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

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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), chlordecone 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,

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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-Perez 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 (Suar 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. 2002; Dogra et al. 2004; Böltner et al. 2005; Ma et al. 2005; Lal et al. 2006; Cérémonie et al. 2006; Manickam et al. 2008; Yamamoto et al. 2009; Dadhwal et al. 2009; Singh and Lal 2009; Bala et al. 2010; Elcey and Kunhi 2010) and anaerobic bacteria (Quintero et al. 2005, 2006; Bhat et al. 2006) (Camacho-Perez et al. 2012). However, only a few of these microorganisms have been phylogenetically identified (Nagata et al. 2007), and some of them belong to the genera *Sphingomonas*, *Rhodanobacter* or *Pandoraea* (Mohn et al. 2006).

Under aerobic conditions, the enzyme γ-hexachlorocyclohexane dechlorinase (LinA) from *Sphingomonas paucimobiliz* UT26 catalyses the elimination of chlorine atoms from the lindane molecule (Nagata et al. 2007). The degradation pathway of γ-HCH has been extensively analysed in *Sphingomonas paucimobiliz* UT26 (Fig. 1). Lindane is transformed to 2,5-dichlorohydroquinone through sequential reactions catalysed by enzymes coded by *LinA*, *LinB* and *LinC*, and the 2,5-dichlorohydroquinone formed is further metabolized by *LinD*, *LinE*, *LinF*, *LinGH* and *LinJ*, leading to succinyl-CoA and acetyl-CoA (succinyl-CoA and acetyl-CoA intermediates), which are metabolized in the citrate/tricarboxylic acid cycle (Phillips et al. 2005; Nagata et al. 2007).

Many studies have reported lindane removal and degradation by aerobic undefined consortia (Elcey and Kunhi 2010; Camacho-Pérez et al. 2010) under a variety of initial lindane concentrations and culture conditions. These studies (by Camacho-Perez et al. 2012) have described average lindane removal between 39 and 96 % in 30 days for lindane-acclimated inocula (Robles-Gonzalez et al. 2008; Camacho-Pérez et al. 2010), *Trametes hirsutus* (Singh and Kuhad 1999) and *Microbacterium* sp. ITCR (Manickam et al. 2006a). Other species and bacterial consortia have shown average lindane removal between 98 and 100 % in 7 to 15 days (Elcey and Kunhi 2010; Manickam et al. 2008; Manickam et al. 2006b), whereas *Arthrobacter citreus* BI-100 has shown 100 % lindane removal in 8 h (Datta et al. 2000).

Some studies (Camacho-Perez 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 slurry microcosms fed with 10 mg/L of a lindane–acetone solution. To assess whether the biodegradation catabolic 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

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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

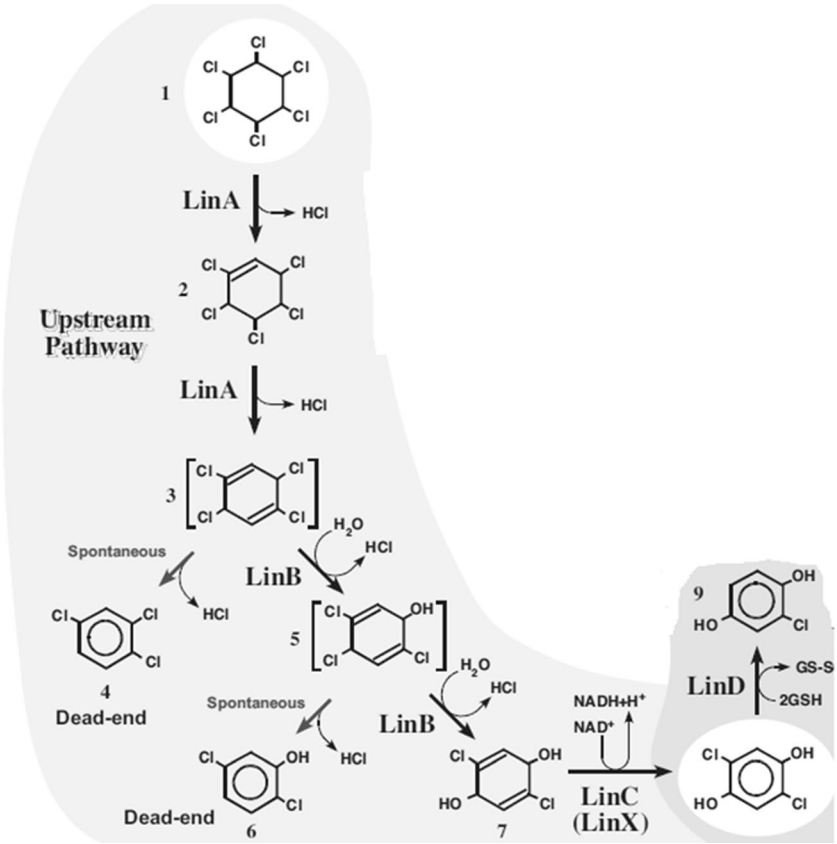

Fig. 1 Proposed aerobic degradation pathways of γ-HCH by *Sphingobium japonicum* UT26 (Nagata et al. 2007). Compounds: (*1*) γ-hexachlorocyclohexane (γ-HCH), (*3*) 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN), (*4*) 1,2,4-trichlorobenzene (1,2,4-TCB), (*5*) 2,4,5-trichloro-2,5-cyclohexadiene-1-ol (2,4,5-DNOL), (*6*) 2,5-dichlorophenol (2,5-DCP), (*7*) 2,5-dichloro-2,5-cyclohexadiene-1,4-diol (2, 5-DDOL), (*8*) 2,5-dichlorohydroquinone (2,5-DCHQ), (*9*) chlorohydroquinone (CHQ), (*10*) hydroquinone (HQ), (*11*) acylchloride, (*12*) γ-hydroxymuconic semialdehyde, (*13*) maleylacetate (MA; 2-maleylacetate, 4-oxohex-2-enedioate), (*14*) β-ketoadipate (3-oxoadipate), (*15*) 3-oxoadipyl-CoA, (*16*) succinyl-CoA, (*17*) acetyl-CoA, (*18*) 2,6-dichlorohydroquinone (2,6-DCHQ), and (*19*) 2-chloromaleylacetate (2-CMA). TCA, citrate/tricarboxylic acid cycle; *GSH*, glutathione (reduced form); *GS-SG*, glutathionne (oxidized form). *Square brackets* show unstable compounds that have yet to be detected. Both of LinE and LinEb have the degradation activities of CHQ and 2,6-DCHQ, but LinE and LinEb are mainly involved in the degradation of CHQ and 2,6-CHQ, respectively, in UT26 (Endo et al. 2006). The *light* and *dark shaded areas* indicate upstream and downstream pathways, respectively (Nagata et al. 2007)

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): $(NH_4)_2HPO_4$ (0.5 g), $CaNO_3{\cdot}4H_2O$ (0.01 g), $MgSO_4{\cdot}H_2O$ (0.2 g), $K_2HPO_4{\cdot}3H_2O$ (0.1 g) and $FeSO_4{\cdot}7H_2O$ (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 γ-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 paucimobiliz* 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 1 Primer sequences for *linA*, *linB* and *linC* genes

Fragment PCR	Primer Name	Position	Sequence	References
Lin A	*lin A1*	+69 to 87	5′-CGT AGA CAA GCG CCA AGA G-3′	Thomas et al. (1996)
	lin A2	+434 to 409	5′-GGT GAA ATA GTT CRT GCA TG-3′	
LinA	*Lin Af*	+69 to 87	5′-CGT AGA CAA GCG CCA AGA G-3′	Cérémonie et al. (2006)
	Lin Ar	+409 to 434	5′-GGT GAA ATA GTT CRT GCA TG-3′	
LinB	*Lin Bf*	+18 to +36	5′-CC ATT TGG CGA GAA GAA-3′	Thomas et al. (1996)
	Lin Br	+712 to 729	5′-GGC GTT GAT GAA GAG TTT-3′	
LinC	*Lin Cf*	+181 to 200	5′-ATC GCA CAG GAA GAG GAC GT-3′	Yamamoto et al. (2009)
	Lin Cr	+513 to 532	5′-GAA TGC CGT GCT TGC CAT AG-3′	

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.

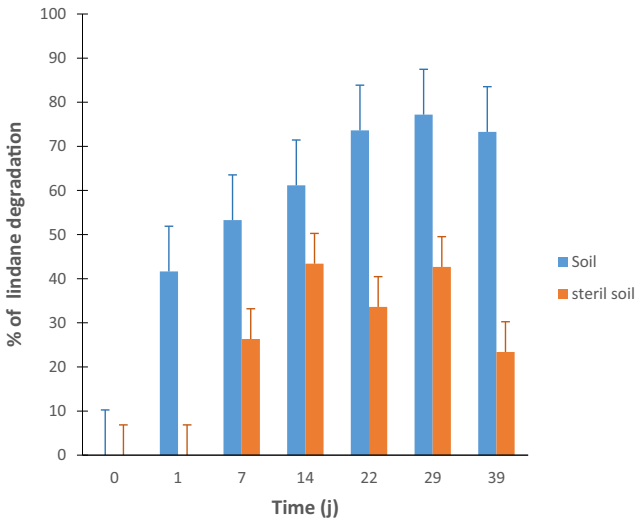

Fig. 2 Kinetics of lindane degradation in agricultural soil slurry (microcosm). The values are mean of three experiments

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

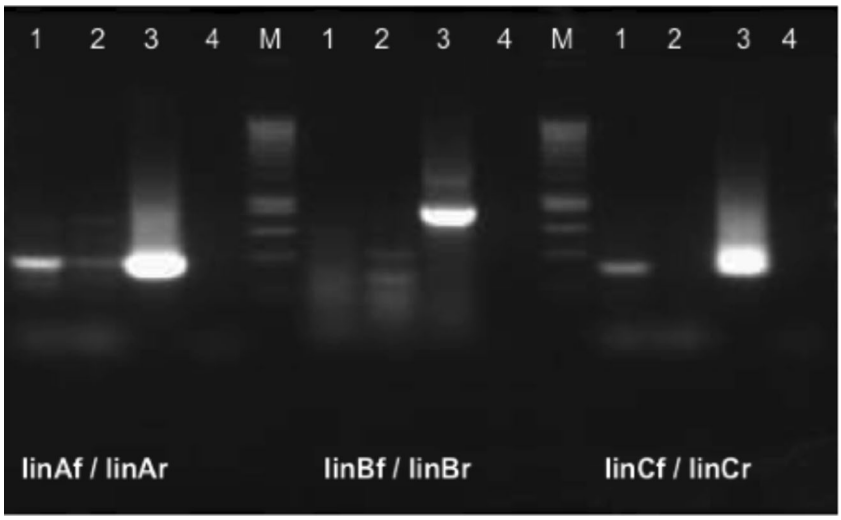

Fig. 3 Presence of *linA*, *linB* and *linC* genes in genomic DNA extracted from soils of Guadeloupe. *M* = marker; *1* = genomic DNA of Fr soil; *2* = genomic DNA of Gc soil; *3* = genomic DNA of *Sphingobium indicum* B90A; *4* = PCR-: negative control for the PCR amplification

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 (Böltner 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.

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References

Abdul Salam J, Das N (2012) Remediation of lindane from environment - an overview. Int J Adv Biol Res 2:9–15

Abhilash PC, Singh N (2009) Seasonal variation of HCH isomers in open soil and plant-rhizospheric soil system of a contaminated environment. Environ Sci Pollut Res 16:727–740

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new

generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Bachmann A, Walet P, Wijnen P, De Bruin W, Huntjens LM, Roelofsen W, Zehnder A (1988) Biodegradation of alpha and betahexachlorocyclohexane in a soil slurry under different redox conditions. Appl Environ Microbiol 54:143–149

Baczynski TP, Pleissner D, Grotenhuis T (2010) Anaerobic biodegradation of organochlorine pesticides in contaminated soil—significance of temperature and availability. Chemosphere 78(1):22–28

Badea LS, Vogt C, Weber S, FlorinteDanet A, Hermann-Richnow H (2009) Stable isotope fractionation of γ-hexachlorocyclohexane (Lindane) during reductive dechlorination by two strains of sulfate-reducing bacteria. Environ Sci Technol 43:3155–3161

Bala K, Sharma P, Lal R (2010) *Sphingobium quisquiliarum* sp. nov., a hexachlorocyclohexane (HCH)-degrading bacterium isolated from an HCH-contaminated soil. Int J Syst Evol Microbiol 60:429–433

Becerra-Castro C, Kidd PS, Rodríguez-Garrido B, Monterroso C, Santos-Ucha P, Prieto-Fernandez A (2013) Phytoremediation of hexachlorocyclohexane (HCH) - contaminated soils using *Cytisus striatus* and bacterial inoculants in soils with distinct organic matter content. Environ Pollut 178:202–210

Bhat P, Kumar MS, Mudliar SN, Chakrabarti T (2006) Biodegradation of tech-hexachlorocyclohexane in a upflow anaerobic sludge blanket (UASB) reactor. Bioresour Technol 97:824–830

Böltner D, Moreno-Morillas S, Ramos J (2005) 16S rDNA phylogeny and distribution of lin genes in novel hexachlorocyclohexane-degrading *Sphingomonas* strains. Environ Microbiol 7:1329–1338

Bonan H, Prime J-L (2001) Inspection générale des affaires sociales et Inspection générale de l'Environnement. Rapport sur la présence des pesticides dans les eaux de consommation humaine

Bonvallot N, Dor F (2004) Institut de veille sanitaire, Rapport Insecticides organochlorés aux Antilles : Identification des dangers et valeurs toxicologiques de références

Boyle AW, Haggblom MM, Young LY (1999) Dehalogenation of lindane (γ-hexaclorocyclohexane) by anaerobic bacteria from marine sediments and by sulfate-reducing bacteria. FEMS Microbiol Ecol 29: 379–387

Cabidoche YM, Lesueur Jannoyer M, Vannière H (2006) Conclusions of the Group for Prospective Studies on Pollution of organic chlorinated compounds at the Antilles, Agriculatural Aspcets. Conclusions du Groupe d'Etude et Prospective "Pollution par les organochlorés aux Antilles", Aspects agronomiques. Contributions CIRAD INRA, March 2006, Montpellier, France

Cabidoche YM, Achard R, Cattan P, Clermont-Dauphin C, Massat F, Sansoulet J (2009) Long-term pollution by chlordecone of tropical volcanic soils in the French West Indies: a simple leaching model accounts for current residue. Environ Pollut 157:1697–1705

Camacho-Pérez B, Ríos-Leal E, Esparza-García F, Barrera-Cortés J, Fava F, Poggi-Varaldo HM (2010) Bioremediation of an agricultural soil polluted with lindane in triphasic, sequential methanogenic-sulfate reducing slurry bioreactors. J Biotechnol 150:S561–S562

Camacho-Perez B, Ríos-Leal E, Rinderknecht-Seijas N, Poggi-Varaldo HM (2012) Enzymes involved in the biodegradation of hexachlorocyclohexane: a mini review. J Environ Manag 95:S306–S318

Camacho-Pérez B, Ríos-Leal E, Solorza-Feria O, Vazquez-Landaverde P, Barrera-Cortés J, Ponce-Noyola MT, Garcia-Mena J, Rinderknecht-Seijas N, Poggi-Varaldo HM (2013). Preformance of an electrobiochemical slurry reactor for the treatment of a soil contaminated with lindane. JNMES 16:217–228

Cérémonie H, Boubakri H, Mavingui P, Simonet P, Vogel TM (2006) Plasmid-encoded γ-hexachlorocyclohexane degradation genes and insertion sequences in *Sphingobium francense* (ex-*Sphingomonas paucimobilis* Sp+). FEMS Microbiol Lett 257:243–252

Dadhwal M, Jit S, Kumari H, Lal R (2009) Sphingobium chinhatense sp. nov., a hexachlorocyclohexane (HCH)-degrading bacterium isolated from an HCH dumpsite. Int J Syst Evol Microbiol 59:3140–3144

Datta J, Maiti AK, Modak DP, Chakrabartty PK, Bhattacharyya P, Ray PK (2000) Metabolism of γ-hexachlorocyclohexane by *Arthrobacter citreus* strain BI-100: identification of metabolites. J Gen Appl Microbiol 46:59–67

Dogra C, Raina V, Pal R, Suar M, Lal S, Gartemann K, Holliger C, Van Der Meer JR, Lal R (2004) Organization of lin genes and IS6100 among different strains of hexachlorocyclohexane-degrading *Sphingomonas paucimobilis*: evidence for horizontal gene transfer. J Bacteriol 186:2225–2235

Elcey CD, Kunhi AM (2010) Substantially enhanced degradation of hexachlorocyclohexane isomers by amicrobial consortium on acclimation. J Agric Food Chem 58:1046–1054

Endo R, Ohtsubo Y, Tsuda M, Nagata Y (2006) Growth inhibition by metabolites of γ-hexachlorocyclohexane in *Sphingobium japonicum* UT26. Biosci Biotechnol Biochem 70:1029–1032

Fuentes MS, Alvarez A, Saez JM, Benimeli CS, Amoroso MJ (2013) Methoxychlor bioremediation by defined consortium of environmental Streptomyces strains. Int J Environ Sci Technol 11: 1147–1156

Halecky M, Spackova R, Paca J, Stiborova M, Kozliak E (2014) Biodegradation of nitroglycerin and ethylene glycol dinitrate by free and immobilized mixed cultures. Water Res 48:529–537

Kumari R, Subudhi S, Suar M, Dhingra G, Raina V, Dogra C, Lal S, Van der Meer JR, Holliger C, Lal R (2002) Cloning and characterization of lin genes responsible for the degradation of hexachlorocyclohexane isomers by *Sphingomonas paucimobilis* strain B90. Appl Environ Microbiol 68:6021–6028

Lal R, Dogra C, Malhotra S, Sharma P, Pal R (2006) Diversity, distribution and divergence of lin genes in hexachlorocyclohexane-degrading *sphingomonads*. Trends Biotechnol 24:121–130

Ma AZ, Wu J, Zhang GS, Wang T, Li SP (2005) Isolation and characterization of a HCH degradation *Sphingomanas* sp. stain BHC-A. Wei Sheng Wu Xue Bao 45:728–732

Manickam N, Mau M, Schlömann M (2006a) Characterization of the novel HCH degrading strain, *Microbacterium* sp. ITRC1. Appl Microbiol Biotechnol 69:580–588

Manickam N, Misra R, Mayilraj S (2006b) A novel pathway for the biodegradation of γ-hexachlorocyclohexane by a *Xanthomonas* sp. strain ICH12. J Appl Microbiol 102:1468–1478

Manickam N, Misra R, Mayilraj S (2007) A novel pathway for the biodegradation of γ-hexachlorocyclohexane by a *Xanthomonas* sp. strain ICH12. J Appl Microbiol 102:1468–1478

Manickam N, Reddy MK, Saini HS, Shanker R (2008) Isolation of hexachlorocyclohexane-degrading *Sphingomonas* sp. by dehalogenase assay and characterization of genes involved in γ-HCH degradation. J Appl Microbiol 104:952–960

Mohn WW, Mertens B, Neufeld JD, Verstraete W, De Lorenzo V (2006) Distribution and phylogeny of hexachlorocyclohexane-degrading bacteria in soils from Spain. Environ Microbiol 8:60–68

Monsalvo VM, McDonald JA, Khan SJ, Le-Clech P (2014) Removal of trace organics by anaerobic membrane bioreactors. Water Res 49: 103–112

Nagasawa S, Kikuchi R, Nagata Y, Takagi M, Matsuo M (1993) Aerobic mineralization of γ-HCH by *Pseudomonas paucimobilis* UT26. Chemosphere 26:1719–1728

Nagata Y, Endo R, Ito M, Ohtsubo Y, Tsuda M (2007) Aerobic degradation of lindane (γ-hexachlorocyclohexane) in bacteria and its biochemical and molecular basis. Appl Microbiol Biotechnol 76:741–752

Nagata Y, Ohtsubo Y, Endo R, Ichikawa N, Ankai A et al (2010) Complete genome sequence of the representative γ-hexachlorocyclohexane-degrading bacterium *Sphingobium japonicum* UT26. J Bacteriol 192:5852–5853

Okeke BC, Siddique T, Arbestain MC, Frankenberger WT (2002) Biodegradation of γ-hexachlorocyclohexane (Lindane) and α-hexachlorocyclohexane in water and a soil slurry by a *pandoraea* species. J Agric Food Chem 50:2548–2555

Phillips TM, Seech AG, Lee H, Trevors JT (2005) Biodegradation of hexachlorocyclohexane (HCH) by microorganisms. Biodegradation 16:363–392

Poggi-Varaldo HM, Rinderknecht-Seijas N, Caffarel-Mendez S (2002) Irreversibility of the adsorptiondesorption behaviour of pollutants in soils and sediments: Quantitative evaluation by means of a differential hysteresis coefficient. Interciencia 27:271–280

Poggi-Varaldo HM, Rinderknecht-Seijas N (2003) A differential availability enhancement factor for the evaluation of pollutant availability in soil treatments. Acta Biotechnol 23:271–280

Quintero JC, Moreira MT, Feijoo G, Lema JM (2005) Anaerobic degradation of hexachlorocyclohexane isomers in liquid and soil slurry systems. Chemosphere 61:528–536

Quintero JC, Moreira MT, Lema JM, Feijoo G (2006) An anaerobic bioreactor allows the efficient degradation of HCH isomers in soil slurry. Chemosphere 63:1005–1013

Quintero JC, Lú-Chau TA, Moreira MT, Feijoo G, Lema JM (2007) Bioremediation of HCH present in soil by the white-rot fungus *Bjerkandera adusta* in a slurry batch bioreactor. Int Biodeter Biodegr 60:319–326

Raina V, Suar M, Singh A et al (2008) Enhanced biodegradation of hexachlorocyclohexane (HCH) in contaminated soils *via* inoculation with *Sphingobium indicum* B90A. Biodegradation 19:27–40

Rama Krishna K, Philip L (2008) Adsorption and desorption characteristics of lindane, carbofuran and methyl parathion on various Indian soils. J Hazard Mater 160:559–567

Ramos J, Gavilán A, Romero T, Ize I (2011) Mexican experience in local, regional and global actions for lindane elimination. Environ Sci Policy 14:503–509

Robles-Gonzalez IV, Fava F, Poggi-Varaldo HM (2008) A review on slurry bioreactors for bioremediation of soils and sediments. Microb Cell Factories 7:1–16

Robles-González IV, Ríos-Leal E, Galindez-Mayer J, Caffarel-Mendez S, Barrera-Cortes J, Esparza-García F, Poggi-Varaldo HM (2006) Adsorptive-desorptive behaviour of lindane in an agricultural soil. Interciencia 31:305–308

Saez JM, Alvarez A, Benimeli CS, Amoroso MJ (2014) Enhanced lindane removal from soil slurry by immobilized Streptomyces consortium. Int Biodeterior Biodegrad 93:63–69

Singh BK, Kuhad RC (1999) Biodegradation of lindane (γ-hexachlorocyclohexane) by the white-rot fungus *Trametes hirsutus*. Lett Appl Microbiol 28:238–241

Singh A, Lal R (2009) *Sphingobium ummariense* sp. nov., a hexachlorocyclohexane (HCH)-degrading bacterium, isolated from HCH-contaminated soil. Int J Syst Evol Microbiol 59:162–166

Suar M, Hauser A, Poiger T (2005) Enantioselective transformation of α-hexachlorocyclohexane by the dehydrochlorinases LinA1 and LinA2 from the soil bacterium *Sphingomonas paucimobilis* B90A. Appl Environ Microbiol 71:8514–8518

Thomas J, Berger F, Jacquier M, Bernillon D, Baud-Grasset F, Truffaut N, Normand P, Vogel TM, Simonet P (1996) Isolation and characterization of a novel γ-hexachlorocyclohexane-degrading bacterium. J Bacteriol 178:6049–6055

Yamamoto S, Otsuka S, Murakami Y, Nishiyama M, Senoo K (2009) Genetic diversity of gamma-hexachlorocyclohexane-degrading *sphingomonads* isolated from a single experimental field. Lett Appl Microbiol 49:472–477