microorganisms have inferior ways to catabolize.

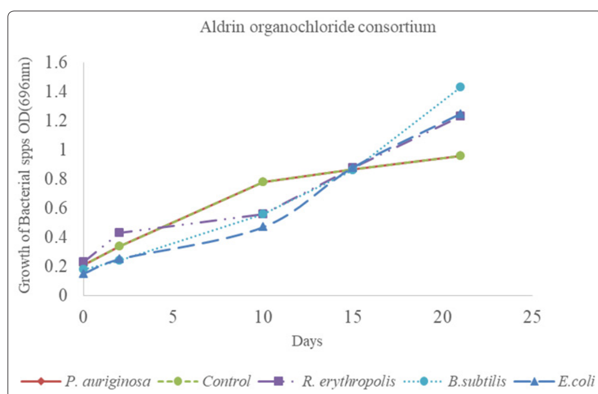

Figure 1(a): Growth rate for *P. auriginosa*, *E. coli, R. erythropolis and B. subtilis* species subjected to aldrin pesticides consortium and the Control (isolates not incubated with any pesticide) augmented with MM media at 15ºC

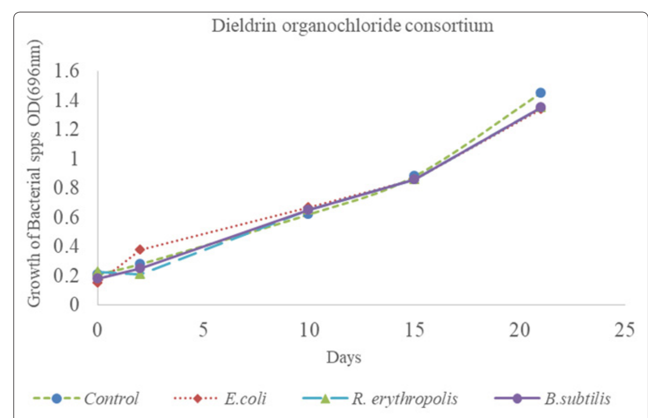

Figure 1(b): Growth rate for *P. auriginosa*, *E. coli*, *R. erythropolis and B. subtilis* species subjected to dieldrin pesticides consortium augmented with MM media at 15ºC

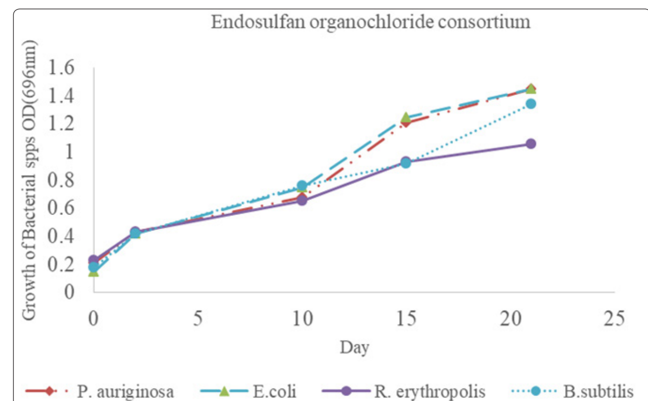

Figure 1(c): Growth rate for *P. auriginosa*, *E. coli*, *R. erythropolis and B. subtilis* species subjected to endosulfan pesticides consortium augmented with MM media at 15ºC

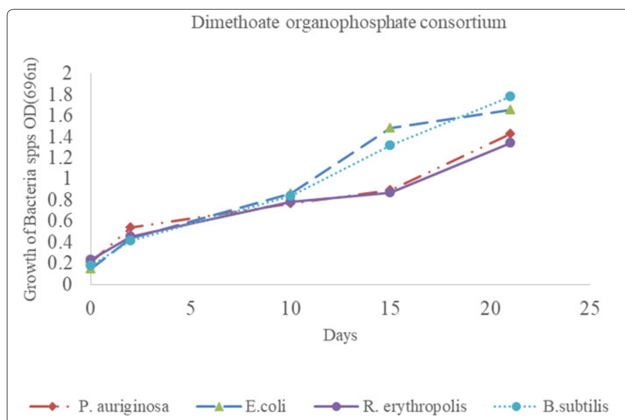

Figure 1(d): Growth rate for *P. auriginosa*, *E. coli*, *R. erythropolis and B. subtilis* species subjected to dimethoate pesticides consortium augmented with MM media at 15ºC

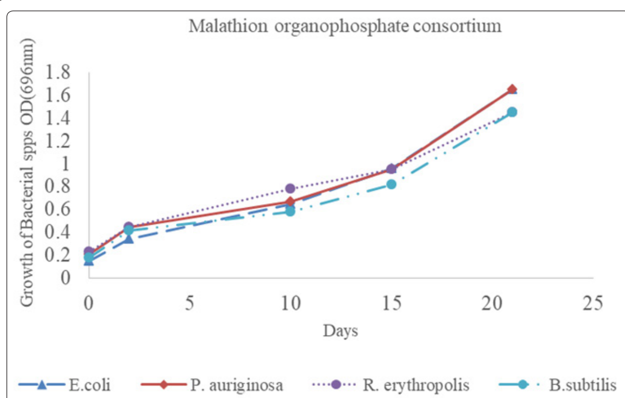

Figure 1(e): Growth rate for *P. auriginosa, E. coli, R. erythropolis and B. subtilis* species subjected to dimethoate pesticides consortium augmented with MM media at 15ºC

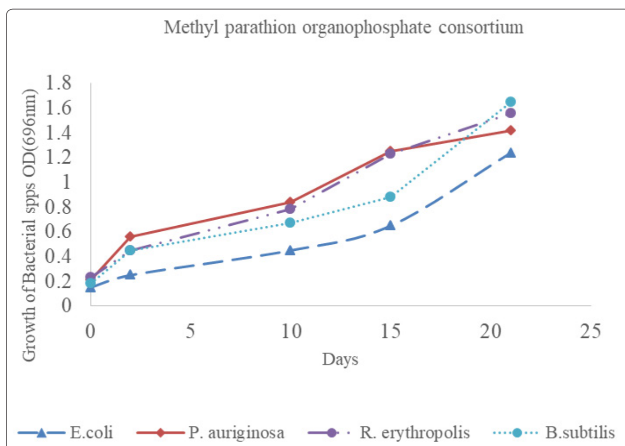

Figure 1(f): Growth rate for *P. auriginosa, E. coli, R. erythropolis and B. subtilis* species subjected to methyl parathion pesticides consortium augmented with MM media at 15ºC

Different bacterial species had different metabolic affinity for each pesticide samples. Differences in metabolic requirements like affinity for oxygen, nutrients and accumulation of metabolic products could have contributed to differences in the growth of the bacterial species in different xenobiotic pesticide consortia. The concentration and accumulation of some metabolites in the consortia might have inhibited some bacterial enzymes involved in the metabolic degradation process. The rate of bacterial growth therefore decreased with time during the incubation period in all the bacterial inoculant. This is due to accumulation of pesticide metabolites in the consortia some of which may be toxic to the bacterial species or may inhibit the enzymatic pathway involved in the biochemical breakdown of xenobiotic organic compounds.

Conclusion and Recommendations

Conclusion

The results obtained in this study provide valuable knowledge on the abilities of *P. auriginosa*, *E. coli*, *R. erythropolis* and *B. subtilis* to colonize soil and might serve as a sound basis for the further exploitation of these species as bacterial inoculants in biological remediation processes. The bacterial consortium isolated from the sample was made up of a group of species whose action was reflected in significant pesticide depletion. However, isolation of bacteria or bacterial consortia that might be used in the cleaning of wastes or polluted environments is very important. The bacteria which were isolated are able to grow in medium in the presence of added pesticide and may therefore be used for bioremediation of pesticide-contaminated soil. Although the bacterial species used in the soil extract broth study were exceptionally efficient in growing in the mixture of pesticides in soil extract broth, in the soil microcosms the growth rates were not has high. This may be as a result of presence of higher level of carbon and phosphate sources in the pesticide mixture consortia as compared to those bacterial inoculates containing one pesticide residue only [18].

Recommendations

Recommended possible further studies based on the findings of this study are:

(a) Study of the microbial populations structure

The current study has shown few differences on microbial populations, therefore a more detailed approach on the effect of this mixture of pesticides, before and after bioremediation, on soil microbial structure, would be very interesting. I would suggest the analysis of polar fatty acids, a method based on the analysis of polar lipids in the biological membranes, which yields a direct quantitative method of the biomass as well as a profile of the microbial community structure. Molecular techniques that rely on the capture and amplification of sequences of interest could also be a good method to characterize the soil microbial communities in microcosm experiments.

(b) Analysis of metabolites by GC-MS

The current study has shown significant decreases in detectable pesticides in soil after incubation with bacterial inoculants. However, these pesticides may have been completely degraded or mineralized; some of it may have been transformed in unknown metabolites. Additional studies on the toxicity of soil after remediation as well as a thorough analysis by GC-MS of the final products would be pertinent, as chemical degradation and microbial co-metabolism may produce toxic intermediates.

(c) Field studies in a contaminated site

The reliability of microcosm studies in the laboratory to interpret field conditions is much debated. Microcosms differ from the field situation concerning the influence of temperature and moisture dynamics, the influence of root presence, and the composition of the

soil biota community. The studies described in this thesis provide an estimate of the possible hazard imposed by this mixture of pesticides on the soil micro flora and the efficiency of the test isolates in the bioremediation of these chemicals. Although the results are very promising, under *in situ* conditions the outcome may be different. First, the exposure to the contaminants may be different since the chemicals are not distributed uniformly, and bioavailability will often vary due to various sorption processes. Second, by contrast, in the laboratory the test bacterial species is under controlled conditions, without secondary stresses, such as cold, drought or excessive rain, which possibly gave a better performance than in the field.

The uncertainties attached to the laboratory-to-field extrapolation can be avoided by conducting experiments under semi-field or field conditions. Therefore, I would suggest further studies to examine the feasibility of using these bacterial species at a larger scale in a contaminated site.

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