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Characterization of chlordecone-tolerant fungal populations isolated from long-term polluted tropical volcanic soil in the French West Indies

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Abstract The insecticide chlordecone is a contaminant found in most of the banana plantations in the French West Indies. This study aims to search for fungal populations able to grow on it. An Andosol heavily contaminated with chlordecone, perfused for 1 year in a soil–charcoal system, was used to conduct enrichment cultures. A total of 103 fungal strains able to grow on chlordecone-mineral salt medium were isolated, purified, and deposited in the MIAE collection (Microorganismes d'Intérêt Agro-Environnemental, UMR Agroécologie, Institut National de la Recherche Agronomique, Dijon, France). Internal transcribed spacer sequencing revealed that all isolated strains belonged to the Ascomycota phylum and gathered in 11 genera: *Metacordyceps*, *Cordyceps*, *Pochonia*, *Acremonium*, *Fusarium*, *Paecilomyces*, *Ophiocordyceps*, *Purpureocillium*, *Bionectria*, *Penicillium*, and *Aspergillus*. Among predominant species, only one isolate, *Fusarium oxysporum* MIAE01197, was able to grow in a liquid culture medium that contained chlordecone as sole carbon source. Chlordecone increased *F. oxysporum* MIAE01197 growth rate, attesting for its tolerance to this organochlorine. Moreover, *F. oxysporum* MIAE01197 exhibited a higher EC_{50} value than the reference strain *F. oxysporum* MIAE00047. This further suggests its adaptation to chlordecone tolerance up to 29.2 mg l^{-1}. Gas chromatography–mass spectrometry (GC-MS) analysis revealed that 40 % of chlordecone was dissipated in *F. oxysporum* MIAE01197 suspension culture. No chlordecone metabolite was detected by GC-MS. However, weak amount of $^{14}CO_2$ evolved from $^{14}C_{10}$-chlordecone and $^{14}C_{10}$-metabolites were observed. Sorption of $^{14}C_{10}$-chlordecone onto fungal biomass followed a linear relationship (r^2=0.99) suggesting that it may also account for chlordecone dissipation in *F. oxysporum* MIAE01197 culture.

Keywords Chlordecone · Andosol · Ecotoxicology · Biosorption · Biodegradation

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Introduction

The cyclodiene insecticide chlordecone (CLD) (1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalen-2-one) was used in the French West Indies (FWI) to control banana weevil, *Cosmopolites sordidus*, until recently. It was applied in FWI banana plantations for 18 years over two distinct periods. During the first one, from 1972 to 1978, the trademark product Kepone was applied and during the second one, from 1982 to 1993, the trademark product Curlone was applied. Over that 18-year period, approximately 300 tons of chlordecone was applied to banana plantations in the French West Indies. Pollution monitoring in the context of the National Action Plan for chlordecone revealed the presence of chlordecone in soils, rivers, springs, and drinking water as well as in food crop products such as root vegetables (Le Déault and Procaccia 2009). Cabidoche et al. (2009) showed that in soils used for banana production (i.e., representing 20,000 ha in the FWI) chlordecone concentrations ranged between 0.2 and 37.4 mg kg^{-1}. In addition, due to its environmental persistence, sorption, and biomagnification potential, this organochlorine

insecticide was classified as a persistent organic pollutant in May 2009 and added to Annex A of the Stockholm Convention. Recently, Multigner et al. (2010) showed a higher prevalence of prostate cancers in FWI populations exposed to chlordecone. In addition, a recent study reports that cognitive, visual, and motor development of young children exposed to chlordecone is impacted (Dallaire et al. 2012). As expected because of its high potential for biomagnification, chlordecone also contaminated aquatic biota, including fish and giant shrimp called *ouassou* (Coat et al. 2006; Coat et al. 2011), and crops, notably locally produced vegetables (Cabidoche and Lesueur-Jannoyer 2012; Clostre and Lesueur-Jannoyer 2012). Consequently, French authorities regulated fishing and marketing of marine wildlife and forbade the consumption of fish and shrimps produced from rivers. They also forbade the consumption of certain species of game birds potentially contaminated with chlordecone in Guadeloupe and defined recommendations for soil usage to avoid vegetable contamination.

Taking into account the recalcitrance of chlordecone to biodegradation, Cabidoche et al. (2009) suggested that leaching is the major process responsible for chlordecone dissipation from contaminated soils. By applying a simple leaching model based on first-order desorption kinetics, taking into account soil organic carbon content (SOC) and the SOC/water partitioning coefficient (Koc) as input parameters, Cabidoche et al. (2009) suggest that chlordecone contamination will last for several decades in Nitisol, centuries in Ferralsol, and half a millennium in Andosol soils of the French West Indies. Faced with this critical situation, we urgently need to search for possible solutions to treat this widespread pollution. Keeping in mind the large contamination area that rules out any ex situ treatments, any practical strategy for decontaminating chlordecone-polluted soils in the French West Indies will have to employ in situ methods. Among possible cleaning-up strategies, bioremediation strategies based on the use of the purifying capabilities of the soil microflora could be considered. Indeed, recently, Dolfing et al. (2012) studied the Gibbs free energy data of chlordecone and concluded that there are no thermodynamic reasons why chlordecone-respiring or chlordecone-fermenting organisms should not exist. However, despite this thermodynamic consideration, there are only a few numbers of studies about chlordecone biodegradation. Under anaerobic conditions *Methanosarcina thermophila*, a methanogenic bacterium that produces methane, transformed up to 86 % of $^{14}C_{10}$-chlordecone within 10 days, leading to the accumulation of polar and nonpolar ^{14}C-labeled metabolites (Jablonski et al. 1996). Although the mechanisms and metabolites of chlordecone transformation have not been further identified, *M. thermophila* was suggested to perform a reductive dechlorination of chlordecone. *Pseudomonas* sp. KO3, a bacterial strain purified from a mixed culture enriched under aerobic conditions from sewage sludge-collected lagoon water, was shown to convert Kepone to monohydro-kepone and to a lesser extent to dihydro-kepone. These findings were criticized by Cabidoche et al. (2009), who considered that the authors did not describe the initial contents in mono-hydro- and di-hydro-chlordecone known to contaminate Kepone due to incomplete chlorination during the production process. This lack of information weakens the findings of that study since both metabolites observed here may derive from an incomplete chlorination of the molecule during the manufacturing process, not from a biodegradation process. A former study of George et al. (1986) reported chlordecone degradation by three *Pseudomonas* spp. strains, with apparent degradation ranging between 15 and 25 % with an error of 10 % on chlordecone HPLC concentration measurements after 2 weeks of incubation. But details were missing in this study casting doubt on the conclusion that chlordecone is degradable under aerobic conditions. More recently, *Pseudonocardia* sp. strain KSF27, a soil bacterium isolated from Japanese agricultural soils frequently treated with endosulfan, was tested using a soil–charcoal perfusion method with aldrin *trans*-diol as a structural analog of dieldrin. *Pseudonocardia* sp. strain KSF27 degraded not only dieldrin, but also other persistent organochlorine pesticides, such as endosulfan sulfate, heptachlor, and chlordecone (Sakakibara et al. 2011). Interestingly, although organochlorine pesticides are mainly degraded by bacterial populations in soil habitats (Porto et al. 2011), several studies evidence their biodegradation by fungal populations. Indeed, Ortega et al. (2011) showed that *Penicillium miczynskii*, *Aspergillus sydowii*, and *Trichoderma* sp. could grow on the insecticide dichlorodiphenyldichloroethane (DDD). *Trichoderma* sp. was found able to degrade DDD, and its biodegradation ability was increased with the addition of H_2O_2. *Aspergillus terreus*, *Aspergillus niger*, and *Cladosporium oxysporum* can biodegrade endosulfan (Bhalerao and Puranik 2007; Mukherjee and Mittal 2005). Fungal enzymatic activities can also degrade several organophosphates. Indeed, *Fusarium oxysporum* cutinase can be involved in the degradation of malathion (Kim et al. 2005) and *A. sydowii*, *A. flavus*, and *F. oxysporum* phosphatases can hydrolyze several pesticides (Hasan 1999). Li et al. (2006) evidenced that *Purpureocillium lilacinum* (formerly *Peacilomyces lilacinus*) could degrade phoxim, an organophosphate insecticide. Although fungal isolates are known to harbor an impressive enzymatic toolbox responsible for pesticide degradation, their involvement in chlordecone degradation is not yet described to our knowledge.

In this context, the purpose of our study was to search for the presence of fungal isolates able to grow on mineral salt medium added with chlordecone as the sole carbon source in heavily contaminated French West Indies soils. To do so, Andosol soil samples contaminated with up to 35 mg kg^{-1} of chlordecone were continuously perfused with a chlordecone solution for 14 months using a soil–charcoal perfusion

system (Takagi and Yoshioka 2000). Enrichment cultures were then initiated using a mineral salt medium added with chlordecone as the sole carbon source. During the time course of the enrichment, aliquots of the enrichment culture were plated onto potato–dextrose–chlordecone solid medium to isolate chlordecone-tolerant fungal species. Fungal isolates were then classified into operational taxonomic units (OTUs) according to their morphological characteristics and further identified using a DNA-based approach. The isolates were then screened for their tolerance to chlordecone and their ability to grow on it. The degrading ability of the fungal isolate able to grow on chlordecone as the sole carbon source was then studied using a radiorespirometry approach and gas chromatography–mass spectrometry (GC-MS) analysis.

Materials and methods

Chemicals

[^{12}C U]-Chlordecone Pestanal® (1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd]pentalen-2-one) was purchased from Sigma-Aldrich (Schnelldorf, Germany) (chemical purity 99.7 %). [^{14}C U]-Chlordecone was purchased from Moravek Biochemicals (Brea, CA, USA) at a specific activity of 1,443 MBq mmol^{-1} (radiochemical purity 99.9 %). The solubility of chlordecone is 2.7 mg l^{-1} at 25 °C (Kilzer et al. 1979).

Media

The mineral salt (MS) medium used in the present study contained 1.6 g of K_2HPO_4, 0.4 g of KH_2PO_4, 0.2 g of $MgSO_4$ $7H_2O$, 0.1 g of NaCl, 0.02 g of $CaCl_2$, and 1 ml of trace element solution per liter. The trace element solution contained $ZnSO_4{\cdot}7H_2O$ (0.2 mg), $MnSO_4{\cdot}H_2O$ (1.8 mg), H_3BO_3 (2 mg), $CuSO_4$ (0.1 mg), and Na_2MoO_4 (0.25 mg) in 1 l. To prepare the agar plates, 15 g of agar (Biokar Diagnostics) per liter was added to the medium. The medium was autoclaved and then supplemented with chlordecone ($C_{10}Cl_{10}O$) as a carbon source using a Bransonic® ultrasonic water bath 221, 10 ml of filter-sterilized $(NH_4)_2SO_4$ solution (100 g l^{-1}) as the sole nitrogen source, 1 ml of filter-sterilized $FeSO_4{\cdot}6H_2O$ solution (5 g l^{-1}), and 1 ml of filter-sterilized vitamin solution per liter. The vitamin solution contained 40 mg of biotin and 100 mg of thiamin–HCl per liter. For tolerance tests, the MS medium was supplemented with 20 ml of filter-sterilized glucose solution (250 g l^{-1}) after autoclaving, as the main carbon source.

The phosphate buffer contained K_2HPO_4 (1 g), KH_2PO_4 (1 g), $MgSO_4$, $7H_2O$ (40 mg), in 1 l adjusted to pH 6.6. The medium was autoclaved and then supplemented with 1 ml of filter-sterilized $FeSO_4{\cdot}6H_2O$ solution (5 g l^{-1}).

The potato dextrose agar (PDA) medium used in the present study contained 39 g of PDA (Fluka Analytical, Sigma-Aldrich) and 5 g of agar per liter. The medium was autoclaved and then supplemented with 30 mg of chlordecone per liter using an ultrasonic water bath. Throughout the cultivation period, all the agar plates were maintained in plastic bags in order to prevent desiccation.

F. oxysporum strains and storage conditions

The strains used in this study were *F. oxysporum* MIAE 00047 (Collection de Microorganismes d'Intérêt Agro-Environnemental, UMR AgroEcologie, Institut National de la Recherche Agronomique, Dijon, France) as the reference strain (Alabouvette 1986) and fungal isolates MIAE01197 to 01208 and MIAE01219 to 01309 isolated from a French West Indies Andosol contaminated with chlordecone (this study). To preserve *F. oxysporum* strains, conidia were collected from the sporulation culture 7 days after inoculation by scraping and using 1 ml of chlordecone–potato–dextrose–broth medium. This suspension was mixed with an equal volume of 50 % (*v*/*v*) sterilized glycerol, distributed in 1 ml aliquots, and stored at −80 °C.

Soil sampling

The soil was sampled in June 2010 from a banana plantation located in Guadeloupe (French West Indies, 61°37′15″W; 16°03′57″N) by the team of Yves-Marie Cabidoche (INRA, Guadeloupe, France). This plantation was regularly treated with chlordecone at 6 kg ha^{-1} year^{-1} from 1982 to 1993. Soil samples were collected in accordance with ISO 10381-6 recommendations (Soil quality–Sampling–Part 6: Guidance on the collection, handling and storage of soil for the assessment of aerobic microbial processes in the laboratory). A composite sample of the surface horizon (0–15 cm) made of at least five random samplings (approximately 5 kg) was constituted. The soil sample was then shipped to INRA Dijon at 4 °C. It was then sieved to 2 mm and stored at 4 °C until use.

Soil physicochemical properties

The soil physicochemical properties were determined by the Laboratory of Soil Analysis (INRA, Arras, France) following standardized procedures. Granulometry analyses showed that it was composed of 128 g kg^{-1} of clay (<2 μm), 99 g kg^{-1} of fine silt (2–20 μm), 60 g kg^{-1} of coarse silt (20–50 μm), 37 g kg^{-1} of fine sand (50–200 μm), and 676 g kg^{-1} of coarse sand (0.2–2 mm). Physicochemical analyses yielded 79.8 g kg^{-1} of organic carbon, 6.11 g kg^{-1} of total nitrogen, a C/N ratio of 13, 138 g kg^{-1} of organic matter, a pH value of 5.21 measured in water, and a pH value

of 4.53 measured in KCl. It was classified as an Andosol (FAO-WRB, 1998) and was rich in allophane, an amorphic clay resulting from the transformation of andesitic rock (volcanic origin). The structural properties and the spatial arrangement of allophane aggregates constitute a trap for the chlordecone molecule, which is thus mechanically retained (Fernandes et al. 2010). This soil was shown to be contaminated with 35.4 mg kg^{-1} of chlordecone and with 0.65 mg kg^{-1} of β-monohydrochlordecone (Martin-Laurent et al. 2013).

Enrichment culture

The enrichment culture was performed using a soil–charcoal perfusion method as initially described by Takagi et al. (2000) and modified later by Takagi et al. (2009). Briefly, 50 g of soil (dry weight equivalent) was mixed with 2.5 g of autoclaved activated charcoal A100 (2 g, grain size 5–10 mm, BET specific surface area of 100 m^2 g^{-1}, pH 7.8; Toyo Denka Kogyo, Kochi, Japan). The soil–charcoal mixture was placed in the perfusion system and continuously perfused with 50 ml of mineral salt solution containing 2 mg l^{-1} of chlordecone. The soil–charcoal mixture was perfused for 14 months in the dark at 28 °C. Over this period, the chlordecone mineral salt solution was replaced once a month.

Soil and charcoal were separated manually. They were then suspended in mineral salt buffer (1/100, *w*/*v*) using a Waring Blendor®. Microbial cultures were then initiated starting from these suspensions by inoculating fresh chlordecone mineral salt medium (1/100, *v*/*v*). Enrichment cultures were conducted over a 4-month period at 28 °C with shaking at 150 rpm. The cultures were renewed once a month by inoculating fresh chlordecone mineral salt medium (1/100, *v*/*v*). For each culture, an aliquot was stored at −20 °C in a glass flask for further chemical analysis.

Fungi were selected on chlordecone–MS agar plates.

Isolation of fungal strains

During the time course of the enrichment cultures, microbes were plated onto MS medium added with chlordecone and incubated at 28 °C. Fungal colonies were retrieved and subcultivated on chlordecone–potato dextrose agar (chlordecone–PDA) medium. Fungal isolates were purified by repeated cultures on chlordecone–PDA medium. Pure fungal isolates were deposited in the "Collection de Microorganismes d'Intérêt Agro-Environnemental" under the accession numbers MIAE01197 to 01208 and MIAE01219 to 01309 (UMR AgroEcologie, Institut National de la Recherche Agronomique, Dijon, France). They were classified according to their morphotypes by considering several parameters such as mycelium characteristics, spore color, and shape. On this basis, the fungal isolates with similar morphotypes were grouped into OTUs. A rarefaction curve was obtained by applying the freeware program aRarefactWin.exe (http://www.uga.edu/~strata/AnRareReadme.html). The diversity of the fungal community isolated from the Andosol was assessed using (a) the Shannon–Wiener index calculated as $H' = -\sum pi \ln pi$, where $pi = ni/N$ and ni is the number of isolates in each morphotype and N the total number of isolates, and (b) the Simpson Diversity index D calculated as $D = \sum (p^2_i)$.

DNA extraction

DNA was extracted from 500 µl of fungal suspension using the DNeasy Plant Mini Kit according to the manufacturer's instructions (Qiagen, France). DNA was extracted using a rapid minipreparation procedure (Edel et al. 2001) when fungal isolates were recalcitrant to DNA extraction. DNA was purified using the Geneclean® Turbo Kit according to the manufacturer's instructions (MPBio). DNA quality was checked by electrophoresis and stored at −20 °C until use.

ITS amplification and sequencing

The internal transcribed spacer (ITS) located between the 18S rRNA and 28S rRNA of the fungal ribosomal gene was amplified by PCR using pure fungal DNA extracts in a final volume of 25 µl containing 1.5 U of Taq DNA polymerase (Q-biogene, France), 2.5 µl of Taq Polymerase buffer 10× containing 1.5 mM $MgCl_2$ (Q-biogene, France), 1.6 µM dNTPs, 1 µl of 10 µM ITS1F primer (5′-CTTGGT CATTTAGAGGAAGTAA-3′ (Gardes and Bruns 1993), and 1 µl of 10 µM ITS4 primer (5′-TCCTCC GCTTATTGATATGC-3′ (White 1990). PCR amplification was performed with a MasterCycler (Eppendorf, Germany) under the following conditions: 94 °C for 3 min and then 35 cycles each composed of 94 °C for 1 min, 50 °C for 1 min, and an elongation step at 72 °C for 1 min. A final elongation at 72 °C for 30 min was performed. ITS amplicon quality was verified by electrophoresis. Each amplicon was then diluted down to 50 to 100 ng $µl^{-1}$. ITS amplicons were sequenced by Beckman Coulter Genomics (Takeley, UK) using Sanger technology. ITS sequences will be deposited in GenBank database from KC786979 to KC787040 accession numbers. They were aligned with reference sequences retrieved from GenBank using ClustalX software. A neighbor-joining tree was computed using NJPlot software package (http://pbil.univlyon1.fr/software/njplot.html).

Chlordecone dissipation ability

One-month-old *F. oxysporum* MIAE01197 cultures grown on chlordecone–MS medium (1.5 mg l^{-1}) were collected. Chlordecone–MS media without fungal inoculums were also analyzed as controls. Three replicates of each treatment

(assay and control) were prepared. *F. oxysporum* MIAE01197 dissipation ability was assessed by GC-MS by modifying the procedure described by Martin-Laurent et al. (2013). Briefly, each culture aliquot was concentrated, and $^{13}C_{10}$-chlordecone as an isotopic tracer and anthracene-d_{10} as an internal standard were added. This mixture was ultrasound treated for 30 min and then filtered. The filtrate was diluted with a solvent and analyzed by GC-MS with an Agilent 7890 A gas chromatograph equipped with a DMPS column coupled to an Agilent 5975 C mass spectrometer. Ionization was performed by electron impact (70 eV). Chlordecone identification was performed by comparing the results with mass spectra in the literature (WILEY257, NIST, Aromalyse databases). $^{12}C_{10}$-chlordecone was quantified against an internal calibration with $^{13}C_{10}$-chlordecone.

Chlordecone mineralization

The capacity of fungal isolates to mineralize chlordecone was tested by radiorespirometry in liquid cultures. The fungal isolates were grown for 1 week at 25 °C with shaking at 150 rpm, in MS medium with glucose as the main carbon source, supplemented with chlordecone (1.5 mg l^{-1}). They were then collected by centrifugation at 10,000 rpm for 5 min. The pellets were washed twice in ultra-pure H_2O and resuspended to 0.1 OD_{600} unit in MS medium containing 1 mg l^{-1} of [^{12}C U]-chlordecone and 33.3 Bq ml^{-1} of [^{14}C U]-chlordecone, with and without glucose. Fungal suspensions were incubated for 3 weeks at 20 °C in a sterile glass jar and shaken (150 rpm). Each jar contained a scintillation vial filled with 5 ml of 0.2 M NaOH to trap CO_2. $^{14}CO_2$ production was monitored all along the incubation period by measuring radioactivity in the NaOH solution by liquid scintillation counting (Wallac 1409), using 10 ml of Ready Safe™ cocktail (Beckman Coulter Inc., USA). Three replicates were performed. Non-inoculated medium was used as control.

At the end of the incubation period, fungal cultures were centrifuged. The collected supernatants were then extracted with dichloromethane (2:1; *v*/*v*). The organic phase was then evaporated and resuspended in 500 μl of hexane/acetone (3:1; *v*/*v*) and spotted onto glass-backed silica gel thin-layer chromatography (TLC) plates (10×20 cm) (Merck). Extracted compounds, including radioactive ones, were separated by migration in hexane/acetone (3:1, *v*/*v*) according to Jablonski et al. (1996). Radioactive compounds were detected by autoradiography (Carestream® Kodak® BioMax® MR film 20.3×25.4 cm, Sigma-Aldrich, France).

Growth test on chlordecone

The ability of *F. oxysporum* strains MIAE01197 and MIAE00047 to grow on chlordecone was tested. The strains were grown on glucose–MS medium (7 days, 25 °C, 180 rpm) to prepare spore suspensions. They were then filtered to remove the mycelium. The filtrates were centrifuged (8,000×*g*, 20 min), the pellets were resuspended in 1 ml of sterilized ultrapure water, and spores were counted using a Malassez counting chamber. The spore suspensions were diluted to obtain a final concentration of 5×10^5 spores ml^{-1}.

The turbidity analysis was performed using a Bioscreen C Microbiological Growth Analyser (Labsystems, Helsinki, Finland). One-hundred-well microplates specifically manufactured for that machine were inoculated with *F. oxysporum* MIAE01197 or MIAE00047 spore suspensions (50 μl) previously loaded with MS medium (200 μl) containing chlordecone (3 mg l^{-1}) as the main carbon source. Five replicates of each condition were prepared.

The OD_{600} was recorded every hour over a 7-day period. The experiments were conducted at 25 °C. Data were recorded using Easy Bioscreen Experiment software (EzExperiment) provided by the manufacturer and then exported to a Microsoft Excel Professional 2010 (Microsoft Corporation, Redmond, Washington, USA) sheet for further analysis.

Tolerance to chlordecone

The tolerance of the fungal isolates to chlordecone was tested by studying their growth in liquid culture. The *F. oxysporum* MIAE01197 and MIAE00047 strains grown on glucose–MS medium (7 days, 25 °C, 180 rpm) were used to prepare spore suspensions following the protocol described for growth test except that cultures were carried out on MS media (200 μl) with glucose (5 g l^{-1}) as main carbon source and with a gradient of chlordecone content (0, 0.1, 1, and 10 mg l^{-1}). Five replicates of each condition were done.

Toxicity of chlordecone for fungal strains

Chlordecone toxicity was assessed in liquid culture by monitoring mycelium biomass production by MIAE01197 and MIAE00047, starting from spore inoculum. Spore suspensions were prepared from mycelium mats grown in petri dishes with agar medium (10 mM KH_2PO_4, 4 mM $MgSO_4$, 50 mM NH_4Cl, 1 mM $CaCl_2$, 7 mM KCl, 10 g l^{-1} glucose, 5 g l^{-1} yeast extract, 10 g l^{-1} agar) adapted from Abadulla et al. (2000). Briefly, spores were collected with sterile water and counted on a Malassez counting chamber. The spore inoculum was diluted to 3×10^4 spores ml^{-1}. Dose–effect experiments were carried out by inoculating 30 μl of spore suspension (1,000 spores) into 10 ml of liquid medium supplemented with gradual quantities of chlordecone ranging from 0 to 30 mg l^{-1}. Whatever the concentration, chlordecone was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich) at 4 % final concentration (*v*/*v*). Three replicates were run for each chlordecone concentration tested,

plus two controls consisting of DMSO alone or sterile deionized water instead of DMSO. Incubation was statically run for 5 days in the dark at 25 °C. Then, cultures were filtered (30 μm pore size nylon filters) and the mycelium from each flask was collected, dried at 80 °C, and weighed to calculate biomass production as an endpoint. For each condition, EC_{50} values (effective concentration of chlordecone that decreases biomass production by 50 % compared to the control) were calculated by performing a nonlinear regression on Hill's model using REGTOX software integrating the optimal EC_{50} value (version EV7.0.5., E. Vindimian, http://eric.vindimian.9online.fr/).

Chlordecone adsorption to fungal biomass

F. oxysporum strain MIAE01197 biomass was produced on MS–glucose and spore suspension prepared as described above. Different spore suspensions were prepared in phosphate buffer to reach 0.1, 0.5, and 1 OD_{600}. All the fungal suspensions were added with 200 Bq of [^{14}C U]-chlordecone. For each fungal suspension, three repeats were done and non-inoculated phosphate buffer was used as control (n_{tot}=12). All the suspensions were incubated at 20 °C under 150 rpm agitation for 72 h. At different times (i.e., 0, 2, 24, and 72 h of incubation), 1 ml aliquots were taken from the different samples. Aliquots were centrifuged at 10,000 rpm for 5 min. The supernatant was removed and kept to quantify the amount of radioactivity remaining in it. Fungal pellet was resuspended in sterile ultrapure water, transferred in a scintillation flask, and added with 10 ml of Ready Safe™ cocktail (Beckman Coulter Inc., USA). Radioactivity was measured by liquid scintillation counting on both supernatant and fungal pellet.

Statistical analyses

Growth curves of fungal isolates were fitted with the Gompertz model modified by Zwietering et al. (1990) using the SigmaPlot 4.0 software. The equation was $y=A\cdot\exp\{-\exp[1+\mu m\cdot\exp(1)(\lambda-t)/A]\}$, where y is the OD_{600} value (arbitrary unit), t is time (hour), μm is the maximum specific growth rate (hour^{-1}), A is the maximum OD_{600} value reached (arbitrary unit), and λ is the lag time (hour). The parameters thus determined were validated by a Student's t test (P<0.005). Tolerance to chlordecone was estimated by comparing the $\mu m_{CLD}/\mu m_{control}$ ratios.

Results

Isolation and morphological characterization of fungal strains isolated from the Andosol

Aliquots of Andosol historically contaminated with 35.5 mg kg^{-1} of chlordecone were perfused for a year with a chlordecone solution (2 mg l^{-1}) in a soil–charcoal system to exert a selection pressure favorable for the development of chlordecone-tolerant microorganisms. Soil samples were then used to perform enrichment cultures. Four successive 1-month long enrichment cultures were performed. For each enrichment culture, the aliquots were plated on chlordecone–PDA solid medium to isolate fungal isolates from the Andosol. A total of 103 fungal isolates were purified. Using the morphological characteristics observed on chlordecone–PDA medium (mycelium shape: form, elevation, border, surface, and spore pigmentation), these isolates were grouped into 18 different morphotypes (Table 1). Rarefaction analysis, which plots the number of fungal strains isolated from the Andosol as a function of the number of morphotypes detected, showed that the curve did not reach the asymptote. This means that the whole diversity of cultivable fungal species was not fully recovered at that sampling effort (Fig. 1). Morphotypes 1, 2, and 3 were dominant and represented up to 76 % of the fungal isolates. The other 15 morphotypes were represented by a few (1 to 4) isolates. The Shannon–Wiener index (H) was equal to 1.94, suggesting a relatively moderate diversity of cultivable fungal isolates retrieved from the contaminated Andosol, as revealed by rarefaction analysis. The Simpson index (D) was equal to 0.22, indicating codominance of several morphotypes within the cultivable fungal community isolated from the chlordecone-contaminated Andosol.

Characterization of the diversity of fungal isolates

In order to estimate the diversity of the fungal isolates, at least one isolate per morphotype was chosen to sequence the 18S–28S ITS of the ribosomal operon. For the three dominant morphotypes, this analysis was performed on several isolates. Altogether 81 ITS sequences were obtained out of which 63 will be deposited in the GenBank database. Comparison of those sequences with known ones available in the database showed similarities ranging from 97 to 100 %. The phylogenetic analysis revealed that all the isolates belonged to the Ascomycota phylum. They were distributed among 11 genera including *Metacordyceps*, *Cordyceps*, *Pochonia*, *Acremonium*, *Fusarium*, *Paecilomyces*, *Ophiocordyceps*, *Purpureocillium*, *Bionectria*, *Penicillium*, and *Aspergillus* (Fig. 2). The three dominant morphotypes were identified as *F. oxysporum* sp. (morphotype 1), *Paecilomyces* sp./*Ophiocordyceps heteropoda*/*P. lilacinum* (morphotype 2), and *Aspergillus* sp. (morphotype 3). Interestingly, the 33 ITS sequences of morphotype 1 were 99 to 100 % similar to *F. oxysporum* isolate 281 (accession no. JN232163.1). Conversely, the 13 ITS sequences of morphotype 2 were 100 % similar to *Paecilomyces* sp. Pa972 (accession no. JQ821350.1), *O. heteropoda* (accession no. AB084157.1), and *P. lilacinum* isolate DF12064 (accession no. JQ863231.1) sequences. The last one was similar to

Table 1 Description of the 103 fungal strains isolated from the Andosol heavily contaminated with chlordecone. The isolates were distributed into 18 morphotypes. Species identification based on ITS analysis is shown. The numbers of isolates for each morphotype observed and for each species are given

Species	Morphotypes																		Total effective per species
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Fusarium oxysporum	33[a]																		33
Paecilomyces sp./Ophiocordyceps heteropoda/ Purpureocillium lilacinum	11+2[a]			1[a]		1	1[a]												16
Aspergillus flavus			11[a]								1[a]								12
Aspergillus nomius			3[a]							1[a]								1[a]	5
Aspergillus terreus			1[a]										1[a]	1[a]			1[a]		4
Metacordyceps chlamydosporia/Cordyceps chlamydosporia/Pochoniachlamydosporia		1[a]		2		1			1[a]										5
Penicillium citrinum					2[a]														2
Penicillium sp.					1														1
Bionectria sp.												1[a]							1
Acremonium															1[a]				1
Pochonia sp.																1[a]			1
n.d.	2	9	7			1	1	2											22
Total effective per morphotype	35	23	22	3	3	3	2	2	1	1	1	1	1	1	1	1	1	1	103

n.d. ITS of morphotype not sequenced

[a] ITS sequences of isolates deposited in GenBank

Metacordyceps chlamydosporia strain NBAII PC55 (accession no. JX918944.1), *Cordyceps chlamydosporia* isolate ID05-F0165 (accession no. AB378545.1) and *Pochonia chlamydosporia* (EU733637.1) sequences. Furthermore, the 15 ITS sequences of morphotype 3 were all similar to *Aspergillus* ITS sequences, namely *A. flavus* (100 % similarity with *A. flavus* accession no. JX157882.1), *A. nomius* (99 to 100 % similarity with *A. nomius* isolate A15A, accession no. JQ781730.1), and *A. terreus* (100 % similarity with *A. terreus* strain SHPP01, accession no. JF738047.1). This morphotype was dominated by *A. flavus*, which represented 50 % of the sequenced fungal isolates. It is noteworthy that several other morphotypes represented by a single isolate gathered in the *Aspergillus* genus (*A. flavus* morphotype 11; *A. nomius* morphotypes 10 and 18; and *A. terreus* morphotypes 13, 14, and 17). Similarly, ITS analysis revealed that identical genera could be found in different morphotypes as *Paecilomyces* sp./*O. heteropoda*/*P. lilacinum* in morphotypes 2, 4, 6, and 7

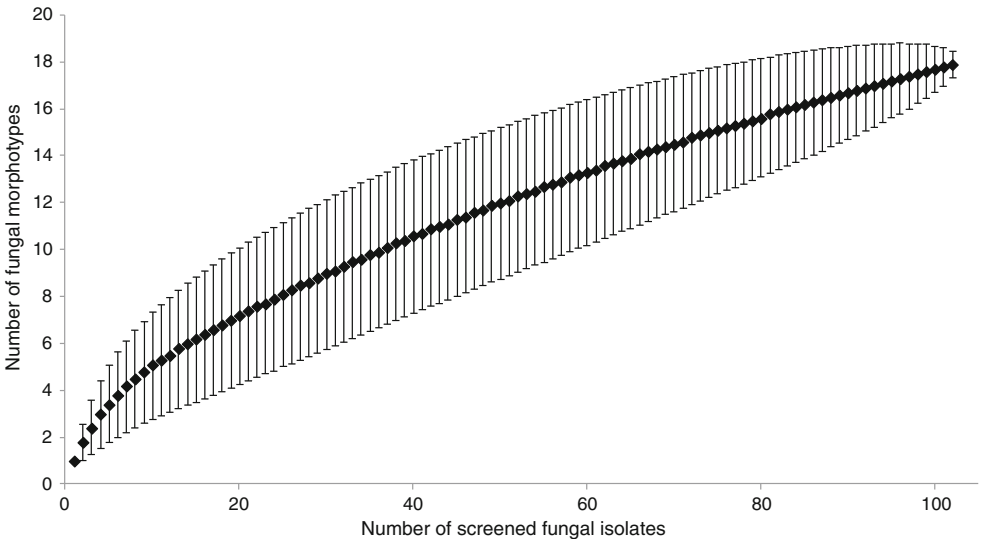

Fig. 1 Rarefaction curve of the observed diversity of the fungal morphotypes as a function of the number of fungal strains isolated from enrichment cultures obtained from the Andosol perfused for 1 year with chlordecone–MS medium in a soil–charcoal device. The rarefaction curve was obtained by applying the freeware program aRarefactWin.exe (http://www.uga.edu/~strata/AnRareReadme.html). *Error bars* represent 95 % confidence intervals calculated from the variance of the number of fungal morphotypes

and *M. chlamydosporia*/*C. chlamydosporia*/*P. chlamydosporia* in morphotypes 2, 4, 6, and 9. At this stage, representative of the morphotype 8 has not been sequenced yet.

Screening of the fungal isolates for growth on chlordecone–MS medium

We focused our attention on the three dominant morphotypes, for which isolates able to grow on chlordecone were searched using a test in Bioscreen. Out of all the isolates tested, only *F. oxysporum* MIAE01197 was able to grow on liquid mineral salt medium with 3 mg l^{-1} of chlordecone added as the sole carbon source (Fig. 3a). As a control, *F. oxysporum* MIAE01197 was unable to grow on liquid mineral salt medium. In addition, the reference strain not previously exposed to chlordecone *F. oxysporum* MIAE00047 grew neither on MS–chlordecone nor on MS medium (Fig. 3b). Further characterization of growth of MIAE01197 and of MIAE00047 addressed by biomass production showed that both of them were able to weakly grow on liquid mineral salt medium (i.e., approximately 2 mg of biomass within 2 weeks of incubation). However, *F. oxysporum* MIAE01197 developed a significantly higher biomass (i.e., 7±1 mg) than *F. oxysporum* MIAE00047 (i.e., 3±1 mg) when grown on liquid mineral salt medium with 10 mg l^{-1} of chlordecone added as the sole carbon source.

Characterization of *F. oxysporum* MIAE01197 tolerance to chlordecone

The tolerance of *F. oxysporum* MIAE01197 and MIAE00047 to chlordecone was addressed by assessing the impact of increasing concentrations of chlordecone (0, 0.1, 1, and 10 mg l^{-1} of chlordecone) on growth parameters. Both strains grew without a lag phase on glucose–MS medium in the presence of chlordecone. However, the growth rate (micrometer) of *F. oxysporum* MIAE01197 was significantly increased in response to chlordecone exposure ($p<0.01$; $n=5$). On the contrary, the growth rate of MIAE00047, the reference strain unexposed to chlordecone, was not affected by chlordecone (Fig. 4).

The tolerance of the two strains to chlordecone was also estimated by calculating the EC_{50}, by quantifying fungal biomass after growth in a liquid culture medium. *F. oxysporum*

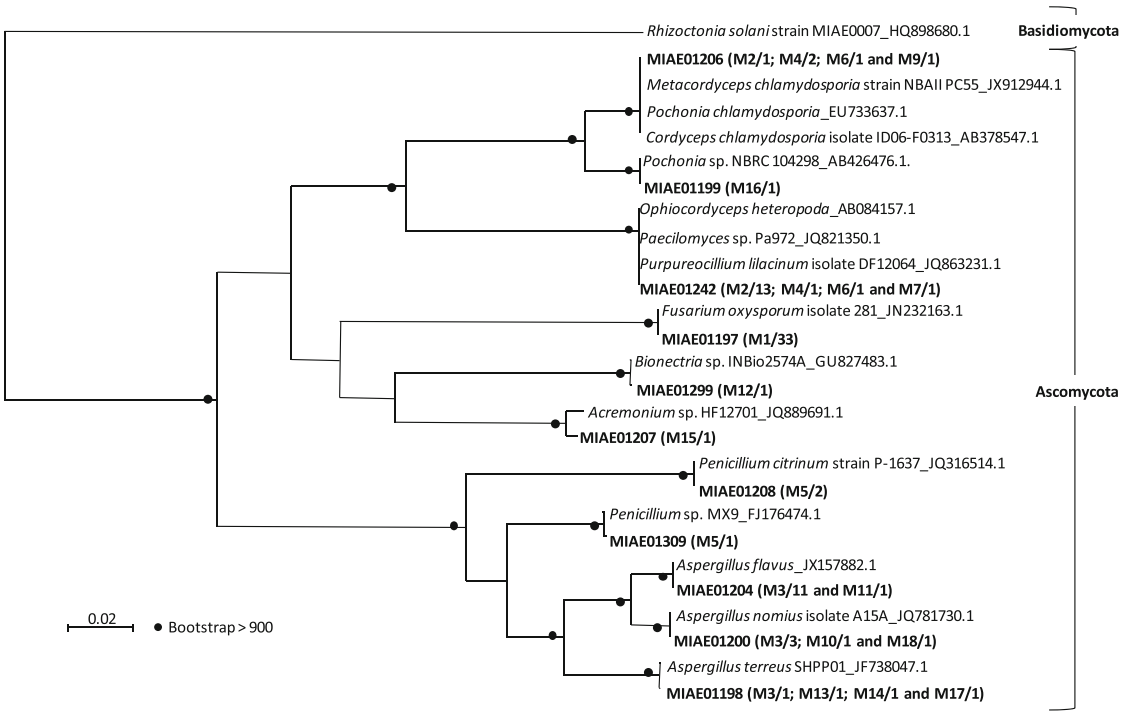

Fig. 2 Phylogenetic neighbor-joining tree of the ITS sequences of the fungal strains isolated from enrichment cultures obtained from the Andosol perfused for 1 year in a soil–charcoal device. The tree was built from the maximum likelihood criteria, using ClustalX software and NJplot program. Branch lengths are proportional to the evolutionary distances between sequences. *Dots* mean that bootstrap values are above or equal to 900 out of 1,000 iterations. Isolates are noted in *bold* and were registered in the MIAE (Microorganismes d'Intérêt Agro-Environnementaux) collection from INRA Dijon (France). In brackets, morphotype number and corresponding abundance are indicated separated by a *dash*. Several ITS sequences were deposited in the GenBank database under the following accession numbers: KC787016 (MIAE01197); KC786984 (MIAE01198); KC603897 (MIAE01199); KC786992 (MIAE01200); KC787004 (MIAE01204); KC787021 (MIAE01207), KC787023 (MIAE01208); KC787001 (MIAE01242); and KC787036 (MIAE01299). One representative of each fungal species is shown. Reference sequences and corresponding accession numbers retrieved from the GenBank database are separated by a *hyphen*

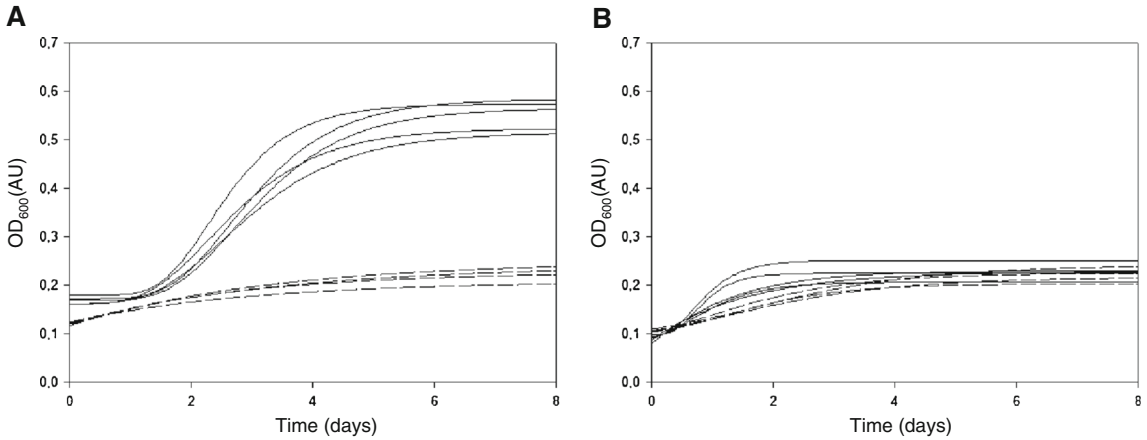

Fig. 3 **a** Growth curves of *F. oxysporum* MIAE01197 recorded from cultures on chlordecone–MS medium (*solid lines*). As a control, the growth of *F. oxysporum* MIAE01197 inoculated MS medium was monitored (*dashed lines*). **b** Growth curves of *F. oxysporum* MIAE00047 grown on MS medium (*solid lines*). As a control, the growth of *F. oxysporum* MIAE00047 inoculated MS medium was monitored (*dashed lines*). For each strain and for each condition tested, five replicates were performed (n_{tot}=20)

MIAE01197 isolated from the chlordecone-contaminated Andosol showed a significantly higher EC_{50} than the reference MIAE00047 strain (i.e., 29.2 and 18.6 mg of chlordecone per liter, respectively, $p<0.01$).

Characterization of *F. oxysporum* MIAE01197 ability to dissipate chlordecone

In order to address *F. oxysporum* MIAE01197 dissipation ability, fungal cultures in liquid MS medium added with chlordecone (1.5 mg l^{-1}) were performed. A control consisting in incubating the chlordecone–MS medium without fungal inoculation was added. GC-MS quantification of the chlordecone that remained in the culture medium at the end of the incubation period showed that 1.06±0.26 mg l^{-1} of chlordecone remained in the control, while 0.60±0.09 mg l^{-1} remained in the MIAE01197 culture. As compared to the control, approximately 40 % of chlordecone was dissipated by *F. oxysporum* MIAE01197. Unfortunately, although our GC-MS method allowed us to detect β-monohydro- and dihydro-chlordecone, no chlordecone metabolites could have been observed on MS spectrum.

To further address *F. oxysporum* MIAE01197 dissipation ability, mineralization experiments using radiorespirometry

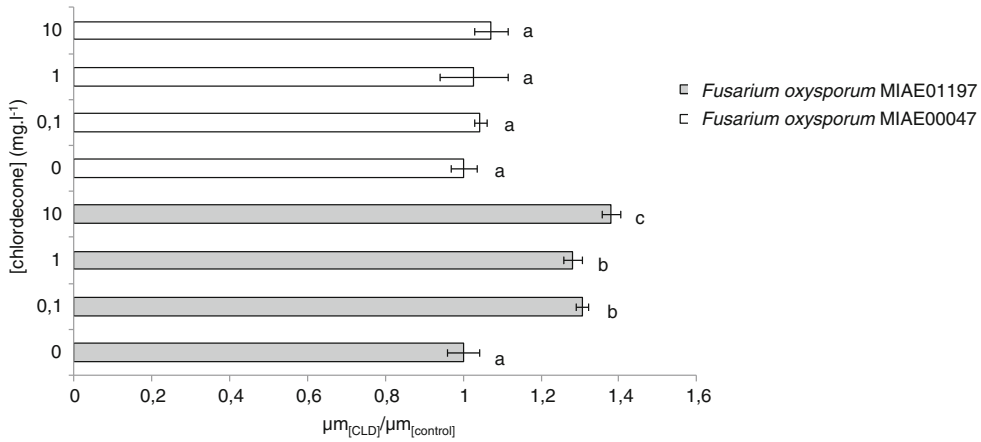

Fig. 4 Tolerance to chlordecone of *F. oxysporum* MIAE01197 isolated from a chlordecone-contaminated Andosol as compared to that of the reference strain *F. oxysporum* MIAE00047 not previously exposed to the insecticide. The growth of the two isolates on glucose–MS medium added with 0.1, 1, and 10 mg l^{-1} of chlordecone or not (control) was monitored over 7 days incubation at 25 °C in the Bioscreen C Microbiological Growth Analyser. For each condition tested, five replicates were performed (n_{tot}=40). Growth rates (micrometer) were estimated by fitting growth curves with the Gompertz model. Tolerance to chlordecone was estimated by calculating the $\mu m_{[CLD]}/\mu m_{[control]}$ ratio. *Gray bars* represent $\mu m_{[CLD]}/\mu m_{[control]}$ values obtained for *F. oxysporum* MIAE01197 and *white bars* those obtained for the *F. oxysporum* MIAE00047 reference strain. Values are the means and standard errors of five replicates. Values labeled with the *same letter* are not significantly different according to the Student's *t* test at $P<0.005$, $n=5$

were carried out with $^{14}C_{10}$-chlordecone on cultures grown on chlordecone–MS or glucose–chlordecone–MS liquid media. A rapid but low emission of $^{14}CO_2$ (i.e., 1.2 % of the initial amount of $^{14}C_{10}$-chlordecone added) was noted in *F. oxysporum* MIAE01197 cultures carried out in glucose-added MS medium, while hardly any emission of $^{14}CO_2$ was recorded in *F. oxysporum* MIAE 01197 cultures carried out in chlordecone–MS medium (Fig. 5). At the end of the incubation period, chlordecone residues were extracted using dichloromethane and separated by thin-layer chromatography (Fig. 6). The autoradiographic analysis of the chromatogram showed that $^{14}C_{10}$-chlordecone was present in the two *F. oxysporum* MIAE01197 cultures. S1 spot was observed close to the deposit line for both cultures and control. S2 and S3/S4 spots were observed below and above the chlordecone spot, respectively. These three spots were detected in both cultures, but not in the control culture medium. In addition, ^{14}C-compounds were weakly present in the migration fronts of the two cultures.

In order to check for the possible adsorption of chlordecone to the fungal biomass, an experiment was carried out by incubating different amounts *F. oxysporum* MIAE01197 in a phosphate buffer presence of ^{14}C-chlordecone. The amount of radioactivity contained in the phosphate buffer and in the fungal biomass was regularly monitored over a 72-h incubation period. A linear relationship was found between the amount of $^{14}C_{10}$-chlordecone adsorption and fungal biomass ($^{14}C_{10}$-chlordecone adsorbed=32,595×[fungal biomass]+0,069, r^2=0.99) suggesting that biosorption could occur with *F. oxysporum* sp. MIAE01197 (Fig. S1).

Discussion

One hundred and three fungal strains were isolated from Andosol contaminated with 35 mg kg^{-1} of chlordecone. These strains were gathered into 18 morphotypes. Rarefaction analysis showed that the whole diversity of cultivable fungal species was not fully recovered. Shannon–Wiener index suggests a relatively moderate diversity of the isolated cultivable fungal strains. However, this observation should be interpreted keeping in mind that most fungal species are not cultivable (Anderson and Cairney 2004; Bridge and Spooner 2001; Manter and Vivanco 2007; Thorn 1997; Zak

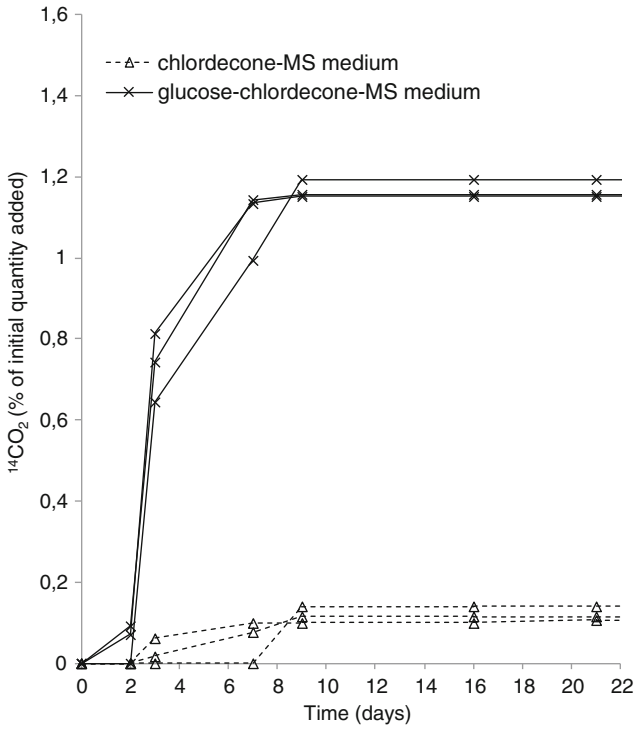

Fig. 5 Kinetics of $^{14}CO_2$ evolution from $^{14}C_{10}$-chlordecone over a 3-week incubation period in cultures of *F. oxysporum* MIAE01197 grown on chlordecone–MS medium (*triangle*, *dashed lines*) or on glucose–chlordecone–MS medium (*cross*, *solid lines*). The amount of accumulated $^{14}CO_2$ is expressed as a percentage of the initial amount of $^{14}C_{10}$-chlordecone added to the medium. Three replicates were performed per treatment

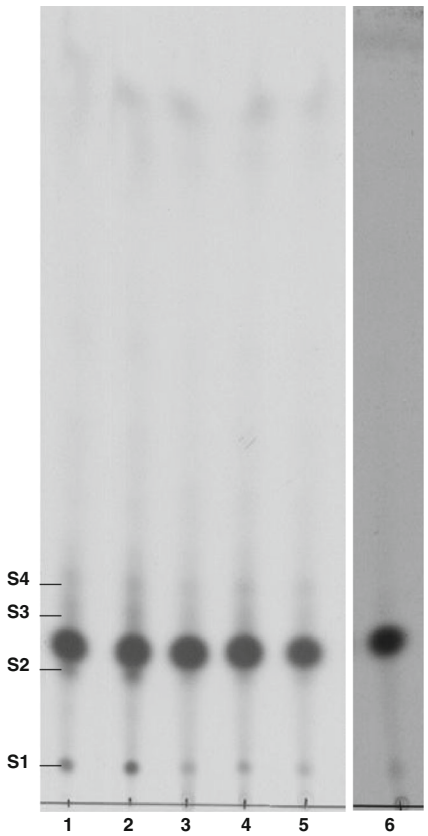

Fig. 6 Search for $^{14}C_{10}$-chlordecone metabolites in *F. oxysporum* MIAE01197 cultures conducted on glucose–chlordecone–MS medium (*lanes 1 and 2*) or on chlordecone–MS medium (*lanes 3 to 5*) using thin-layer chromatography. Non-inoculated chlordecone–MS medium was used as a control (*lane 6*). ^{14}C-metabolites extracted from the cultures were separated in a glass-backed silica gel layer. Radioactive compounds were detected by autoradiography

and Visser 1996). Consequently, the Andosol may have harbored higher fungal diversity. In addition, incubating the Andosol in a soil–charcoal system and isolating fungal strains under chlordecone selection pressure may also have yielded a moderate diversity of fungal species. As revealed by the Simpson index, several morphotypes (1, 2, and 3) codominated the cultivable fungal community and represented up to 76 % of the total effective. Molecular analysis based on 18S–28S ITS sequencing revealed that morphological classification led to a slight overestimation of the diversity of the fungal strains. Indeed, although the 103 isolates gathered in 18 morphotypes, they were distributed into only 11 genera (*Fusarium*, *Paecilomyces*, *Ophiocordyceps*, *Aspergillus*, *Purpureocillium*, *Metacordyceps*, *Pochonia*, *Cordyceps*, *Penicillium*, *Bionectria*, and *Acremonium*). This discrepancy was mainly due to whitish color isolates clustered in morphotype 4 and morphotypes 6 to 14 which did not display any spore pigmentation. Being classified as a separate morphotype according to mycelium shape, ITS sequencing revealed that some of them belonged to the *Aspergillus* genus explaining why diversity was overestimated. All the fungal strains isolated from the Andosol belonged to the Ascomycota phylum. This observation is in agreement with Rohilla and Salar (2012) who reported that Ascomycota was the dominant phylum (represented by seven genera) among fungal strains isolated from 23 agricultural soils contaminated with different pesticides. In addition, two of our three dominant morphotypes, namely *Fusarium* sp. (morphotype 1) and *Aspergillus* sp. (morphotype 3), were also found as predominant genera in pesticide-contaminated agricultural soils (Rohilla and Salar 2012).

Strains gathered in morphotype 1 belonged to *Fusarium* species which are ubiquitous organisms widely distributed in the environment (Nelson et al. 1994). They are known as plant (Nelson et al. 1994) and occasionally as animal pathogens (Evans et al. 2004) including humans causing broad spectrum of infections (Gorman et al. 2006; Nucci and Anaissie 2002, 2007).

Strains gathered in morphotype 2 belonged to the Hypocreales order including *Paecilomyces*, *Ophiocordyceps*, and *Purpureocillium* genera. Unfortunately, ITS amplification with ITS1F/ITS4 primers failed to distinguish between these genera. To give these strains a clearer affiliation, other molecular markers such as elongation factor or beta-tubuline will have to be investigated. Strains belonging to these genera are known as entomopathogenic fungi infecting emerald ash borers (Johny et al. 2012), whitefly nymphs (Wraight et al. 1998), and cicada larvae (Kobayasi 1938).

Strains gathered in morphotype 3 belonged to the *Aspergillus* genus which is ubiquitous, including saprophytes living in diverse habitats. They are known as a mycotoxin producer (Caira et al. 2012; Hedayati et al. 2007; Lass-Flörl et al. 2005) such as aflatoxin that impacts human health (Kurtzman et al. 1987). According to a biogeographical study, *A. flavus* and *A. terreus* strains occur at expected frequencies in soil and litter in the tropical latitudes (Guadeloupe latitude is 16°09′00) (Klich 2002).

In order to identify a chlordecone-tolerant fungal strain, the strains gathered in the three dominant morphotypes were screened in liquid cultures by monitoring their growth on chlordecone–MS medium using the Bioscreen. Among all the strains we tested, only *F. oxysporum* MIAE01197 grew on MS medium supplemented with chlordecone as the sole carbon source. *F. oxysporum* MIAE00047, which had never been exposed to chlordecone, did not grow on chlordecone–MS medium at least using a Bioscreen. The measurement of biomass production showed that both reference and MIAE01197 strains were able to weakly grow on MS medium. This discrepancy between the Bioscreen and biomass production experiments is most likely due to technical limitations of Bioscreen not allowing the detection of weak biomass production. However, only MIAE01197 produced a significantly higher biomass in chlordecone MS medium than MIAE00047. In order to further assess the adaptation of *F. oxysporum* MIAE01197 to chlordecone, its tolerance to chlordecone was studied in vitro following a method typically used to assess the impact of different pesticides on organism growth (Bhalerao and Puranik 2007; Dritsa et al. 2007; Ortega et al. 2011). *F. oxysporum* MIAE01197 and MIAE00047 were exposed to a chlordecone gradient in liquid glucose–MS medium. *F. oxysporum* MIAE01197 growth rate (micrometer) was significantly increased in response to exposure to increasing concentrations of chlordecone ($p<0.01$; $n=5$), whereas the reference strain growth rate was not affected. *F. oxysporum* MIAE01197 and MIAE00047 tolerance was also assessed by calculating their EC_{50} values following the procedure used by different authors for different pesticides (Dorigo et al. 2007; Pesce et al. 2010). *F. oxysporum* MIAE01197 showed a significantly higher EC_{50} value than the reference strain MIAE00047 (i.e., 29.2 mg l^{-1} for MIAE01197 vs 18.6 mg l^{-1} for MIAE00047). As compared to the reference strain MIAE00047, it even seems to be stimulated by chlordecone exposure up to 10 mg l^{-1}, suggesting its adaptation in response to chlordecone exposure.

To characterize *F. oxysporum* MIAE01197 degrading ability, chlordecone dissipation was monitored by GC-MS. Approximately 40 % of chlordecone was dissipated by *F. oxysporum* MIAE01197. Unfortunately, no known chlordecone metabolites (β-monohydro- and dihydrochlordecone) could have been observed on the MS spectrum. In addition, no trace of intermediary metabolites recently suggested by Dolfing et al. (2012) was detected either. Therefore, at this stage, chlordecone dissipation cannot be clearly related to a degradation process. In order to investigate this possibility, radiorespirometry analyses were conducted. *F. oxysporum* MIAE01197 did not mineralize

$^{14}C_{10}$-chlordecone when chlordecone was added as the sole carbon source. However, in the presence of glucose a low amount of $^{14}C_{10}$-chlordecone evolved to $^{14}CO_2$ (i.e., 1.2 % of the initial amount of $^{14}C_{10}$-chlordecone added). Although several radioactive spots were detected in the *F. oxysporum* MIAE01197 culture by TLC analyses, most of the radioactivity remained in the $^{14}C_{10}$-chlordecone spot, suggesting poor transformation. These last two observations contradict the fact that *F. oxysporum* MIAE01197 was able to grow on chlordecone as the sole carbon source in liquid medium. Indeed, although chlordecone dissipation was observed by GC-MS, hardly any evolution to $^{14}CO_2$ was recorded by radiorespirometry, except in the presence of glucose. One could not exclude that MIAE01197 could grow on some contaminants of chlordecone, but this hypothesis seems very unlikely because chlordecone pure at 99.7 % was used at low concentration (i.e., not more than 3 mg l^{-1}) not furnishing enough C to fuel its growth. Keeping in mind the poor ability of *F. oxysporum* MIAE01197 to transform $^{14}C_{10}$-chlordecone, we could explain chlordecone dissipation by biosorption. Indeed, adsorption onto fungal mycelium and/or absorption into the fungal cells has been observed by several authors for different xenobiotics (Aksu 2005; Benoit et al. 1998; Dritsa et al. 2007; Juhasz et al. 2002; Lievremont et al. 1996; Shin et al. 1970; Wu and Yu 2006). Fungal biosorption was even recommended as a water-cleaning strategy to remove heavy metals or organic pollutants by environmental bioremediation (Aksu 2005; Kapoor and Viraraghavan 1995; Yan and Viraraghavan 2003). We evidenced for a linear relationship between the amount of $^{14}C_{10}$-chlordecone adsorption and fungal biomass (r^2=0.99) suggesting that biosorption could occur with *F. oxysporum* sp. MIAE01197. However, in our dissipation experiment, fungal biomass was too low to account for 40 % chlordecone removal by biosorption alone.

Conclusion

This study led to the isolation and characterization of 103 fungal strains from a historically contaminated Andosol. All these isolates were affiliated to the Ascomycota phylum known to be present in soils frequently exposed to pesticides. Among them, *F. oxysporum* MIAE01197 was the only one able to grow on chlordecone while the reference strain, *F. oxysporum* MIAE00047, not previously exposed to chlordecone did not grow on it. *F. oxysporum* MIAE01197 was shown to not only tolerate high concentration of chlordecone but also growth to be stimulated by chlordecone. This suggests that in response to long-term exposure to chlordecone in contaminated French West Indies soils, fungal species adapted processes involved in their adaptation. Among possible processes, *F. oxysporum* MIAE01197 biosorption and degradation abilities were shown to be involved in the dissipation of chlordecone. Chlordecone degradation ability was low and further work is needed to improve it. Biosorption may give insight in cleaning water from chlordecone contamination by developing fungal filter.

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