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Biodegradation of γ-Hexachlorocyclohexane (Lindane) and α-Hexachlorocyclohexane in Water and a Soil Slurry by a *Pandoraea* Species

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Isomers of 1,2,3,4,5,6-hexachlorocyclohexane (HCH) were some of the most widely used pesticides. Despite reduction in their production and use, HCH isomers present a serious environmental hazard. In this study, two bacterial isolates (LIN-1 and LIN-3) that can grow on γ-HCH as a sole source of carbon and energy were isolated from an enrichment culture. In liquid cultures of LIN-1 and LIN-3, 25.0 and 45.5% removal of γ-HCH, respectively, were achieved in 2 weeks. LIN-3 was identified as *Pandoraea* sp. by 16S rRNA gene sequence analysis (99% identity). *Pandoraea* sp. substantially degraded both γ- and α-HCH isomers at concentrations of 10−200 mg L^{-1} in liquid cultures. After 8 weeks of incubation in liquid culture, 89.9 and 93.3% of the γ- and α-HCH isomers declined, respectively, at an initial concentration of 150 mg L^{-1}. In soil slurry cultures of *Pandoraea* sp., simulating a soil slurry phase bioremediation treatment, substantial decreases in the levels of the HCH isomers were observed at concentrations of 50−200 mg L^{-1}. After 9 weeks, 59.6 and 53.3% biodegradations of γ- and α-HCH isomers, respectively, were achieved at 150 mg L^{-1}. Using two 23-mer oligonucloetide primers targeting the 330 bp region of the 16S rRNA gene of *Pandoraea* sp., an approximately 330 bp PCR product was successfully amplified from DNA templates prepared from bacterial colonies and soil slurry culture. This system provides a direct and rapid PCR-based molecular tool for tracking *Pandoraea* sp. strain LIN-3 in water and soils. These results have impplied implications for the treatment of soils and water contaminated with HCH isomers.

KEYWORDS: Biotechnology; bioremediation; pesticides; hexachlorocyclohexane isomers; lindane; *Pandoraea* sp.; PCR detection

INTRODUCTION

Extensive usage of pesticides has caused significant pollution of natural water bodies, soils, and the atmosphere. Isomers of 1,2,3,4,5,6-hexachlorocyclohexane (HCH) were some of the most widely used pesticides. They were discovered to display insecticidal activity against a wide variety of insects during the Second World War (*1*). Important agricultural applications include insect control in agronomic grain, vegetable crops, and forestry, vector control, and seed treatment (*2*).

There are eight isomers of HCH designated β, γ, δ, ϵ, η, and θ and two α-enantiomers (*3*). They differ only in their relative orientation of the chlorine atoms bound to the different carbons (*1*, *4*). Industrial production of HCH is by photochemical chlorination of benzene, and the product is generally known as technical grade lindane (*5*). Technical grade HCH is composed of the following isomers: α-HCH (60−70%), β-HCH (5−12%), γ-HCH (10−15%), δ-HCH (6−10%), and ϵ-(3−4%) (*6*). The α-isomer is the principal constituent of technical grade HCH followed by the γ-isomer, which is the most potent insecticidal ingredient of the technical product. Because the γ-isomer is the active ingredient of technical grade HCH, it has been common to refine it from the mixture and market it under the name lindane. Other HCH isomers are, however, present in lindane in trace amounts (*5*). Thus, in the international market, there are two commercial formulations of HCH: the technical grade (an isomeric mixture) and the pure grade lindane (γ-HCH, 99%) (*7*).

Technical grade HCH was extensively used worldwide (*8*, *9*). Although the widespread use of lindane and technical grade HCH has been discontinued for a long time, the problem of their residues, due to the lengthy persistence of these chemicals in many soils, exists (*10*). Although the use of HCH isomers as a chemical defense in crop production has been banned in a number of developed countries, it is still used in many

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developing countries (*9*). Furthermore, unused stockpiles of both technical grade HCH and lindane still exist (*5*). A total of 2785 metric tons of technical grade HCH, 304 metric tons of lindane, and 45 metric tons of unspecified HCH material were found scattered in Africa and the Near East (*11*).

Lindane has been found in air, water, soil, snow, and tissue samples throughout the world (*12*). Atmospheric concentrations ranging from 0.006 to 3.5 ng m^{-3} of γ-HCH have been reported (*13*). HCH isomers have the tendency to bioaccumulate in the food chain (*9*). Consequently, concerns have been raised about its potential adverse health effects on humans and other living systems. Adverse health effects include neurological problems and immunosuppression in humans and liver cancer in rats and mice (*3*, *5*). The α-isomer possesses the most carcinogenic activity and has been classified together with technical grade HCH as a group B2 probable human carcinogen by the U.S. Environmental Protection Agency.

HCH isomers can be biologically degraded under aerobic and anaerobic conditions (*14*). Studies on microbially mediated biodegradation of lindane and other HCH isomers include the use of *Phanerochaete chrysosporium*, BKM-F-1767 (*15*), mixed native soil microbial population incubated under different redox conditions (*16*), an enrichment culture (*17*), sewage sludge (*18*), methanogenic conditions (*19*), *Clostridium* spp., and members of the Bacillaceae and Enterobacteriaceae families (*20*). Soil amendments such as the addition of peptone (*21*), glucose, cellulose plus rice straw (*22*), and rice straw alone (*23*) have accelerated the degradation of HCH isomers. Abiotic degradation of lindane by porphyrins and corrins has also been reported (*24*). However, biological degradation offers the most attractive remediation strategy (*25*, *26*) which is considered to be cost-effective.

In the present study, we report the discovery of a lindane-degrading bacterium, *Pandoraea* sp. strain LIN-3, isolated from an enrichment culture. As the α-isomer is the principal constituent of technical grade HCH, we investigated co-degradation of the γ- and α-isomers in water and a soil slurry by the *Pandoraea* sp.

EXPERIMENTAL PROCEDURES

Reagents and Chemicals. α-Hexachlorocyclohexane (99% pure) and γ-hexachlorocyclohexane (97% pure) were purchased from Aldrich, Milwaukee, WI. Stock solutions were prepared in ethanol and an acetone/ethanol mixture (50:50) for γ- and α-HCH, respectively. Pesticide calibration standard mixture (608 calibration mix) was purchased from VWR Scientific Products, San Diego, CA. Other chemicals were of analytical grade and purchased from commercial sources.

Enrichment Culture. An enrichment culture for HCH-degrading bacteria was set up using a high-density bacterial cocktail (HDBC) obtained from the Center for Environmental Microbiology Inc. (Riverside, CA). FTW medium (*27*) used is composed of the following (in g L^{-1}): K_2HPO_4, 0.225; KH_2PO_4, 0.225; $(NH_4)_2SO_4$, 0.225; $MgSO_4 \cdot 7H_2O$, 0.05; $CaCO_3$, 0.005; $FeCl_2 \cdot 4H_2O$, 0.005; and 1 mL of trace elements solution (*28*). The medium was sterilized by autoclaving (121 °C, 15 min), before lindane was aseptically added to a final initial concentration of 100 mg L^{-1}. Five milliliters of HDBC was inoculated onto 75 mL of sterile FTW mineral salts solution. The aerobic culture was incubated at 30 °C with orbital shaking (150 rpm) for 2 weeks (round 1 enrichment culture). Thereafter, 1 mL of the culture was transferred to 75 mL of fresh sterile FTW enrichment medium, containing 100 mg L^{-1} lindane and further incubated for 2 weeks (round 2 enrichment culture).

Lindane-Degrading Monocultures. To isolate single strains from the enrichment culture, 1 mL aliquots of round 2 enrichment cultures were centrifuged (8000 rpm, 10 min) using a Beckman microfuge II. The supernatant was removed and cell residues were resuspended in 50 μL of sterile FTW mineral elements medium by vortexing. Aliquots of this suspension were plated on FTW/lindane mineral elements agar (FTW/lindane agar) by streaking. This solid medium was prepared by adding 2% washed agar to the enrichment basal medium followed by autoclaving (121 °C, 15 min). Thereafter, lindane was aseptically added after the molten agar had been cooled to ~50 °C, to a final initial concentration of 100 mg L^{-1}. Agar plates were incubated under aerobic condition at 30 °C. On the basis of differences in colony morphology, four isolates were obtained. Isolates were further purified by streaking on FTW/lindane agar. Washed agar was prepared by suspending 10 g of agar powder (Difco) in 500 mL of deionized water and thoroughly mixed with a glass rod and then allowed to stand at room temperature for 15 min. The supernatant was carefully decanted. This washing procedure was repeated twice with the FTW mineral elements solution. The washed agar residue was then made up to 500 mL with FTW mineral elements solution before autoclaving.

Screening of Isolates for Lindane Degradation in Liquid Culture. Two bacterial isolates (LIN-1 and LIN-3), which grew luxuriantly on the lindane agar, were selected and tentatively classified as prolific lindane degraders. The isolates were pregrown in FTW mineral salts medium containing 100 mg L^{-1} lindane (FTW/lindane broth). Cultures were incubated (30 °C, 150 rpm, 1 week). Cells were harvested by centrifugation (5000 rpm, 15 min) and washed twice in 40 mL of FTW mineral salts solution. Cells were thereafter resuspended in the same medium to an initial OD_{600} of ~0.92. Five hundred microliters of the cell suspension was then used to inoculate 50 mL of FTW/lindane broth in a 250 mL Erlenmeyer flask. Cultures were incubated (30 °C, 150 rpm) for 14 days.

Molecular Identification of Bacterial Isolate. One bacterial isolate, tentatively designated LIN-3, was identified by analysis of the 16S rRNA gene sequence (Midi labs, Newark, DE). Briefly, primers corresponding to the 16S rRNA gene of *Escherichia coli* positions 005 and 531 were used to amplify the 16S rRNA gene by Polymerase Chain Reaction (PCR). The template was genomic DNA isolated from bacterial colonies. Excess primers and dNTPs were removed from the PCR product using Amicon 100 (Millipore, Bedford, MA) molecular mass cutoff membranes. Purity of the PCR product was checked by agarose gel electrophoresis. Cycle sequencing of the PCR product was carried out using an ABI Prism 377 DNA sequencer. Sequence data were analyzed using PE/Applied Biosystem's Microseq microbial analysis software and database. Neighbor-joining phylogenetic trees were generated (*29*) using the top 10 alignment matches.

Preparation of Bacterial Inoculum for Biodegradation Studies. LIN-3 was pregrown in FTW nutrient solution containing 150 mg L^{-1} γ-HCH and incubated (30 °C, 150 rpm) for 4 days. The inoculum was then centrifuged (5000 rpm, 20 min). To remove residual nutrients and HCH, cells were washed twice by centrifugation (5000 rpm, 20 min) using 40 mL of FTW nutrient solution. Washed cells were resuspended in FTW nutrient solution.

Hexachlorocyclohexane Biodegradation Kinetics in Liquid Culture. Biodegradation studies in liquid culture were performed in 250 mL Erlenmeyer flasks containing 50 mL of FTW nutrient solution, in triplicate. Flasks were autoclaved (121 °C, 20 min), and the final pH was 7.2. After cooling to ambient temperature, the flasks were spiked with γ- and α-HCH stock solutions to give final concentrations of 10, 50, 100, 150, and 200 mg L^{-1} of γ- and α-HCH. Flasks were then inoculated with 600 μL of the inoculum (OD_{600} = 0.87). The same amount of FTW nutrient solution was added to uninoculated controls. The flasks were closed with sterile rubber stoppers and incubated (30 °C, 160 rpm).

HCH Degradation Kinetics in Soil Slurry. The biodegradability studies in a soil slurry were conducted in 50 mL Erlenmeyer flasks containing 10 mL of FTW nutrient solution and 1.5 g of a sandy loam soil (15% soil slurry), in triplicate. Flasks were autoclaved for 20 min at 121 °C. After cooling to room temperature, the flasks were then spiked with different concentrations (10, 50, 100, 150, and 200 mg L^{-1}) of γ- and α-HCH. Flasks were inoculated with 350 μL of inoculum (OD_{600} = 3.18). The same volume of nutrient solution was added to the uninoculated controls. The flasks were closed using a sterile rubber stopper and incubated (30 °C, 160 rpm).

Analysis of Cultures. Bacterial density in liquid cultures was determined spectrophotometrically by measuring the absorbance at 600 nm. For the soil slurry experiments, HCH-degrading bacterial biomass was measured by counting the colony forming units (CFU). FTW nutrient solution hardened with washed agar was autoclaved (121 °C, 20 min) and kept warm at 50 °C in a hot water bath. γ-HCH (200 mg L^{-1}) was added to the molten agar and thoroughly mixed before transfer to sterile plates. Soil slurry culture samples were serially diluted (10^{-1} to 10^{-8}), and 100 μL aliquots were plated on the FTW lindane agar plates. Plates were incubated at 30 °C, and CFU were counted after 7 days. HCH in cultures was extracted by the addition of an equal volume of acetone followed by 1 h of shaking using a reciprocal shaker. One milliliter was then transferred to 9 mL of hexane and further shaken for 30 min. The sample was dehydrated by passing it through anhydrous Na_2SO_4 and concentrated using a rotary evaporator. Appropriate dilutions of the sample extract were then analyzed using a Hewlett-Packard gas chromatograph equipped with an electron capture detector (GC-ECD), an autosampler, and a DB-5MS capillary column of 0.25 mm i.d. and 0.25 μm film thickness (J&W Scientific, Folsom, CA). Hydrogen was the carrier gas at a flow rate of 0.8 mL min^{-1}.

Data Analysis. The means and standard deviations ($n-1$) of replicates were computed. Biodegradation (percent) was calculated on the basis of the difference between remaining HCH in controls and treated samples. Biodegradation in liquid culture was subjected to a first-order decay model, and the rate constant (k) and half-life ($t_{1/2}$) were estimated.

Construction of a PCR-Based Molecular Tool for Detection of the *Pandoraea* Species. *PCR Primers.* Based on the 16S rRNA, two 23-mer oligonucleotide primers (LIN3FO, 5′-AGCACGGGTGCTTG-CACCTGGTG-3′, $T_m = 77.9$ °C, as the forward primer; and LIN3RE, 5′-CGCGGCATTGCTGGATCAGGCTT-3′, $T_m = 78.1$ °C, as the reverse primer) were designed for the amplification of a 0.33 kb specific region of the 16S rRNA.

Target DNA Template. Suspension of colonies from 6-week-old liquid cultures and a 9-week-old soil slurry culture, plated on FTW/lindane agar, and a 9-week-old soil slurry culture were used as DNA templates. Colonies of a bacterial isolate from the soil sample served as a control organism. Five hundred microliters of DNA templates (bacterial colony suspension in sterile water or soil slurry) was heated in a boiling water bath for 15 min and centrifuged (8000 rpm, 5 min).

PCR Procedure. PCR was performed in a reaction mixture of 50 μL containing 200 μM each of deoxynucleoside triphosphate, 25 pmol of each primer, a 4 μL aliquot of each DNA template, and 1 μL of Taq polymerase (Promega, Madison, WI). A 35 PCR cycle was carried out as follows: initial denaturation (95 °C, 5 min), subsequent denaturation (95 °C, 1 min), annealing (60 °C, 1 min), elongation (72 °C, 1 min), and final elongation (72 °C, 5 min).

Agarose Gel Electrophoresis of PCR Product. Aliquots of the PCR amplification were analyzed on 2% agarose gel prepared in 1× TAE buffer. A 100 bp DNA ladder (Promega) was used to identify the expected size of the PCR product.

RESULTS

HCH-Degrading Monocultures from Enrichment Cultures. Both round 1 and round 2 enrichment cultures showed substantial turbidity, indicating the utilization of lindane as the sole source of carbon and energy. Round 2 was therefore used for the isolation of lindane-degrading monocultures. Two bacterial isolates (LIN-1 and LIN-3) growing luxuriantly on γ-HCH as the sole source of carbon and energy were selected for further studies. Both isolates substantially degraded γ-HCH in liquid culture. With LIN-1 and LIN-3, approximately 25.0 and 45.5% removal of γ-HCH, respectively, was achieved in 2 weeks. LIN-3 was, therefore, selected for further studies. Minor interconversion of γ-HCH to other isomers was detected. Approximately 0.08, 0.50, and 0.02 mg L^{-1} of α-, β-, and δ-HCH isomers, respectively, were detected in liquid cultures of LIN-3 spiked with 100 mg L^{-1} γ-HCH.

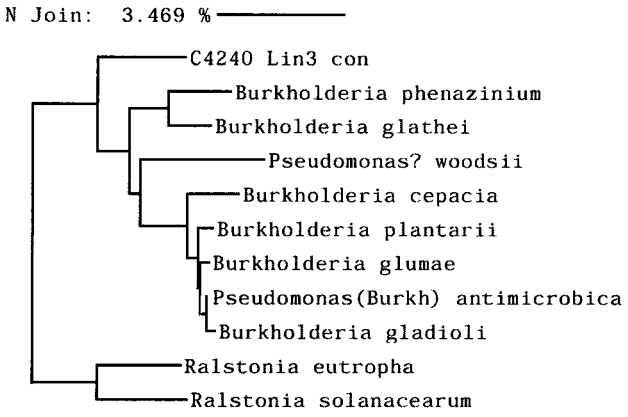

Figure 1. Neighbor-joining phylogenetic tree of *Pandoraea* sp. LIN-3 (C4240 Lin3 con).

Phylogenetic Identity of LIN-3. The lindane-degrading bacterial isolate (LIN-3) is a Gram-negative rod that grows aerobically on lindane as the sole source of carbon. The bacterium was identified as *Pandoraea* sp. by 16S rRNA gene sequence analysis. On the basis of the first 500 base pairs of the 16S rRNA gene, the isolate is most closely related to *Pseudomonas* (*Burkh*) *antimicrobia* (94.8% identity), followed by *Burkholderia glumae*, *Burkholderia cepacia*, and *Burkholderia gladioli* (94.7% identity). The isolate is 7.66, 7.85, and 8.59% dissimilar to *Ralstonia eutropha*, *Pseudomonas woodsii*, and *Ralstonia solanacearum*, respectively. The phylogenetic neighbor joining tree of *Pandoraea* sp. LIN-3 is presented in **Figure 1**.

Biodegradation of HCH Isomers in Liquid Culture. *Effect of Initial Concentration.* The decline of different concentrations of γ- and α-HCH isomers in liquid culture by *Pandoraea* sp. LIN-3 was examined. **Table 1** presents residual readings of γ- and α-HCH isomers in the liquid culture and control flasks. A substantial decline of the γ-isomer and α-isomers at different concentrations (10−200 mg L^{-1}) (**Figure 2**) was observed within 2−4 weeks of incubation. Decline of the γ- and α-HCH isomers increased with increasing initial concentration of HCH. Maximal depletion was observed at 150 mg L^{-1} and thereafter decreased substantially at 200 mg L^{-1}. The lowest percent biodegradation of the γ- and α-HCH isomers in liquid culture was observed at 10 mg L^{-1}. Recoveries of γ- and α-HCH in liquid cultures were 59−100 and 72−100%, respectively.

HCH Biodegradation Dynamics. The dynamics of γ- and α-HCH biodegradation in liquid culture of *Pandoraea* sp. was examined at an initial concentration of 150 mg L^{-1}. Residual γ- and α-HCH isomers in cultures and control flasks over an 8 week incubation period are summarized in **Table 2**. The rates of biodegradation of γ- and α-HCH isomers, relative to the controls, in liquid culture are presented in **Figure 3**. Rapid depletion of both HCH isomers was observed in the first 5 weeks. About 50% of the HCH isomers were removed from the cultures in 3 weeks. After 8 weeks of incubation, 89.9 and 93.3% biodegradations were achieved with the γ- and α-HCH isomers, respectively. The rate constants (k) for γ- and α-HCH were estimated to be 0.28 and 0.32, respectively. The half-lives ($t_{1/2}$) for γ- and α-HCH were estimated to be 2.51 and 2.19 weeks, respectively. Minor abiotic decreases in HCH concentrations in control flasks were observed.

Bacterial Density and Culture pH. Bacterial density in the cultures increased rapidly in 2 weeks and thereafter increased slowly in weeks 2−4 (**Figure 3**). This was followed by a stationary phase from 4 to 6 weeks of incubation and,

Table 1. Residual γ- and α-HCH Isomers in Culture and Control Flasks

HCH isomer	incubation time (weeks)	10 mg L^{-1} control	10 mg L^{-1} treated	50 mg L^{-1} control	50 mg L^{-1} treated	100 mg L^{-1} control	100 mg L^{-1} treated	150 mg L^{-1} control	150 mg L^{-1} treated	200 mg L^{-1} control	200 mg L^{-1} treated
γ	2	7.03 (±0.88)[a]	5.85 (±0.007)	29.6 (±3.82)	21.9 (±3.61)	71.7 (±0.71)	48.6 (±1.56)	139.4 (±6.72)	93.4 (±2.26)	202.8 (±8.77)	145.5 (±18.1)
	4	7.38 (±1.36)	4.8 (±0.42)	29.6 (±6.08)	15.7 (±3.82)	67.5 (±5.87)	31.1 (±1.77)	115.5 (±0.64)	41.3 (±8.77)	202.3 (±8.06)	131.9 (±6.93)
α	2	7.71 (±0.02)	6.85 (±0.07)	43.9 (±1.63)	32.0 (±2.69)	99.3 (±7.85)	70.3 (±9.40)	145.1 (±6.72)	91.2 (±5.37)	183.5 (±0.71)	149.7 (±0.21)
	4	7.26 (±1.70)	6.05 (±0.06)	46.4 (±10.1)	25.3 (±3.11)	110.5 (±8.91)	55.8 (±4.03)	141.5 (±2.69)	49.3 (±0.21)	201.6 (±7.00)	163.4 (±2.69)

[a] Values in parentheses show standard deviation.

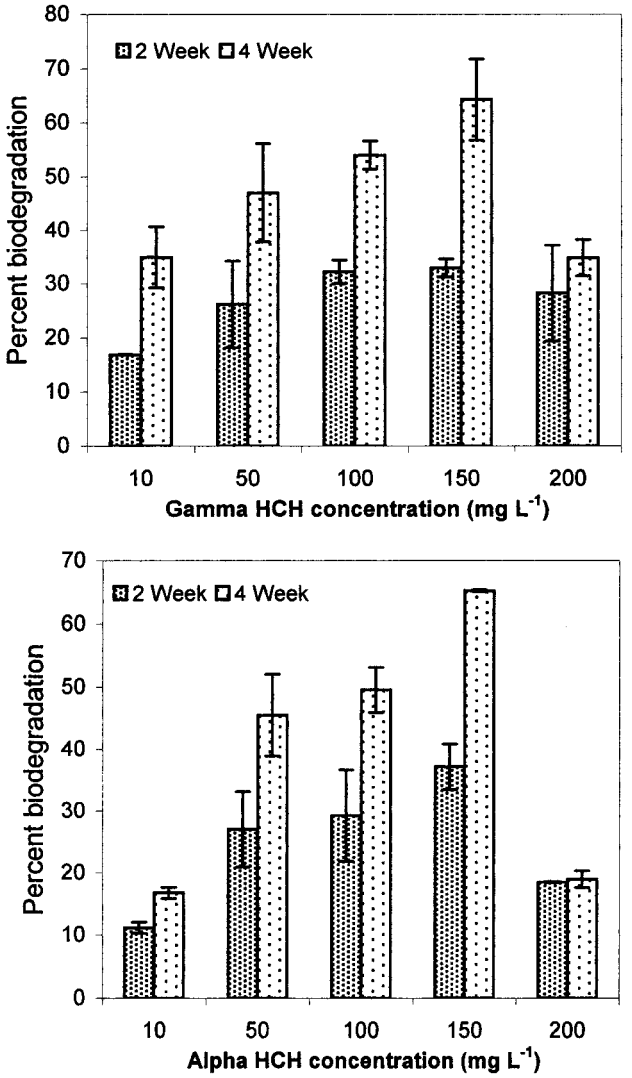

Figure 2. Biodegradation of different concentrations of γ- and α-HCH isomers in liquid culture by *Pandoraea* sp. LIN-3.

subsequently, a decline was observed. Culture pH decreased substantially to the acidic range. After 1, 2, 3, 4, 5, 6, 7, and 8 weeks of incubation, the pH readings were approximately 6.5, 5.8, 5.3, 5.0, 4.7, 4.4, 4.4, and 4.4, respectively.

Biodegradation of HCH Isomers in Soil Slurry. *Effect of Initial Concentration.* **Table 3** presents residual concentrations of γ- and α-HCH isomers in soil slurry cultures of *Pandoraea* sp. and controls spiked with 10−200 mg L^{-1} of each HCH isomer. The bacterial isolate degraded different concentrations of γ- and α-HCH isomers to a significant extent. At 50−150 mg L^{-1}, a substantial decline in the levels of HCH isomers was observed in 3 weeks. However, no significant decrease in HCH concentration was observed in 3 weeks at 200 mg L^{-1}. Recoveries of γ- and α-HCH in soil slurry cultures were 58−77 and 71−77%, respectively, at 50−200 mg L^{-1}. HCH recovery was very low in soil slurry cultures containing 10 mg L^{-1}.

HCH Biodegradation Dynamics. **Figure 4** illustrates the biodegradation dynamics of different concentrations of γ- and α-HCH isomers in the soil slurry cultures over a 9 week incubation period. At an initial concentration of 50−200 mg L^{-1}, substantial biodegradation of both HCH isomers was observed after 3 weeks and proceeded rapidly with further incubation up to 9 weeks. After 9 weeks, 64.3, 57.4, 59.6, and 43.0% biodegradation of γ-HCH was achieved at initial concentrations of 50, 100, 150, and 200 mg L^{-1}, respectively. With the α-HCH isomer, 63.2, 51.6, 53.3, and 48.8% biodegradation was achieved at initial concentrations of 50, 100, 150, and 200 mg L^{-1}, respectively. In soil slurry cultures spiked at 10 mg L^{-1} of each HCH isomer, no biodegradation was observed in 9 weeks. The pH of the soil slurry cultures remained relatively stable (7.2−7.4) during the 9 week incubation period.

Enumeration of Pandoraea sp. LIN-3 in Soil Slurry Culture. **Table 4** presents bacterial counts of *Pandoraea* sp. LIN-3 transforming different concentrations of γ- and α-HCH isomers in soil slurry culture over a 9 week period. Bacterial counts (CFU mL^{-1}) increased with increasing HCH concentration up to 100 mg L^{-1} in the soil slurry. Relatively lower bacterial counts were detected in the soil slurry cultures containing 150−200 mg L^{-1} HCH. At an initial concentration of 10 mg L^{-1}, no increase in bacterial count was observed in 3−9 weeks. At 50−100 mg L^{-1}, the bacterial count increased up to 6 weeks and thereafter declined. At 150−200 mg L^{-1}, the bacterial count was initially low but increased with incubation time.

Molecular Tracking (Detection) of Pandoraea sp. LIN-3 in Soil. Two 23-mer oligonucloetide primers targeting a 330 bp region of the 16S rRNA gene of *Pandoraea* sp. were designed. By PCR, the primers successfully amplified the expected PCR product of approximately 330 bp from both bacterial colonies isolated from cultures and soil slurry templates (**Figure 5**). No PCR product was achieved with the control templates (a suspension of a bacterial isolate from the uninoculated soil slurry) and in the absence of a template.

DISCUSSION

Although large-scale agricultural use of the HCH insecticide has been discontinued in many countries, the theory of "global distillation", which suggests that organic pollutants will be volatilized from warmer regions of the globe where they are still in use and transported to colder regions where they condense (*30*), warrants international remediation efforts. Biological remediation strategies are environmentally desirable and considered a cost-effective option. In this study, we have demonstrated substantial biological removal of γ- and α-HCH isomers from liquid and soil slurry cultures.

Pandoraea sp. metabolized both γ- and α-HCH isomers, but at different rates and extents of biodegradation in liquid and soil slurry cultures. In liquid cultures, the rates and extent of HCH biodegradation increased with increasing concentrations

Table 2. Residual γ- and α-HCH Isomers in Culture and Control Flasks over an 8 Week Incubation Period with Initial Concentration of 150 mg L^{-1} of Each Isomer

HCH isomer		week 1	2	3	4	5	6	7	8
γ (mg L^{-1})	control	141.9 (±0.92)	139.4 (±6.72)	130.3 (±4.67)	115.9 (±0.07)	110.9 (±0.92)	106.2 (±1.56)	112.7 (±0.42)	112.1 (±8.49)
	treated	106.6 (±2.90)	93.4 (±2.26)	63.7 (±2.19)	41.3 (±8.77)	28.7 (±7.42)	23.6 (±6.49)	18.5 (±5.13)	11.4 (±1.34)
α (mg L^{-1})	control	148.4 (±11.0)	145.1 (±6.72)	145.0 (±13.4)	141.5 (±2.69)	144.3 (±2.90)	145.2 (±4.24)	139.2 (±6.51)	136.2 (±1.34)
	treated	115.3 (±5.87)	91.2 (±5.37)	68.9 (±3.82)	49.3 (±0.22)	40.6 (±0.92)	30.6 (±2.47)	17.3 (±6.43)	9.15 (±0.92)

Table 3. Residual γ- and α-HCH Isomers in Soil Slurry Cultures and Control Flasks

HCH isomer	incubation time (weeks)	10 mg L^{-1} control	10 mg L^{-1} treated	50 mg L^{-1} control	50 mg L^{-1} treated	100 mg L^{-1} control	100 mg L^{-1} treated	150 mg L^{-1} control	150 mg L^{-1} treated	200 mg L^{-1} control	200 mg L^{-1} treated
γ	3	1.05 (±0.21)[a]	1.75 (±0.21)	29.0 (±1.41)	19.0 (±2.83)	59.5 (±3.54)	43.0 (±4.24)	116.0 (±5.66)	92.5 (±4.95)	154.5 (±7.78)	152.0 (±18.4)
	6	0.55 (±0.07)	0.50 (±0.14)	16.0 (±4.24)	8.0 (±1.41)	45.0 (±8.49)	23.0 (±4.24)	101.5 (±3.54)	64.0 (±8.49)	123.5 (±17.7)	96.0 (±8.49)
	9	0.31 (±0.02)	0.36 (±0.42)	7.0 (±1.41)	2.5 (±0.71)	27.0 (±1.41)	11.5 (±2.12)	54.5 (±2.12)	22.0 (±2.83)	79.0 (±2.83)	45.0 (±4.24)
α	3	0.75 (±0.21)	1.15 (±0.21)	36.0 (±1.41)	29.0 (±1.41)	71.0 (±2.83)	55.5 (±7.78)	116.0 (±8.49)	98.5 (±6.36)	143.5 (±4.95)	143.5 (±2.12)
	6	0.45 (±0.07)	0.45 (±0.07)	22.5 (±0.71)	11.5 (±3.54)	57.5 (±4.95)	32.0 (±4.24)	100.0 (±2.83)	67.0 (±4.24)	129.0 (±12.7)	105.0 (±9.90)
	9	0.33 (±0.007)	0.35 (±0.028)	9.5 (±0.71)	3.5 (±0.71)	46.5 (±0.71)	22.5 (±3.54)	68.5 (±3.54)	32.0 (±2.83)	87.0 (±8.49)	44.5 (±7.78)

[a] Values in parentheses show standard deviation.

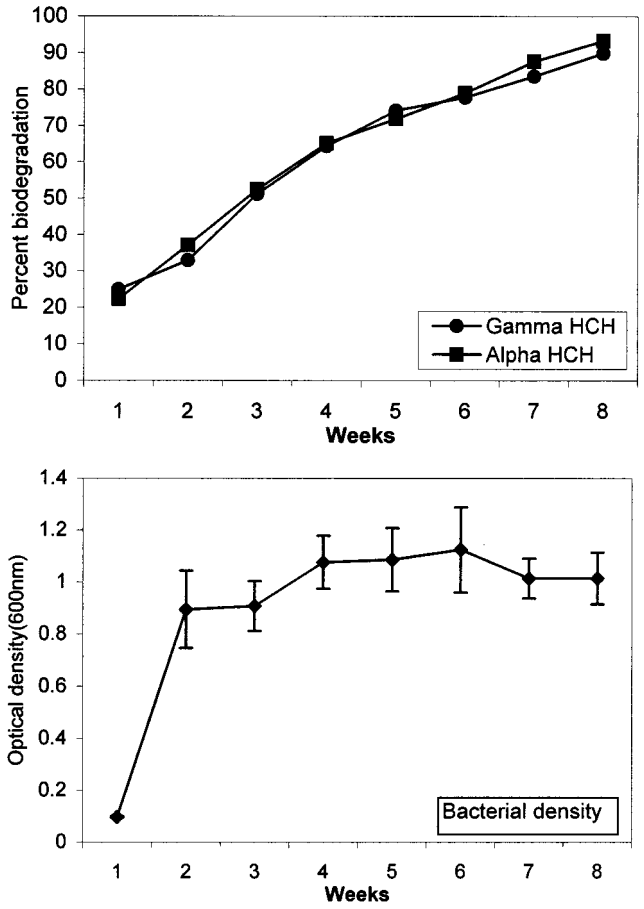

Figure 3. Relationship between biodegradation of γ- and α-HCH isomers and bacterial density in liquid culture of *Pandoraea* sp. LIN-3 over an 8 week period at an initial concentration of 150 mg L^{-1} of each isomer.

up to 150 mg L^{-1} but declined at 200 mg L^{-1}. *Pandoraea* sp. degraded ~50% of the HCH isomers in liquid culture in 3 weeks, at 150 mg L^{-1}. Similar findings on HCH degradation were reported by Singh and Kuhad (*31*), who studied the ability of white-rot fungus, *Trametes hirsutus*, to degrade lindane (γ-isomer) in liquid culture. Their results indicated that most of the lindane in the growth medium was degraded after 28 days of incubation.

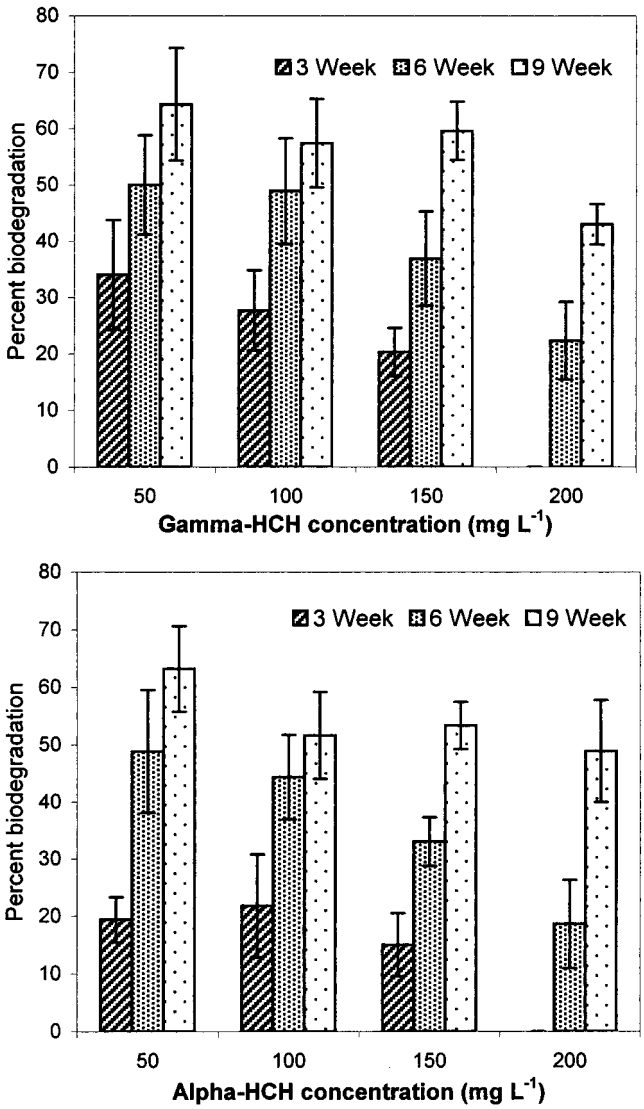

Figure 4. Biodegradation of γ- and α-HCH isomers in a soil slurry culture inoculated with *Pandoraea* sp. LIN-3.

The *Pandoraea* sp. promoted rapid biodegradation of both HCH isomers (γ and α) in soil slurry cultures, at an initial

Table 4. Viable Bacterial Count in Soil Slurry Culture

incubation time (weeks)	bacterial count (cfu mL^{-1})				
	10 mg L^{-1}	50 mg L^{-1}	100 mg L^{-1}	150 mg L^{-1}	200 mg L^{-1}
3	7.4×10^6 ($\pm 0.85 \times 10^6$)[a]	3.48×10^7 ($\pm 0.39 \times 10^7$)	4.4×10^7 ($\pm 0.57 \times 10^7$)	1.37×10^7 ($\pm 0.18 \times 10^7$)	5.4×10^6 ($\pm 1.9 \times 10^6$)
6	7.25×10^6 ($\pm 0.35 \times 10^6$)	6.35×10^7 ($\pm 0.92 \times 10^7$)	7.15×10^7 ($\pm 1.62 \times 10^7$)	3.85×10^7 ($\pm 1.20 \times 10^7$)	8.15×10^6 ($\pm 0.35 \times 10^6$)
9	6.55×10^6 ($\pm 0.78 \times 10^6$)	5.38×10^7 ($\pm 0.88 \times 10^7$)	6.14×10^7 ($\pm 0.22 \times 10^7$)	5.65×10^7 ($\pm 0.64 \times 10^7$)	9.1×10^6 ($\pm 0.42 \times 10^6$)

[a] Values in parentheses show the standard deviation.

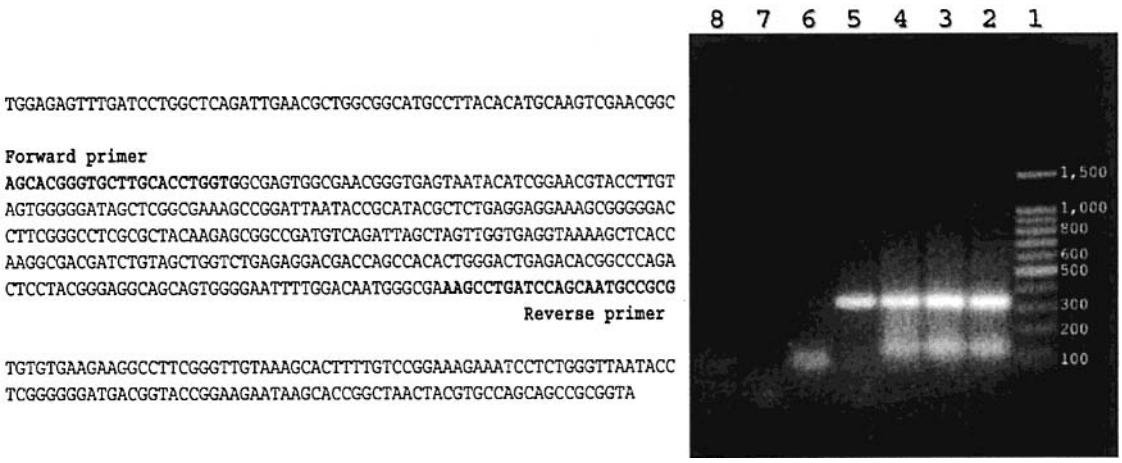

Figure 5. PCR detection of *Pandoraea* sp. using two 23-mer oligonucloetide primers targeting a 330 bp region of the 16S rRNA gene sequence. The adjacent nucleotide sequence represents the first 524 bp of the 16S rRNA gene sequence. Agarose gel shows successful amplification of the targeted 330 bp region of the 16S rRNA gene sequence: Promega 100 bp DNA ladder (lane 1); DNA templates of bacterial colonies isolated from duplicate 6-week-old liquid cultures (lanes 2 and 3); DNA template of bacterial colonies isolated from a 9-week-old soil slurry culture (lanes 4); soil slurry culture directly used as DNA template (lane 5); DNA templates of a control bacterium isolated from the soil (lane 6); sterile soil slurry (15%) control template (lane 7); PCR reaction mixture without a DNA template (lane 8).

concentration of 100 mg L^{-1}. Kennedy et al. (*15*) reported that lindane was readily mineralized by *Phanerochaete chrysosporium* in soil and liquid matrices. Approximately 23% of lindane was degraded in 30 days in liquid cultures and in 60 days in soil−corncob cultures inoculated with *P. chrysosporium.* Bachmann et al. (*16*) also observed α-HCH mineralization in soil under aerobic conditions. This xenobiotic compound was mineralized at the rate of 23 mg kg^{-1} of soil per day by the soil native microbial population.

Increasing concentrations of HCH isomers beyond 100 mg L^{-1} showed initial lower percent biodegradation in soil slurry cultures. During the first 3 weeks, about 22% of γ-HCH and 25% of α-HCH isomers were degraded with an initial concentration of 100 mg L^{-1} of each isomer. Substantial biodegradation was, however, achieved after 9 weeks of incubation. The rates of biodegradation of the HCH isomers were similar at 150 and 100 mg L^{-1}, after 9 weeks. Appreciable biodegradation was also noted in the flasks spiked with 200 mg L^{-1} after 9 weeks of incubation. These results reflect the phenomenon of acclimation (*32*, *33*). Acclimation periods extend with increasing concentrations of substrate (*32*).

No biodegradation was observed in the soil slurry culture spiked with 10 mg L^{-1}. This concentration may be far below the biodegradation threshold for the γ- and α-HCH isomers in soil slurry. Generally the rate of aerobic biodegradation of an organic compound is frequently proportional to the effective concentration of the compound, which decreases as biodegradation progresses with a parallel decrease in the rate of biodegradation (*34*). The structure of a xenobiotic and such factors as molecular size, charge and functional group, solubility in water, concentration, toxicity, and adsorption/sorption characteristics in soil are properties that determine availability for biodegradation (*35*, *36*). Thus, the differences in extent of biodegradation in water and soil slurry are attributable to adsorption.

With growth of *Pandoraea* sp. and HCH degradation in liquid culture, acidity of the culture medium increased, whereas the pH of the uninoculated control was stable at ~7. This indicates that the production of acidic metabolic intermediates had overwhelmed the buffering capacity of the medium. Contrarily, the pH of the soil slurry culture was relatively stable compared to that of the control. This is possibly due to the high buffering capacity of the soil constituents.

Viable bacterial count of the soil slurry culture indicated growth and survival of *Pandoraea* sp. However, biodegradation of the HCH isomers was low in the first 3 weeks. This is not surprising because the soil contained readily available organic nutrients that the bacterium may prefer for growth. Survival and growth of the *Pandoraea* sp. in soil were further confirmed by PCR using oligonucleotide primers targeting a specific region of its 16S rRNA gene. This provides a rapid and specific molecular tool for tracking *Pandoraea* sp. in the soil environment. Interestingly, boiling the soil slurry for 15 min was sufficient to extract enough DNA template. The PCR technique is currently a very useful technique for studying microbial community analysis and fingerprinting (*37*, *38*).

Transformations of γ-HCH isomer to very low levels of α-, β-, and δ-isomers by the *Pandoraea* sp. were observed in some cases. Others have reported the isomerization of lindane (γ-HCH) to other HCH isomers, α-HCH in particular (*5*). Field applications of lindane to soil produced only trace amounts of other HCH isomers (*39*). Similarly, Buser and Muller (*18*) found that only a small percentage of γ-HCH was slowly converted into α- and δ-isomers in studies using anaerobic sewage sludge. This interconversion accounts for the higher than expected ratios

of α/γ-isomers found in the environment (*5*). The γ-isomer can be transformed into the α-isomer by sunlight (*40*) or UV light (*41*), a process generally known as photoisomerization. Also, the γ-isomer can be transformed into the α-isomer by microbial activity (bioisomerization) in soil and sediments (*39*, *42*).

In summary, we have described the simultaneous biodegradation of γ- and α-HCH isomers by a *Pandoraea* sp. in liquid and soil slurry cultures. A molecular procedure for tracking this organism in the environment is presented. Results of this study have applications in the removal of HCH from contaminated wastewater and in the treatment of soils in slurry phase bioreactors. Further studies will be undertaken to understand the influence of environmental parameters on γ- and α-HCH biodegradation and other bioremediation techniques using the *Pandoraea* sp.

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