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ORIGINAL PAPER

Evidence of α-, β- and γ-HCH mixture aerobic degradation by the native actinobacteria *Streptomyces* sp. M7

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Abstract The organochlorine insecticide γ-hexachlorocyclohexane (γ-HCH, lindane) and its non-insecticidal α- and β-isomers continue to pose serious environmental and health concerns, although their use has been restricted or completely banned for decades. In this study we report the first evidence of the growth ability of a *Streptomyces* strain in a mineral salt medium containing high doses of α- and β-HCH (16.6 mg l^{-1}) as a carbon source. Degradation of HCH isomers by *Streptomyces* sp. M7 was investigated after 1, 4, and 7 days of incubation, determining chloride ion release, and residues in the supernatants by GC with μECD detection. The results show that both the α- and β-HCH isomers were effectively metabolized by *Streptomyces* sp. M7, with 80 and 78 % degradation respectively, after 7 days of incubation. Moreover, pentachlorocyclohexenes and tetrachlorocyclohexenes were detected as metabolites. In addition, the formation of possible persistent compounds such as chlorobenzenes and chlorophenols were studied by GC–MS, while no phenolic compounds were detected. In conclusion, we have demonstrated for the first time that *Streptomyces* sp. M7 can degrade α- and β-isomers individually or combined with γ-HCH and could be considered as a potential agent for bioremediation of environments contaminated by organochlorine isomers.

Keywords Aerobic degradation · Bioremediation · HCH isomers · β-HCH · *Streptomyces* sp.

Introduction

Organochlorine pesticides (OPs) have been widely used for agriculture and medical purposes; however the use of these compounds has been prohibited because of their toxicity, environmental persistence, and bioaccumulation in the food chain. Thus in 2009, OPs were added as new persistent organic pollutant to the UNEP Stockholm Convention list (Vijgen et al. 2011).

The hexachlorocyclohexanes are commercially available in two formulations: technical HCH (a mixture of isomers) and lindane (γ-HCH, the only isomer which possesses insecticidal activity). During the production of lindane up to 85 % of the final product consists of α-, β- and δ-isomers. These stereoisomers are then separated out and frequently dumped as waste at various locations on the production sites, causing a negative environmental impact (Heeb et al. 2014). In addition, it was estimated that for each ton of lindane, between 8 and 12 tons of the other isomers were produced (Vijgen 2006). Although the use of lindane has been strictly forbidden in many countries since the 1990s, γ-HCH and its non-insecticidal isomers (α-, β-, and δ-HCH) continue to pose a real risk to the environment and to human health (Pavlíková et al. 2012). Furthermore, abiotic and biotic degradation of HCH isomers in nature lead to accumulation of β-isomer, which is the most persistent and resistant to bacterial attack (Phillips et al. 2005).

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All the HCH isomers are lipophilic compounds and therefore tend to accumulate and concentrate in the body fat of animals and humans (Johri et al. 2000). Lindane is considered a potential carcinogen and listed as a priority pollutant by the US EPA (Cuozzo et al. 2009). The β-HCH isomer has been classified in group 2B as possibly carcinogenic to humans by the International Agency Research and Cancer (IARC) (Ingelido et al. 2009).

Aerobic metabolism of HCHs has been reported for several species of bacteria (Camacho-Pérez et al. 2012; Nagata et al. 1999; Phillips et al. 2005; Raina et al. 2008; Tabata et al. 2011). Biodegradation of HCH isomers consists of progressive elimination of the chlorine and hydrogen atoms and the subsequent formation of double bonds; the chlorine atoms are possibly replaced by hydroxyls (Raina et al. 2008; Wu et al. 2007). Owing to their molecular geometry, α-, β-, and γ-HCH show different degradation pathways; therefore not all the degradation mechanisms are present in all the organisms studied to date (Heeb et al. 2015; Raina et al. 2007).

Pentachlorocyclohexenes are often products of the first step of HCH isomers degradation and can be further degraded via a variety of pathways, depending upon the isomeric form and the bacterial strain involved (Singh and Kuhad 1999). Complete aerobic degradation of HCH isomers has been reported for the Gram-negative bacterium *Sphingomonas* (Heeb et al. 2014, 2015; Nagata et al. 2007) and for the white-rot fungi *Trametes hirsutus*, *Phanerochaete chrysosporium, Cyathus bulleri*, and *Phanerochaete sordida* (Mougin et al. 1999; Singh and Kuhad 1999, 2000). Chlorobenzenes and chlorophenols have been reported as the most persistent metabolites in aerobic bacterial degradation of HCH and in some cases these were dead-end products. Some hydroxylated metabolites are also resistant to further bacterial degradation, as they have been detected in soils and water near pesticide production sites (Raina et al. 2007). It is therefore necessary to search for versatile microorganisms able to degrade mixtures of HCH isomers without producing dangerous sub-products.

In particular, *Streptomyces* sp. M7, previously isolated by Benimeli et al. (2003) was able to remove organic and inorganic toxic compounds (Benimeli et al. 2008; Cuozzo et al. 2009; Polti et al. 2014) and the mechanisms that govern the lindane degradation process in this strain are currently under study. However, the ability of *Streptomyces* sp. M7 to remove different HCH isomers and the intermediate metabolites produced remains unknown.

It has also been determined that environmental factors such as temperature, pH, and salinity have a strong influence on microbial activity, as well as on the bioavailability of target chemicals. Therefore, the optimization of these parameters is necessary in order to achieve substantial degradation of pollutants. In this context, the aim of the present work is to determine the optimum conditions for α- and β-HCH aerobic removal by indigenous *Streptomyces* sp. M7, to characterize their potential degradation in mix with lindane, and to investigate the production of metabolites from α- and β-HCH. We believe this study represents a key step towards understanding aerobic degradation of α- and β-HCH isomers by actinobacteria.

Materials and methods

Microorganisms, culture media and chemicals

The strain *Streptomyces* sp. M7 was previously isolated in our laboratory from sediment samples contaminated with heavy metals and pesticides, collected in the province of Tucumán, Argentina (Benimeli et al. 2003).

HCH isomers (99.8 % pure) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and stock solutions were prepared in dimethyl sulfoxide. Solvents were of pesticide grade. All other chemicals used in this study were analytical grade and were purchased from standard manufacturers.

Minimal medium (MM), containing in g l^{-1}: $(NH_4)_2SO_4$, 4.00; K_2HPO_4, 0.50; $MgSO_4 \cdot 7H_2O$, 0.20; and $FeSO_4 \cdot 7H_2O$, 0.01 (Hopwood et al. 1985), was used throughout the study. The pH was adjusted using either NaOH 0.1 N or H_2SO_4 2 N, as appropriate, prior to sterilization. It was sterilized by autoclaving at 121 °C for 20 min. Stock solutions of HCH isomers were filter-sterilized (0.22 µm pore size Millipore filter) and then aseptically added to the autoclaved MM.

Streptomyces sp. M7 cultures at different physico-chemical conditions in minimal medium with HCH isomers

A spore suspension (150 µl) of *Streptomyces* sp. M7 (10^9 CFU ml^{-1}) was inoculated in 125 ml flasks containing 30 ml of MM at pH 7, supplemented with 1.66 mg l^{-1} of α- or β-HCH isomers as the carbon source. All cultures were incubated on a rotatory shaker (200 rpm) at 30 °C for 7 days. Samples were taken at 0, 1, 4, and 7 days. The supernatants were separated by centrifugation (9900×*g*, 30 min, 4 °C) and used to determine the residual HCH isomers by gas chromatography (GC) and chloride ion release. Biomass was estimated after centrifugation by washing the pellets with 25 mM Tris–EDTA buffer (pH 8) and then drying to constant weight at 105 °C.

In order to determine the ability of *Streptomyces* sp. M7 to remove α-/β-HCH isomers at different pH levels, the pH of the MM was adjusted to 5, 7, or 9 depending upon the assay, and the incubation temperature was 30 °C. Removal

of the compounds was also studied at three different incubation temperatures (25, 30, and 35 °C), keeping the pH of the MM at 7. The effects of varying initial concentrations of α-/β-HCH isomers on the removal ability was also studied by adding an appropriate volume of HCH stock solution to each Erlenmeyer flask in order to reach the following concentrations of the isomers: 1.66, 8.33, and 16.6 mg l^{-1}, with an initial pH of 7 and an incubation temperature of 30 °C. The lowest concentration (1.66 mg l^{-1}) was chosen based on previous works where it was determined as the optimum for lindane removal by different strains of regional actinobacteria (Benimeli et al. 2003; Fuentes et al. 2011). In order to study the effect of five and tenfold higher concentrations of the different isomers, the resulting concentrations were 8.33 and 16.6 mg l^{-1}.

The removal capacity of *Streptomyces* sp. M7 was evaluated in a mixture of α-, β-, and γ-HCH. Each isomer was added to the MM (pH 7) at a concentration of 1.66 mg l^{-1}. Incubations were carried out at 30 °C.

For the purpose of identifying the intermediate metabolites of HCH isomers degradation, 200 ml of MM (pH 7) containing 1.66 mg l^{-1} of α- or β-HCH isomers were inoculated with 2 ml of spore suspension (10^9 CFU ml^{-1}) of *Streptomyces* sp. M7 and incubated at 30 °C and 150 rpm for 72 h. Cells were then harvested by centrifugation (9900×*g* for 10 min) and washed with sterile distilled water twice. The pellet was suspended in a buffer [50 mM Tris–sulphate (pH 7.5), 1 mM EDTA, and 1 mM dithiothreitol (DTT)] and the cell suspensions were broken in a French Press at 20,000 psi. The resulting cell-disrupted suspensions were centrifuged (9900×*g*, 30 min, 4 °C). The free-cell extracts obtained and the supernatants of the culture media were used for identification of intermediate metabolites.

All experiments were carried out in triplicate and the results were expressed as the means from the three tests. Inoculated MM without HCH isomers (designed as "biotic control") and non-inoculated MM with HCH isomers (designed as "abiotic control") were also included.

Gas chromatography analysis

Residual HCH isomers in the supernatants were determined by GC with micro electron capture detector (μECD). For this purpose solid phase extraction (SPE) using a C18 column (Agilent Technologies, USA) was performed, then evaporated to dryness under reduced pressure and finally the residue was re-suspended in hexane. These extracts were then analysed in a gas chromatograph (Agilent Technologies 7890A) equipped with an HP5 capillary column (30 m × 0.53 mm × 0.35 μm), a ^{63}Ni μECD, a split/splitless injector (Agilent 7693B), and Agilent ChemStation software. The chromatographic conditions were as follows: carrier gas (nitrogen) flow rate, 25 cm s^{-1}; initial oven temperature, 90 °C increasing to 180 °C at 30 °C min^{-1}, then increasing further to 290 °C at 20 °C min^{-1}; detector temperature, 320 °C; and injection volume, 1 μl. Quantitative analyses were performed using appropriate calibration standards (AccuStandard, New Haven, CT, USA). The detection limit for HCH isomers was 0.07 μg l^{-1}.

In order to identify the intermediates metabolites of α-/β-HCH degradation by GC–MS; the extractions of these metabolites from supernatant and cell-free extracts were performed using SPE with a C18 column. The extracts obtained were analysed in a gas chromatograph (Agilent Technologies 6890) equipped with a Network Mass Selective Detector (Agilent 5973), a HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm), and a split/splitless injector (Agilent 6850). The chromatographical conditions were as follows: inlet temperature 280 °C, carrier gas (helium) with constant flow rate at 1.0 ml min^{-1}; oven temperature was programmed to increase from 50 to 125 °C at 3 °C min^{-1}, and then at 17.5 °C min^{-1} to 300 °C remaining at this temperature for 10 min. The Mass Detector was used in Single Ion Monitoring mode. We therefore performed tentative identification of metabolites by comparing their MS spectra with those reported in the NIST (mass spectra library).

Determination of chloride ions released

Cell-free supernatant samples were used for estimating the level of chloride ions released into the media from the HCH molecules. For this purpose, a modification of the procedure described by Bidlan and Manonmani (2002) was used. Briefly, 500 μl of each 0.15 N HNO_3 and 0.10 N $AgNO_3$ were added to 500 μl of the supernatant samples. Turbidity was measured at 600 nm after standing for 20 min of incubation at room temperature. The concentration of chloride was determined using a standard calibration curve prepared with NaCl. The physico-chemical conditions assayed were compared in terms of release of chloride ions, considering the highest release determined as 100 % (Fuentes et al. 2010).

Statistical analysis

The data were analyzed using the ANOVA parametric test and differences between treatments were detected using the Tukey HSD test at the 0.05 level. Statistical analyses were performed using *Infostat* statistical software.

Results

Determination of the ability of *Streptomyces* sp. M7 to use α- and β-HCH as carbon source

Streptomyces sp. M7 was able to grow in MM with 1.66 mg l^{-1} of α- or β-HCH, showing similar growth profiles in both cases (Fig. 1a, b). The maximum biomass values for *Streptomyces* sp. M7 were obtained at 4 days of incubation in presence of the α- and β-isomers, with 0.32 and 0.33 mg ml^{-1}, respectively. Furthermore, no microbial growth inhibition was observed in the presence of α-/β-isomers, since these biomass values represented twice the biomass of the biotic control (0.15 mg ml^{-1}).

A marked decrease in the concentration of α-HCH was observed after the first day of incubation (50 %). Then, at 7 days of incubation, another significant decrease drop was observed, reaching an isomer removal of 79 %. For the β-HCH isomer, at the first day of incubation a drop around 78 % was observed, remaining constant until the end of incubation time. The concentration of both isomers remained constant in the abiotic controls, clearly demonstrating that the compounds were removed by the microbial activity (Fig. 1a, b).

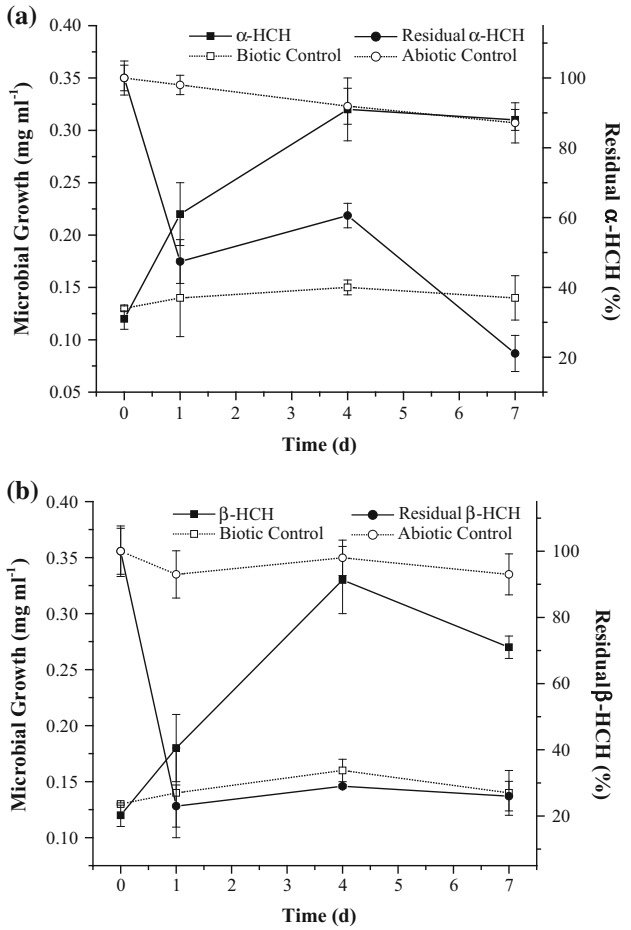

Fig. 1 Residual HCH isomers (*circle symbols*) and microbial growth (*square symbols*) in MM supplemented with: α-HCH (**a**) and β-HCH (**b**) as carbon source. Inoculated MM without HCH isomers (designed as "biotic control") and non-inoculated MM with HCH isomers (designed as "abiotic control") were also included

Optimization of conditions for the removal of α-/β-HCH isomers: effects of temperature, initial pH, and isomer concentration

Considerable differences were observed in the removal of α-HCH at different temperatures, with the highest removal percentage at 30 °C (79 %) (Table 1). The highest release of chloride ions (94 %) was detected after 7 days of incubation at 30 °C, indicating that it was the optimum temperature (Fig. 2a).

Taking into account the different initial pH of the culture medium (5, 7, and 9), significant differences ($p < 0.05$) were observed for α-HCH removal between pH 5 and 7–9: 56, 79, and 83 %, respectively, at 7 days (Table 1). The highest release of chloride ions (94 %) and microbial biomass (0.32 mg l^{-1}) were both observed at pH 7; however the lowest duplication time (18 h) was observed at pH 5 (data not shown).

Based upon the above results, α-HCH removal at pH 7 and 30 °C was assayed in increasing concentrations of the isomer (1.66, 8.33, and 16.6 mg l^{-1}) (Table 1). The removal percentages at 7 days were 79, 85, and 83 % for the different concentrations, with chloride ion release percentages of 94, 100, and 63 % (Fig. 2a), respectively.

When β-HCH removal was evaluated at different incubation temperatures, significant differences were observed only between 25 and 30 °C (Table 1). The highest β-HCH removal percentage was achieved at the 7th day at 30 °C. Additionally, the release of chloride ions at 30 °C was 100 %, namely four times higher than the levels reached at incubation temperatures of 25 and 35 °C (Fig. 2b). On the other hand no significant differences ($p < 0.05$) were observed for β-HCH isomer removal at pH 5 and 7 (79 and 78 %, respectively), although it was significantly lower at pH 9 (72 %) (Table 1). The release of chloride ions showed values of 23, 100, and 24 % for the initial pH assayed (5, 7, and 9). Therefore, based on these chloride ions release, it was concluded that the best removal performance for *Streptomyces* sp. M7 was obtained at pH 7 (Fig. 2b).

The β-HCH isomer degradation at the different concentrations (1.66, 8.33, and 16.6 mg l^{-1}) led to removal percentages of 78, 71, and 63 % (Table 1), with chloride ions release percentages of 100, 67, and 32 %, respectively (Fig. 2b).

Table 1 Removal percentage of α-HCH and β-HCH by *Streptomyces* sp. M7 at different culture conditions: incubation temperature, initial pH and HCH isomers concentration. Incubation time, 7 days

	HCH removal (%)								
	Temperature (°C)			Initial pH			Isomer concentration (mg l^{-1})		
	25	30	35	5	7	9	1.66	8.33	16.6
α-HCH	22.8 ± 1.6^{a}	78.9 ± 0.7^{b}	36.8 ± 0.8^{c}	55.6 ± 1.7^{b}	78.9 ± 0.7^{a}	82.7 ± 1.6^{a}	78.9 ± 0.7^{a}	85.6 ± 6.3^{ab}	83.1 ± 1.4^{b}
β-HCH	73.1 ± 3.6^{a}	77.8 ± 0.6^{b}	76.1 ± 2.5^{ab}	78.9 ± 5.25^{a}	77.8 ± 0.6^{a}	71.8 ± 4.2^{b}	77.8 ± 0.6^{a}	71.2 ± 3.7^{b}	62.7 ± 2.7^{c}

The data were subjected to analysis of variance (ANOVA Oneway), considering significant probability level of $p < 0.05$ (Tukey-post test)
Means with different letters are significantly different ($p < 0.05$)

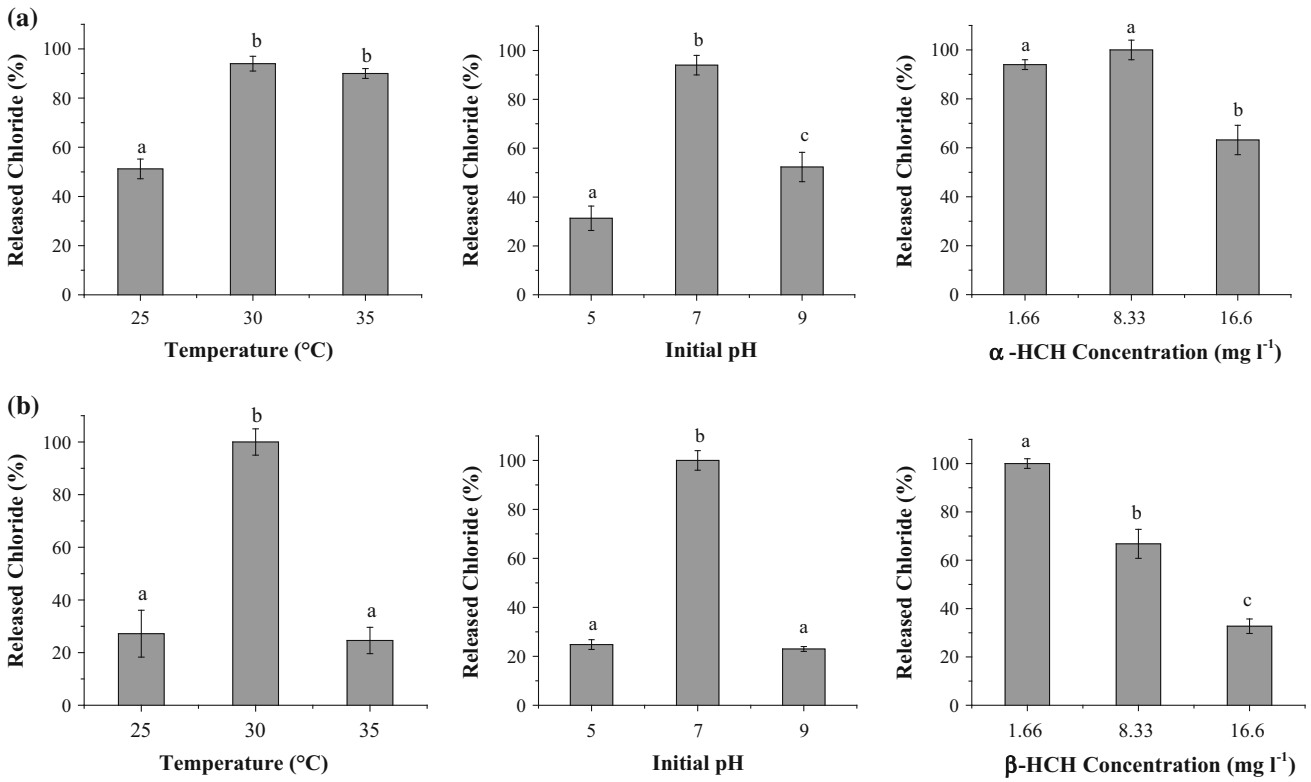

Fig. 2 Percentage of chloride ions released into culture medium at different conditions: incubation temperature, initial pH and isomers concentrations: α-HCH (**a**) and β-HCH (**b**); incubation time, 7 days

Effect of a mixture of α-, β- and γ-HCH isomers on *Streptomyces* sp. M7 growth and its removal ability

The maximum biomass value (0.55 mg ml^{-1}) was obtained at the first day of incubation, followed by a decrease along the time of 0.35 and 0.25 mg ml^{-1} at 4 and 7 days of incubation, respectively (Fig. 3).

When the isomers were tested individually, 79 and 78 % of the α- and β-isomers were removed, respectively (Fig. 1), whereas when these two isomers where combined with lindane, their removal percentages were 46, 39, and 45 % for α-, β-, and γ-HCH, respectively (Fig. 3). These results demonstrate the ability of *Streptomyces* sp. M7 to remove the three isomers together. However, the isomers removal resulted in a decrease of 33 and 39 % respect to the removal of the isomers individually.

Identification of metabolites produced during α- and β-HCH isomers degradation

When *Streptomyces* sp. M7 was cultured with the α-HCH isomer, the GC–MS results from the culture supernatant obtained at 72 h of incubation revealed the appearance of

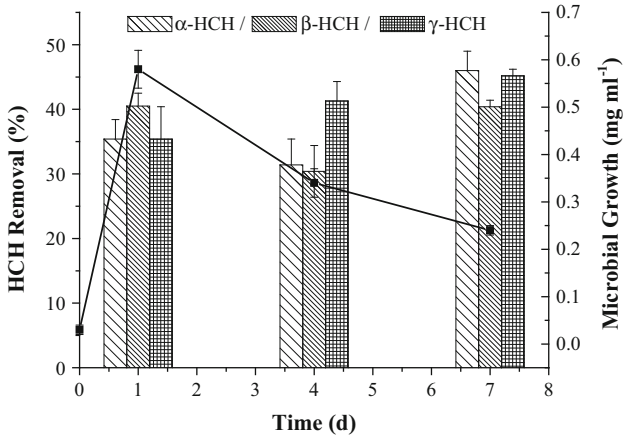

Fig. 3 Microbial growth of *Streptomyces* sp. M7 in MM supplemented with a mixture of α-, β- and γ-HCH (1.66 mg l^{-1} each one) and removal percentage of α-, β- and γ-HCH

three peaks in the chromatogram (Table 2). These were identified as (1) 1,2-dichlorobenzene (1,2-DCB) with 97 % identity (Rt 9.8 min); (2) either 1,4-dichlorobenzene (1,4-DCB) or 1,3-dichlorobenzene (1,3-DCB) with 97 % identity (since the equipment used cannot differentiate these two compounds) (Rt 10.23 min); and (3) trichorobenzenes (1,2,3-trichlorobenzene, 1,2,4-trichlorobenzene, or 1,3,5-trichlorobenzene) with 97 % identity in the three cases (Rt 17.3 min).

In the case of *Streptomyces* sp. M7 cultured in the presence of β-HCH, dichlorobenzene isomers were identified in the culture supernatant with 82 % identity. In both cultures, the isomer used as carbon source (α- or β-HCH) was also identified, which indicates that these compounds were not completely degraded during the 72 h of incubation.

The intermediate products from α-HCH degradation found in the cell-free extract were pentachlorocyclohexene (PCCH) with 90 % identity (Rt 29.5 min) and dichlorobenzene isomers with 81 % identity (Rt 12.2 min). The products identified from β-HCH degradation were tetrachlorocyclohexene (TCCH) with 59 % identity (Rt 26.6 min) and dichlorobenzene isomers with 81 % identity (Rt 12.2 min) (Table 2).

Discussion

The results would indicate that the use of the xenobiotics α- and β-HCH as carbon source is not toxic to the bacterial cells at the tested concentration (1.66 mg l^{-1}), and may also suggest that the intermediate metabolites identified were not producing inhibitory effects on microbial growth of *Streptomyces* sp. M7, as demonstrated in a previous work using a consortium of actinobacteria for lindane biodegradation (Saez et al. 2015).

Similar results were reported for the optimization of the conditions for the removal of α-/β-HCH isomers by Siddique et al. (2002), who found that an incubation temperature of 30 °C was optimum for the aerobic removal of α-HCH isomer by a *Pandoraea* specie in liquid medium, obtaining 62.5 % removal. Also, Benimeli et al. (2007) and Bourguignon et al. (2014) reported that 30 °C was the optimum temperature for the removal of lindane and methoxychlor by a variety of *Streptomyces* strains.

Table 2 Intermediate metabolites identified during the biodegradation of α- and β-HCH by *Streptomyces* sp. M7, determined by GC–MS

Carbon source	Suggested compound[a]	Rt (min)	Identity (%)	Monitored ion in SIM mode
α-HCH				
Culture supernatant	α-HCH	30.2	99	181
	1,2-dichlorobenzene	9.98	97	146
	1,3 or 1,4-dichlorobenzene	10.2	95	146
	Trichlorobenzene isomers	17.1	97	180
Cell-free extract	α-HCH	30.2	99	181
	Pentachlorocyclohexene	29.5	90	181
	Dichlorobenzene isomers	10.2	81	146
β-HCH				
Culture supernatant	β-HCH	31.1	95	146
	Dichlorobenzene isomers	10.2	81	146
Cell-free extract	β-HCH	31.1	95	146
	Tetrachlorocyclohexene	26.6	59	147
	Dichlorobenzene isomers	10.2	81	146

Nomenclature of compounds is according to IUPAC (International Union of Pure and Applied Chemistry)

SIM selected ion monitoring

[a] Identification is based on mass spectrum compared with the WILEY7 NIST library

In this study, the removal of α-/β-HCH isomers were optimum at pH 7 for both cases. Additionally it should be emphasized that pH 7 is generally considered the optimum for metabolic activities of the genus *Streptomyces* (Cuozzo et al. 2009). In addition, Robinson et al. (2009) observed that an acidic environment was not favourable for aerobic bacterial dehalogenation.

It is important to highlight that the removal process not only involve the biodegradation, but also a combination of mechanisms such as adsorption, absorption and desorption (Abromaitis et al. 2016). Therefore, the physico-chemical conditions of the environment influence greatly in the removal of xenobiotic compounds.

The increasing isomer concentration showed that *Streptomyces* sp. M7 was able to remove even the maximum β-HCH concentration from the culture medium, although it was 20 % less compared to the removal obtained with α-HCH at 16.6 mg l^{-1}. These results may indicate a possible inhibitory effect on the β-HCH removal capacity of *Streptomyces* strain, since this is the most recalcitrant isomer, as reported in the literature (Johri et al. 1998; Phillips et al. 2005). Similarly, Rajashekara Murthy and Manonmani (2007), who evaluated the effects of increasing initial concentrations of technical lindane on its degradation, found that increasing the substrate concentration caused a decrease in the degradation rates for α- and β-HCH isomers of about 20 and 76 %, respectively by a defined microbial consortium consisting of ten bacterial isolates (*Pseudomonas, Burkholderia*, *Flavobacterium,* and *Vibrio*).

Moreover, *Streptomyces* sp. M7 was able to remove the three isomers present in the mixture. However, a decrease of approximately 40 % compared to the removal obtained for each individual isomer was observed. Furthermore, the β-HCH showed higher resistance to degradation than the other two isomers, therefore its removal was approximately 5 % lower at 7 days of incubation. In another work focused on the effect of a mixture of α-/β-HCH isomers and lindane, Rajashekara Murthy and Manonmani (2007) found that degradation of lindane was not affected by the presence of any other isomer. However those experiments were performed in the presence of defined microbial consortium which could produce a synergistic effect in the presence of the isomers mixture.

The intermediate metabolites of α- and β-HCH were identified for the first time in culture supernatants and cell-free extracts. Singularly the presence of intermediates in the supernatant would indicate that they are produced by the strain, as demonstrated by Cuozzo et al. (2009) in plates of *Streptomyces* sp. M7 through the presence of a clear halo around the colony which indicated the capacity of this strain to degrade lindane. Other intermediates such as PCCH and TCCH have been previously detected in α- and β-HCH degradation by *Arthrobacter fluorescens* and *Arthrobacter giacomelloi*, respectively (De Paolis et al. 2013). On the other hand, PCCH and TCCH are products identified through the first and second chloride ion elimination reactions in the degradation pathways proposed by Nagata et al. (2005) for *Sphingobium japonicum* UT26.

The α-HCH isomer is known to exist in two enantiomeric forms, which are converted to its respective β-PCCH enantiomer: (+)-α-HCH is converted to β-(3S,4S,5R,6S)-1,3,4,5,6-PCCH, and (−)-α-HCH becomes β-(3R,4R,5S,6R)-1,3,4,5,6-PCCH. Interestingly, these two β-PCCH enantiomers are then metabolized to 1,2,4-TCB (trichlorobenzene) (Lal et al. 2010). The presence of these intermediate compounds suggests that the TCDN (tetrachlorocyclohexadiene) path could be followed, and two further rounds of dehydrochlorination appear to produce dead-end products in *Streptomyces* sp. M7. Few studies have reported on the toxic effects of HCH metabolites. For instance, it is known that 1,2,4-TCB is moderately to highly toxic to aquatic organisms and is not classified as a human carcinogen, unlike lindane which is in fact classified as carcinogen and neurotoxic. Moreover, its persistence in soil is fivefold shorter than that of lindane. In the case of 1,3-DCB it is not classifiable as carcinogenic to humans (Robles-González et al. 2012).

In both cases, phenolic compounds were not detected after 72 h of incubation, indicating that the process would continue and the benzene rings could not be the final degradation products.

Finally, the microbial degradation of hexachlorocyclohexanes involves the removal of chlorine atoms from these molecules by the action of enzymes with dechlorinase activity. The identification of some of the metabolic intermediaries from this process indicates the tendency of a microorganism to biodegrade these compounds (Datta et al. 2000; Manickam et al. 2008; Raina et al. 2008).

Conclusions

The use of microorganisms to clean up polluted environments is rapidly changing and expanding the field of environmental biotechnology. Although much work continues to be done, our limited understanding of the potential contributions of biological approaches and their impact on ecosystems where they are applied has been an obstacle to making these technologies safer and more reliable.

This study demonstrates for the first time that an indigenous actinobacterium strain, *Streptomyces* sp. M7, has the ability to use α- and β-HCH isomers as carbon sources and it can remove them from the culture medium. In addition, optimum temperature and pH for the isomers removal were established. It has also been demonstrated

that *Streptomyces* sp. M7 has the ability to remove high α-HCH isomer concentrations and its growth is not inhibited by its initial concentration. Moreover, this microorganism was able to remove a mixture composed of α-, β- HCH and lindane, which is important considering that in contaminated sites multiple isomers are normally present together. Finally, pentachlorocyclohexene, tetrachlorocyclohexene, trichlorobenzene, and dichlorobenzene were identified for the first time as metabolic intermediates during α- and β-HCH degradation by *Streptomyces* sp. M7.

The results of this study have highlighted the ability of *Streptomyces* sp. M7 to remove α- and β-HCH isomers from a liquid system under a diverse set of physicochemical conditions. This strain should be considered as a potential agent to bioremediate environments contaminated with organochlorine pesticides.

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