Biodegradability of HCH in agricultural soils from Guadeloupe (French West Indies): identification of the lin genes involved in the HCH degradation pathway

L. Laquitaine1 · A. Durimel1 · L. F. de Alencastro2 · C. Jean-Marius1 · O. Gros3 · S. Gaspard1

Received: 16 March 2015 / Accepted: 24 November 2015 / Published online: 19 December 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract Banana has been a main agricultural product in the French West Indies (Guadeloupe and Martinique) since the 1960s. This crop requires the intensive use of pesticides to prevent attacks by insect pests. Chlorinated pesticides, such as hexachlorocyclohexane (HCH), chlordane and dieldrin, were used until the beginning of the 1990s, resulting in a generalized diffuse contamination of the soil and water in the areas of banana production, hence the need to develop solutions for cleanup of the polluted sites. The aims of this work were (i) to assess lindane degradation in soil slurry microcosms treated with lindane at 10 mg/L and (ii) to detect the catabolic genes involved in the HCH degradation pathway. The soil slurry microcosm system showed a 40 % lindane degradation efficiency at the end of a 30-day experiment. Lower lindane removal was also detected in the abiotic controls, probably caused by pesticide adsorption to soil particles. Indeed, the lindane concentration decreased from 6000 to 1330 ng/mL and from 800 to 340 ng/mL for the biotic and abiotic soils, respectively. Nevertheless, some of the genes involved in the HCH degradation pathway were amplified by polymerase chain reaction (PCR) from crude deoxyribonucleic acid (DNA) extracted from the Guadeloupe agricultural soil, suggesting that HCH degradation is probably mediated by bacteria closely related to the family Sphingomonadaceae.

Keywords Lindane degradation · Soil slurry microcosm · lin genes · Sphingobium · Soil bacteria

Abbreviations

HCH Hexachlorocyclohexane
PCR Polymerase chain reaction
DNA Deoxyribonucleic acid or deoxyribose nucleic acid
SM medium Mineral salt medium
SM-GP medium Mineral salt medium supplemented with glucose and peptone
GC-ECD Gas chromatography with an electron capture detection
EDTA Ethylenediaminetetraacetic acid
TAE Tris–acetate–EDTA
BLAST Basic Local Alignment Search Tool
DAPI 4′,6′-Diamidino-2-phenylindole
FWI French West Indies

Introduction

The economy of Guadeloupe is based mainly on fast-growing banana crops, which have been known since the 1960s as the country’s first agricultural product and the principal product for export (Bonan and Prime 2001). However, the warm and humid environment of the tropics favours the development of fungal diseases (e.g. yellow cercosporiose) and many pests (e.g. ‘Cosmopolite sordidus’ and nematodes) (Bonvallot and Dor 2004). These pests are why organochlorine pesticides,
such as chlordecone (Kepone), aldrin, dieldrin and hexachlorocyclohexane (HCH), have been used intensively in the French West Indies (Guadeloupe and Martinique) since their commercialization in the 1950s (Bonan and Prime 2001). Gamma-hexachlorocyclohexane (also called lindane) is a highly halogenated organic pesticide that has been used worldwide and for many decades for crop protection and the prevention of vector-borne diseases (Quintero et al. 2005; Camacho-Pérez et al. 2012). A mixture of HCH isomers called ‘technical HCH’ was often spread. Indeed, technical ‘HCH’ contains the eight possible stereoisomers of HCH, of which, four predominate: α-, β-, γ- and δ-HCH. Lindane is the sole effective insecticide component of HCH, but it accounts for only 9–18 % of the technical HCH (Staar et al. 2005; Quintero et al. 2007). In a large number of countries in Europe, Africa, Asia and South America and in the USA, between 4 and 7 million tonnes of wastes of toxic, persistent and bioaccumulative lindane residues has been produced and discarded during the last six decades (Abhilash and Singh 2009). The use of lindane has been banned because it is considered as one of the priority organic pollutants under the Stockholm Convention on Persistent Organic Pollutants (Abdul Salam and Das 2012). Although most countries have prohibited these compounds, lindane was used in Guadeloupe until the 1990s for economic reasons, and some other countries still use this pesticide; consequently, many agricultural lands are still contaminated (Cabidoche et al. 2006; Saez et al. 2014).

Different organisms have been found capable of using halogenated compounds as a growth substrate. In particular, several microorganisms are able to degrade γ-HCH. Examples include fungi, cyanobacteria and aerobic (Kumari et al. 2006) (Camacho-Pérez et al. 2012). Some studies (Camacho-Pérez et al. 2012) have reported possible anaerobic lindane degradation with an average lindane removal between 47 and 98 % in 30 days of incubation (Robles-Gonzalez et al. 2008; Camacho-Pérez et al. 2010), 90 to 100 % in 20 days (Boyle et al. 1999; Badea et al. 2009; Baczynski et al. 2010) and a more efficient 100 % lindane removal in 3 days (Quintero et al. 2005 and 2006).

The aims of this study were (i) to determine the abilities of polluted Guadeloupe agricultural soils to degrade lindane and (ii) to determine whether these HCH-polluted banana-producing lands harbour bacteria containing some of the lin genes involved in the γ-HCH degradation pathway. In this study, lindane degradation experiments were conducted in soil slurries microcosms fed with 10 mg/L of a lindane–acetone solution. To assess whether the biodegradation catalytic genes for HCH metabolism are present in soil samples, polymerase chain reaction (PCR) amplification of the dehalogenase genes, followed by sequencing, was conducted using whole-genome deoxyribonucleic acid (DNA) from the soil.

Materials and methods

Materials and methods

Chemicals and soils

Lindane, or γ-HCH (98 % of purity), was provided by Sigma-Aldrich. For the experiments, a stock solution at a concentration of 5 g/L was prepared by dissolving lindane in pure acetone. The soil used in this research was an agricultural soil of the Andosol type (Cabidoche et al. 2006, 2009), 0–20-cm depth, collected from three HCH-polluted banana fields in Capesterre-Belle-Eau, in the south of Guadeloupe (French West Indies). The main physicochemical characteristics of the
agricultural mineral soil are as follows: pH 7.7, organic matter 4.83 %, organic carbon 2.81, nitrogen content 0.27 and phosphate content 4.04 for Fromager soil; pH 6.8, organic matter 4.67 %, organic carbon 2.71, nitrogen content 0.25 and phosphate content 2.54 for Grand Café soil; and pH 7.2, organic matter 2.60 %, organic carbon 1.51, nitrogen content 0.15 and phosphate content 3.88 for Blondinière soil.

**Bacterial strains**

*Sphingobium indicum* B90A was obtained from Pr. Holliger, Switzerland. The B90A strain was cultured for 48 h at 28 °C in 10 mL of Luria broth (10 g of bacto tryptone, 5 g of yeast extract and 10 g/L of sodium chloride, pH 7). The cells were then harvested during the exponential growth phase and used for DNA extraction with a DNeasy® Blood & Tissue Kit (QIAGEN).

**Lindane biodegradation in aerobic soil slurry system**

The biodegradation experiments were conducted in 150-mL flasks containing the soil slurry, which comprised 25 mL of soil and 80 mL of mineral salt medium (SM) (30 % soil/water ratio). The SM medium contained the following (per litre): \((\text{NH}_4)_2\text{HPO}_4\) (0.5 g), \(\text{Ca(NO}_3\text{)}_2\cdot 4\text{H}_2\text{O}\) (0.01 g), \(\text{MgSO}_4\cdot \text{H}_2\text{O}\) (0.2 g), \(\text{K}_2\text{HPO}_4\cdot 3\text{H}_2\text{O}\) (0.1 g) and \(\text{FeSO}_4\cdot 7\text{H}_2\text{O}\) (0.01 g). The pH of the medium (before mixing with soil) was 7. An abiotic control was carried out with sterilized soil in the same culture conditions. The soil used for the abiotic controls was sterilized by autoclaving (three times at 120 °C for 60 min) with a 24-h period of incubation between treatments. All soil slurry flasks were contaminated with a \(\gamma\)-HCH solution (for a final concentration of 10 mg/L) as the sole carbon source. The slurry was incubated under aerobic conditions and at room temperature. One millilitre of sample was taken out
at 0, 3, 7, 15, 25 and 30 days of incubation, and HCH and its metabolites were manually extracted with 10 mL of an 85:15 hexane–acetone solution. The concentration of γ-HCH was measured by gas chromatography with electron capture detection (GC/ECD).

**GC/ECD protocol**

All HCH isomer standard materials, which had a certified purity of more than 98 %, were supplied by Dr. Ehrenstorfer GmbH. Stock solutions were prepared by dissolving the pesticides in isooctane to obtain 'mother solutions', and further dilutions were also prepared in isooctane.

The gas chromatography–electron capture detector (GC-ECD) system was a Model 6890 by Hewlett-Packard, equipped with a 63Ni ECD and with a DB-5 column (60 m × 0.25 mm × 0.25 μm). The carrier gas was helium (23 cm/s). The oven temperature programme was 80 °C, with a 0.5-min hold; increased by 50 °C/min to 150 °C with a 1-min hold; and then increased by 5 °C/min to 280 °C with an 11-min hold. After 1 min, it was increased to 280 °C at 5 °C per minute. The injector temperature programme was 85 °C with a 0.5-min hold and then increased to 100 °C/min to 280 °C with a 38-min hold. The detector temperature was 300 °C with nitrogen as the makeup gas.

An autosampler with an on-column injection volume of 1 μL was used with the GC system. The GC data output and processing system was a Varian Star Chromatography Workstation, Version 5.3A. Alpha, beta, gamma and delta HCH were quantified in the samples by external standards and calibration curves.

DNA manipulation

The genomic DNA of *Sphingobium indicum* B90A was extracted using the DNeasy® Blood & Tissue Kit (QIAGEN) according to the manufacturer instructions from cells pelleted from 10-mL cultures of LB medium. The genomic DNA from the agricultural soil was extracted using a Power Soil™ DNA Kit (MO BIO Laboratories, Inc). The primer sets (Table 1) used for the polymerase chain reaction (PCR) were based on a lin gene sequence of *Sphingomonas paucimobilis* UT26 and had been determined and published previously (Thomas et al. 1996; Yamamoto et al. 2009; Cérémonie et al. 2006).

The PCR reaction contained 200 μL of dNTP, 0.25 μM of each primer, 1.25 U/50 μL reaction of GoTaq enzyme (Promega) and 0.1 ng/μL of DNA. Thermal cycling was performed under the following conditions: hot start at 95 °C (5 min), followed by 35 cycles consisting of denaturation at 95 °C for 1 min, then primer annealing at 60 °C for 1 min and elongation at 72 °C for 1 min. The final extension of the amplification was 10 min at 72 °C. Gel electrophoresis of the PCR products was performed in Tris/acetate/ethylenediaminetetraacetic acid (EDTA) buffer (TAE) in a 1.5 % agarose gel.

DNA sequencing and analysis

Sequencing was performed by GATC Biotech SARL (France). Comparison searches were performed with the basic local alignment search tool (BLAST) program provided by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Primer sequences for linA, linB and linC genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fragment PCR</strong></td>
<td><strong>Primer Name</strong></td>
</tr>
<tr>
<td>Lin A</td>
<td>lin A1</td>
</tr>
<tr>
<td></td>
<td>lin A2</td>
</tr>
<tr>
<td>Lin A</td>
<td>Lin A f</td>
</tr>
<tr>
<td></td>
<td>Lin A r</td>
</tr>
<tr>
<td>Lin B</td>
<td>Lin B f</td>
</tr>
<tr>
<td></td>
<td>Lin B r</td>
</tr>
<tr>
<td>Lin C</td>
<td>Lin C f</td>
</tr>
<tr>
<td></td>
<td>Lin C r</td>
</tr>
</tbody>
</table>

PCR polymerase chain reaction
Results and discussion

Lindane degradation in soil slurry microcosms

Figure 2 shows that, after 15 days, lindane removal reached 60 and 40 % for the active soil and the abiotic control, respectively. The slurry microcosm system showed an 80 % lindane degradation efficiency at the end of a 30-day experiment (Fig. 2). For the abiotic controls, 40 % lindane removal was obtained at the end of the 30-day experiment. Moreover, lindane removal in the abiotic control remained stable (30 to 40 %) through time (Fig. 2). This indicates that biotic removal contributed up to 40 % of the overall lindane reduction. Our results might be considered relatively low compared to the lindane removal previously observed in some experiments in a slurry bioreactor, for example, with an external source of carbon and energy. Indeed, some studies have previously described lindane removal averages between 54 and 94 % in 30 days (Abhilash and Singh 2009; Ramos et al. 2011; Robles-Gonzalez et al. 2008; Camacho-Pérez et al. 2010, 2012, 2013).

Nevertheless, the lindane removal observed in our soil slurry system was similar to the lindane removal average values of 16 to 50 % that have been reported for standard slurry bioreactors in the literature (Ramos et al. 2011; Robles-Gonzalez et al. 2008; Okeke et al. 2002; Raina et al. 2008). For example, Ramos (2011) reported a 41 % lindane removal in a 30-day incubation experiment for a lindane-acclimated inoculum in a soil slurry (soil with 8 % organic matter) system.

Perhaps, better lindane degradation efficiency for our soil slurry system would require longer incubation periods to achieve higher values (Bachmann et al. 1988; Saez et al. 2014). Indeed, Bachman (1988) described a 100 % lindane degradation in 100 days for a native microbial population mix in a soil slurry system.

Apart from the low availability of the pesticide due to adsorption to soil matter, one of the greatest difficulties encountered in the microbial treatment of toxic compounds is the substrate inhibition of microbial growth and the concomitant hindrance in the biodegradation (Halecky et al. 2014). This substrate inhibition could explain the relatively low lindane degradation ability of our slurry microcosms.

Notably, lindane removal was also detected in the abiotic control, reaching removal values of 40 %. This could be due to the physical phenomenon of adsorption of lindane to soil particles, especially due to Van der Waals forces and hydrogen bonding (Rama Krishna and Philip 2008). Indeed, these types of phenomena have already been reported in many studies (Poggi-Varaldo et al. 2002; Poggi-Varaldo and Rinderknecht-Seijas 2003; Cabidoche et al. 2006, 2009; Robles-González et al. 2006, 2008; Fuentes et al. 2013; Monsalvo et al. 2014; Saez et al. 2014). The organic carbon fraction of soil tends to decrease the bioavailability of organic compounds. The soil assayed contained 4.8 % organic matter; thus, the lindane removal observed in the abiotic controls may correspond to instances where the lindane has sorbed to soil organic matter (Becerra-Castro et al. 2013; Saez et al. 2014). Similarly, Saez et al. (2014) showed a 25 % lindane removal for an abiotic control in a soil slurry system with an organic matter content of 2.6 %.

Microorganism growth was also determined by measuring changes in the number of bacteria using DAPI staining. Bacterial growth reached a maximum after 25 days of culture and then was stable (data not shown). This bacterial growth phase between 0 and 25 days is correlated with the HCH-degrading stage observed between 0 and 30 days. Moreover, the absence of bacterial growth after 30 days could explain why the lindane concentration did not decrease after a 1-month incubation period.

Identification of the lin genes in the total DNA from tropical soils

Total DNA extracted from the Blondinière, Grand Café and Fromager soils was used for PCR amplification of the linA, linB and linC genes using three sets of specific primers. The PCR products were electrophoresed on a 1 % agarose gel to ascertain their size and quality. The fragments exhibited a size close to what we expected when compared with the positive control. A 300-bp fragment (Fig. 3) was amplified from the crude DNA extracted from the Fromager (Fr) and Grand café (Gc) soils using the PCR primers linAf/linAr (Table 1). A 600-bp and a 300-bp fragment were amplified by PCR, using the linBr/linBf and linCr/linCf primer pairs, respectively, for the Fr soil DNA only, suggesting the presence of linB and linC genes in the Fromager soil. PCR experiments performed on
the genomic DNA extracted from the Blondinière (Bl) and Gc soils did not reveal the presence of these lin genes for the primer sets used under the specified PCR conditions (data not shown).

When Sphingobium indicum B90A genomic DNA was used as a positive control, the same size amplicons were yielded as obtained for the respective linA, linB and linC primers used with soil crude DNA (Fig. 3). This suggests that a linB and/or a linC gene might not have been amplified for the Bl and Gc soils under our PCR conditions.

Nucleotide sequences obtained from the sequencing of PCR products showed 94 to 98 % identity to the respective lin genes present in the HCH-degrading Sphingomonas species, as shown by a BLAST search (Altschul et al. 1997) in the GenBank (http://www.ncbi.nlm.nih.gov/blast) database. Partial nucleotide sequence (300-bp fragment) of linC and linA genes amplified from the Fr soil genomic DNA showed, respectively, 98 % homology with the linC genes of Sphingobium indicum B90A (Dogra et al. 2004; Kumari et al. 2002) and 94 % homology with both the linA gene from Sphingobium japonicum UT26S (Nagata et al. 2010) and the linA gene of Sphingomonas sp. alpha 1,2 (Böttner et al. 2005).

These results describe the presence and involvement in Fromager soil of the lin genes in an early stage of the lindane degradation pathways (linA, linB and linC), which is in agreement with the lindane removal efficiency of our soil slurry system.

Conclusions

The results presented in this paper describe the lindane removal efficiency (40 % in 30 days) of our soil slurry microcosms; the presence of lin genes, which are involved in the aerobic lindane degradation pathway; and, consequently, the presence of bacteria able to degrade HCH in the agricultural soil of Guadeloupe. The percentage of lindane removed was lower than expected, which is probably due to the presence of relatively important amounts of organic matter (4.83 %), which is able to sorb lindane, in the tested Guadeloupe agricultural soil.

This is the first report showing that Guadeloupe agricultural soils have HCH-degrading abilities and that this capacity might involve bacteria containing genes related to the lin genes of the Sphingomonadaceae family.

Furthermore, other experiments will be conducted in different conditions of culture to enable study of the influence of various parameters, such as the temperature, pH, buffer medium and xenobiotic concentration, on γ-HCH biodegradation. Many studies mentioned that some HCH-degrading bacteria are not able to degrade all the HCH isoforms (Nagasawa et al. 1993; Phillips et al. 2005; Manickam et al. 2007). Therefore, a determination of the tested soil’s abilities to degrade α-, β- or δ-HCH would be of interest. Further enrichment and metagenomic experiments must be performed to isolate and identify the bacterial strain(s) able to degrade other HCH isomers.

Acknowledgments The financial support that was provided by the French Overseas Ministry (MOM), Regional Council of Guadeloupe and The Syndicat intercommunal d’alimentation en eau et d’Assainissement de la Guadeloupe (SIAEAG), Direction of Agriculture (DAF) and Regional Council of Guadeloupe for this work is gratefully acknowledged.

References


