Preliminary Investigation of Actinomycetes as a Chlordane Remediation Agent

Jocelyn Bidlack, James Green, Felicia Osburn, BrieAunna Webster, and Paul Olson
Department of Biology, University of Central Oklahoma, Edmond, OK 73034

Abstract

Actinomycetes bacterium are promising remediation agents capable of dechlorination and degradation of many organochlorine pesticide contaminants. In this study, Actinomycetes strains were isolated from soil samples collected from a chlordane-contaminated site. The Actinomycetes strains were subjected to a mixture of α-chlordane and γ-chlordane in solution to assess their tolerance to, and ability to degrade, the isomeric compounds. Cellular biomass measurement were collected as a determinant of cellular growth and the chlordane product residues were extracted from the solution. The residues are currently being analyzed by gas chromatography mass spectrometry to determine the extent of degradation and the identity of the metabolic degradation products.

Introduction

Chlordane (Octachloro-4,7-methanohydroindane) is a neurotoxic pesticide used extensively to eradicate termite infestation in housing additions through the 1980's. The organochlorine is heavily substituted and non-polar persisting in the environment in high concentrations in relatively localized areas (Colt *et al.* 2009). Although chlordane use is now prohibited under the United Nations Environmental Program, contaminant is still detected at significant levels across North America. Current protocol for treatment of contaminated sites involves excavation and incineration, this treatment is expensive and labor intensive. As an alternative, remediation by biological organisms may provide a cost effective method of rehabilitating contaminated sites.

Actinomycetes is a gram positive, filamentous bacteria ubiquitous in soils and sediments. Many genera of actinobacteria have been indicated as promising biodegradation agents of organic compounds (Fuentes *et al.* 2010). Actinomycetes have been shown to thrive with organochlorines as the sole carbon source (Cuozzo et al. 2009). These organisms demonstrate the unique ability to oxidize and dechlorinate many organochloride pesticides such as metolachor (Liu *et al.* 1990), Lindane (Benimeli *et al.* 2006) and Atrazine (Radosevich *et al.* 1995).

The current study was conducted to determine if Actinomycetes strains isolated from a chlordane contaminated site were capable of dechlorination and degradation of chlordane utilized as a sole carbon source.

Sampling: Site Characterization and collection strategy

In 2011, the Oklahoma Department of Environmental Quality (ODEQ) identified a 100 m^2 plot at UTM 14 S 63860.46 m E 3947334.65 m as an area contaminated with chlordane. Soil samples were collected from an area 0-15 cm in depth using a sterilized stainless steel borer or trowel. Soil samples were collected in sterilized glass jars and dried to a constant weight at 30° C. Pesticide residues were extracted from the soil samples according to procedure outlined by Quintero et al. (2005). Three ml of soil was transferred to a 15 ml centrifugation tube and 6 ml of 1:1 hexane-acetone solution was added. To extract the organic phase, the tubes were sealed and shaken in a vortex for 10 minutes. To separate the organic and aqueous phases, the tubes were centrifuged at 2500 rpm for 10 minutes. An aliquot of the organic phase was removed for gc/ms analysis.

Figure 1. Contaminated site

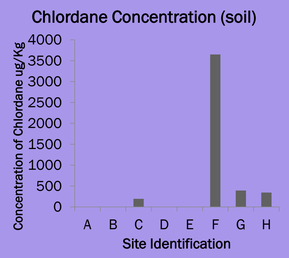

Figure 2. Documented Chlordane levels

Isolation of Actinomycetes from the soil samples

The dried soil samples were homogenized in a (1:10 w/v) 150 mM NaCl solution and 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} , 10^{-5}, 10^{-6}dilutions were inoculated on a starch-casein (SC) agar designed for the isolation of Actinomycetes. The SC agar contained: 10.0 g L^{-1} starch, 1.0 g L^{-1} casein, 0.5 g L^{-1} K_2HPO_4 at a final pH of 7.0, 15.0 g L^{-1} agar, 0.01 g L^{-1} nalidixic acid and 0.01 g L^{-1} cycloheximide for the inhibition of gram negative bacteria growth and fungi contamination (Ravel *et al.* 1998). Plates were incubated for 7 days at 30° C. To determine the approximate density of culturable Actinomycetes in the soil samples, the number of colonies were counted and the dilution factor referenced to determine the number of colony forming units (CFUs) for each replicate soil sample. Distinct colonies were extricated from the plates and streaked on fresh SC agar plates.

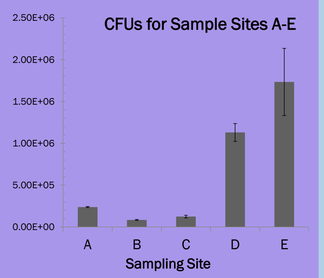

Figure 3. CFUs for sample sites

Table 1. Strains identified from sample site A, B, and C

Strain ID	Site A	Site B	Site C
Streptomyces spp.			
S. albus	*		*
S. aureofaciens		*	
S. californicus		*	
S. coelicolor	*		*
S. cyaneus	*		*
S. erythrochromogenes	*	*	*
S. griseus	*	*	*
S. oidospores		*	
S. resistomycificus	*		
S. reticuli		*	
S. viridis		*	*

* Indicates positive identification from site

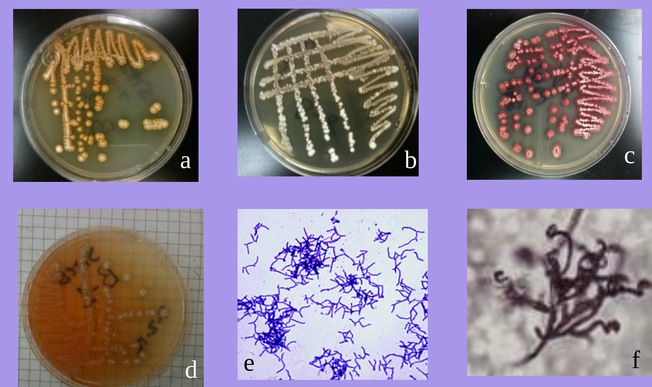

Figure 4. (a-c) example isolated strains, (d) soluble pigment , (e) gram stain, (f) spiral spore formation

Screening of actinomycetes strains as efficacious remediation agents

A liquid culture medium was utilized to evaluate the Actinomycetes strains ability to tolerate and successfully degrade chlordane residues in solution. A minimum medium was prepared consisting of 0.5 g L^{-1} $(NH_4)_2SO_4$, 0.5 g L^{-1} K_2HPO_4 pH 7.0, 0.2 g L^{-1} $MgSO_4 \cdot 7H2O$, and 0.01 g L^{-1} $FeSO_4 \cdot 7H2O$. The liquid medium was autoclaved at 121° C (15 psi) for 20 minutes to destroy contaminating organisms. The α-chlordane and γ-chlordane were dissolved in methanol and added to the medium in a concentration of 1.66 mg L-1 (control solutions received methanol in an equivalent volume). The medium was inoculated with a medium (.2cm<X<1cm) Actinomycetes colony. The solution were incubated at 30° C in a shaking water bath (100 rpm) for 1 week. Following 7 days incubation, an aliquot of the solution was sampled and analyzed for bacterial growth and chlordane metabolism.

The solutions were centrifuged at 9000 x g for 10 minutes at 4° C. The supernatant was retained for GC analysis of chlordane metabolic residues. The pellet containing the bacterial growth was washed with a phosphate buffered saline solution and drier to a constant weight for biomass determination.

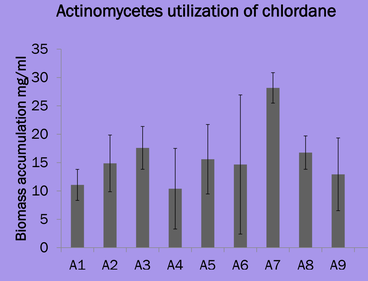

Figure 5. Biomass of Actinomycetes treated samples

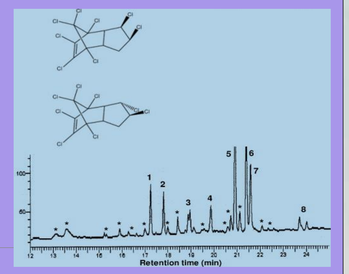

Figure6. Chlordane Chromatogram

GC-MS Determination of chlordane degradation

Chlordane degradation products are currently being analyzed by gas chromatography mass spectrometry (Sovocool *et al*. 1977). Chlordane products were extracted from the cell-free supernatant by solid phase extraction (SPE) using C18 columns, controls and chlordane standards were processed in the same manner as treatment samples. Extracts will be injected into an Agilent Tech Gas Chromatograph model 7890A with a mass selective detector model 5975C. The oven temperature will be programmed as follows: hold at 35° C for 6 minutes, ramp 3° /minute to 180° C, ramp 8° /minute to 270° C. Chlordane degradation products will be identified by mass spectrometry in consulate with the NIST08 quadripole mass spec database.

Discussion and Impact

Preliminary data indicates that Actinomycetes species distribution varies significantly across the site however, correlation with chlordane contaminant has not been verified. All isolated species have been tentatively identified as members of the genus Streptomyces, Studies have indicated that several species in this genus possess dehalogenase activity (Fuentes *et al.* 2010). Several strains isolated in this study have demonstrated a capacity to utilize chlordane in solution as a sole carbon source indicated by an accumulation of biomass. However, chlordane residues isolated from the medium are still being analyzed for degradation products. Actinomycetes bacteria have the potential to be powerful bioremediation agents however, more research is necessary to determine the optimal physiological conditions (temperature, pH, and biotic interactions) to facilitate the highest rate of organochlorine degradation.

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Acknowledgements

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