Microorganisms capable to degrade organochlorine pesticides

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The γ-HCH is an organochlorine pesticide used in agriculture and medicine to world level. It has a big tendency to bioaccumulate into the environment. There are many reports indicating that hexachlorocyclohexane (γ-HCH) is present in soil, water, air, plants, agricultural products, animals, food, microbial environments etc. Considered a potential carcinogen and listed as a priority pollutant by the US EPA, γ-HCH is a lipophilic compound and therefore tends to accumulate and concentrate in the body fats of animals and humans. In Salí River, Tucumán, Argentina, lindane was detected 10-fold in relation to the traces permitted concentrations. Hence the development of new technologies to remediate these sites using microorganisms is every time more necessary.

The actinomycetes are Gram positive bacteria with great potential to bioremediate xenobiotics. One strain, *Streptomyces* sp. M7, isolated from organochlorine pesticides (OPs) contaminated sediment was selected for its capacity to grow in presence of lindane as only carbon source. This microorganism was cultured in soil extract medium added of lindane 100 μg L^{-1}, obtaining a maximal growth of 0.065 mg mL^{-1}, similar to the control, with a highest lindane remotion of 70.4% at 30 ºC and pH 7.

When different initial pesticide concentrations (100, 150, 200 and 300 μg L^{-1}) were added in soil medium, an increment of the microbial growth was detected in all the concentrations tested. Also a diminution of the residual lindane concentration was determined in the soil samples in relation to the abiotic controls (29.1; 78.03; 38.81 and 14.42% respectively).

Besides it was determined the optimum *Streptomyces* sp. M7 inoculum when lindane 100 μg Kg^{-1} soil was added to the soil sample. It was 2 g Kg^{-1} soil for obtaining the most efficiently bioremediation process, the lindane removal in these conditions was 67.8% at 28 days of incubation.

Later it was considered necessary to know the pesticide effects on maize plants seeded in lindane-contaminated soil previously inoculated with *Streptomyces* sp. M7. Lindane concentrations of 100, 200, and 400 mg kg^{-1} soil did not affect the germination and vigor index of maize plants seeded in contaminated soils without *Streptomyces* sp. M7. When this microorganism was inoculated at the same conditions a better vigor index was observed and 68% of lindane was removed. These results confirm the potential lindane-contaminated soil bioremediation of *Streptomyces* sp. M7.

There are some reports regarding aerobic degradation of γ-HCH by Gram-negative bacteria like *Sphingomonas* and by the white-rot fungi *Trametes hirsutus*, *Phanerochaete chrysosporium*, *Cyathus bulleri* and *Phanerochaete sordida*. However, little information is available on the ability of biotransformation of organochlorine pesticides by Gram positive bacteria and particularly by actinomycete species. It was demonstrated that *Streptomyces* sp. M7 possesses the LinA enzyme that catalyzes dehydrochlorination of γ-HCH to 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN) via γ-pentachlorocyclohexene (γ-PCCH). The increase of γ-PCCH was detected in the time by Gas Chromatography (GC).

On the other side two actinomycetes defined consortium with lindane biodegradation capacity, were isolated from soil samples from a deposit of organochlorine pesticides in the locality of Santiago del Estero, Argentina. These consortia: Streptomyces sp.: A2-A5-M7-A11 and A2-A5-A8 produced a significant increment of the specific dechlorinase activity (SDA), compared to the individual culture with 1.66 mg L-1 of lindane as the only carbon source. Therefore actinomycete strains could be considered one of the most promising bacterial groups for lindane biodegradation in contaminated environment.

Keywords Bioremediation, Actinomycetes, Pesticides

1. Introduction

Organochlorine pesticides have been used extensively all over the world for public health and agricultural purposes. Currently, their use is being phased out because of their toxicity, environmental persistence and accumulation in the food chain. Hexachlorocyclohexane (HCH) is one of the most extensively used organochlorine pesticides for both agriculture and medical purposes. Though the use of technical mixture containing eight stereoisomers was banned in several advanced countries in the 1970s, many developing countries continue to use lindane (γ-HCH) for economic reasons. Thus, new sites are continuously being contaminated by γ-HCH and its stereoisomers [1-2]. Although only lindane is insecticidal, HCH as a group are toxic and considered as potential carcinogens [3].

For the supply of γ isomer, the other stereoisomers are separated from γ-HCH and dumped as waste at different spots on the production sites causing serious soil pollution [4]. HCH continues to pose a serious toxicological problem at industrial sites where post-production of lindane along with unsound disposal practices has led to serious contamination

and HCH contamination continues to be global issue. These compounds have moderate volatility and can be transported by air to remote locations [5]. Therefore, a possible pathway for bioremediation of contaminated soils is the use of indigenous microorganisms. It knows that the microbial degradation of chlorinated pesticides such as HCH is usually carried out by using either pure or mixed culture systems. There have been some reports regarding aerobic degradation of γ-HCH by Gram-negative bacteria like *Sphingomonas* [6] and by the white-rot fungi *Trametes hirsutus, Phanerochaete chrysosporium, Cyathus bulleri* and *Phanerochaete sordida* [7-9]. However, little information is available on the ability of organochlorine pesticide biotransformation by Gram-positive microorganisms and particularly by actinomycete species, the main group of bacteria present in soils and sediments [10]. These Gram-positive microorganisms have a great potential for biodegradation of organic and inorganic toxic compounds, and also could remove different organochlorine pesticides when other carbon sources are present in the medium as energy source [11-13]. Therefore, the ability of actinomycetes to transform organochlorine pesticides has not been widely investigated, despite studies demonstrating that actinomycetes, specifically of the genus *Streptomyces*, have been able to oxidize, partially dechlorinate and dealkylate aldrin, DDT and herbicides like metolachlor or atrazine [14-17]. In addition to their potential metabolic diversity, strains of *Streptomyces* may be well suited for soil inoculation as a consequence of their mycelial growth habit, relatively rapid rates of growth, colonization of semi-selective substrates and their ability to be genetically manipulated [18]. One additional advantage is that the vegetative hyphal mass of these microorganisms can differentiate into spores that assist in spread and persistence; the spores are a semi-dormant stage in the cycle life that can survive in soil for long periods [19] and impart resistance to low nutrient concentrations and water availability [20].

The aim of this work was to study the bioremediation capacity of indigenous actinomycete strains.

2. Lindane removal by *Streptomyces* sp. M7 in a Soil Extract Medium

Benimeli et al. [21] studied the growth of *Streptomyces* sp. M7 in a Soil Extract Medium (SE) with and without lindane addition. Carbon and Nitrogen composition of SE were 0.5 and 0.01 g L^{-1}, respectively. Nevertheless and despite the pour organic matter in SE, *Streptomyces* sp. M7 was able to grow in this medium at limited time.

When the effect of the temperature (25, 30 and 35 ºC) on the growth of *Streptomyces* sp. M7 in SE was analyzed, it was observed that 25 ºC was the optimal temperature of microbial growth. When *Streptomyces* sp. M7 was cultured in SE supplemented with lindane 100 μg L^{-1}, at different incubation temperatures (Figure 1), a maximum growth of 0.11 mg mL^{-1} was observed at 25 ºC. Significant differences in the biomass were not observed at 30 and 35 ºC. These results would indicate that the optimal temperature for the growth of *Streptomyces* sp. M7 in SE, in presence as well as in absence of the pesticide, is 25 ºC.

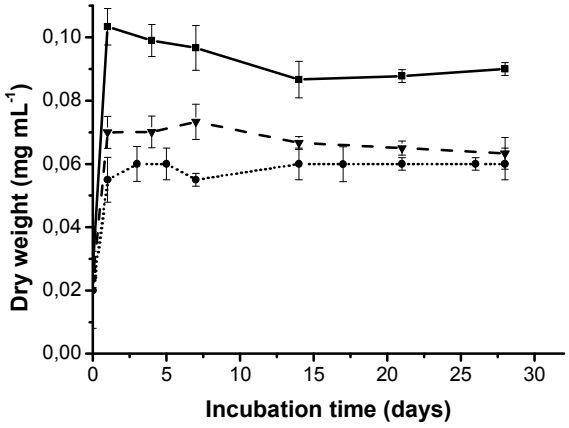

Figure 1. Effect of temperature on the bacterial growth of *Streptomyces* sp. M7 in SE medium amended with lindane 100 μg L^{-1}. 25 ºC (■), 30 ºC (●), 35 ºC (▼). Error bars represent standard deviations. Benimeli et al. [21]

It is important to observe that the presence of lindane in culture medium did not inhibit the growth of *Streptomyces* sp. M7, since significant differences in bacterial growth in SE with and without the pesticide were not observed ($p < 0.05$). Similar results were obtained previously [22], when *Streptomyces* sp. M7 was cultured in Minimal Medium supplemented with lindane 100 μg L^{-1}, suggesting that the pesticide could not been toxic for this microorganism and that would not either be accumulated toxic intermediary metabolites that had an inhibiting effect on the growth.

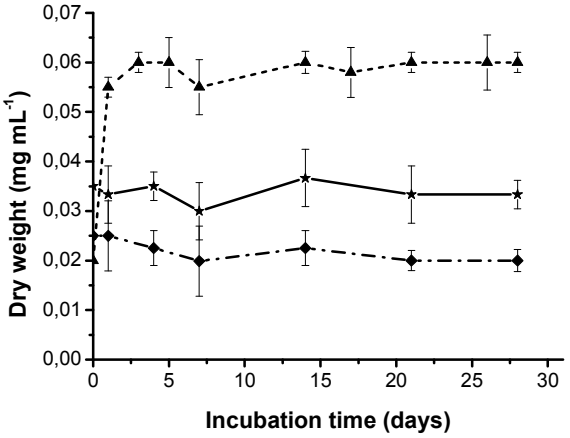

Figure 2. Effect of pH on the bacterial growth of *Streptomyces* sp. M7 in SE amended with lindane 100 μg L^{-1}. pH 5 (★), pH 7 (▲), pH 9 (◆). Error bars represent standard deviations. Benimeli et al. [21].

Streptomyces sp. M7 was able to grow in SE over a relatively wide range of initial pH. No significant differences were observed when the microorganism was cultured at initial pH of 5, 7 or 9 ($p< 0.05$). When *Streptomyces* sp. M7 was cultured in SE added with lindane 100 μg L^{-1}, at different initial pH, a maximum growth of 0.06 mg mL^{-1} was observed at pH 7, nevertheless the microorganism was not able to grow at pH 5 and 9 (Figure 2). The obtained results would indicate that the optimal initial pH for the growth of *Streptomyces* sp. M7 in SE with lindane was 7.
Figure 3 shows the impact of the incubation temperature on the lindane removal by *Streptomyces* sp. M7. The maximum lindane removal was 70.4%, when the microorganism was incubated in SE at 30 ºC. Although the optimum temperature for *Streptomyces* sp. M7 growth, with and without lindane, was 25 ºC, the optimal temperature for the pesticide removal was 30 ºC.

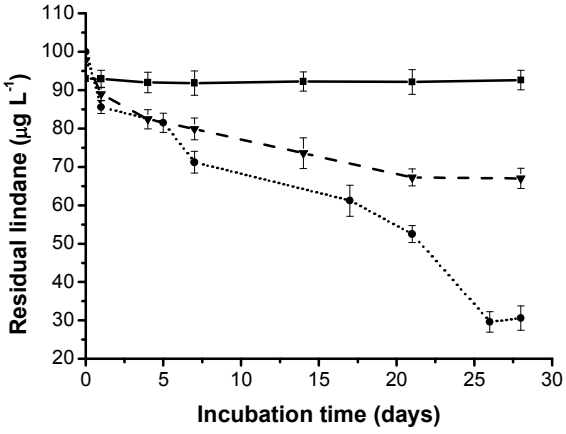

Figure 3. Effect of temperature on the lindane removal by *Streptomyces* sp. M7 in SE amended with lindane 100 μg L^{-1}. 25 ºC (■), 30 ºC (●), 35 ºC (▼).Error bars represent standard deviations. Benimeli et al. [21]

Bachmann et al. [23] reported that temperature of 30 ºC was most favourable for the biodegradation of α-HCH in soil slurry by the mixed native microbial population of the soil. Arisoy and Kolankaya [24] observed that the suitable incubation temperature for maximum growth and degradation activity of lindane by the fungus *Pleurotus sajor-caju* was 30 ºC. Manonmani et al. [25] also observed the degradation of the α-HCH isomer by a microbial consortium under a wide range of temperatures (4-40 ºC) in a liquid culture medium, and 30 ºC was the optimum for α-HCH degradation. Siddique et al. [26] obtained similar results studying the effect of incubation temperature in the biodegradation of lindane by *Pandoraea* sp.; an incubation temperature of 30 ºC was optimum for degradation of γ-HCH (57.7%) in liquid culture and soil slurry (51.9%).

The effect on lindane removal by *Streptomyces* sp. M7 in SE at initial pH of 5, 7 and 9 are presented in Figure 4. Removal of pesticide (47.2 and 38.0%) was observed at initial pH of 5 and 9 respectively at 28 days of incubation. The highest removal ability of *Streptomyces* sp. M7 (70.4%) was noted at an initial pH 7. The fate of organic pollutants in

the environment is influenced by environmental factors, such as pH and temperature, affecting the activity of microorganisms. *Streptomyces* sp. M7 was able to remove lindane over a wide range of pH in SE.

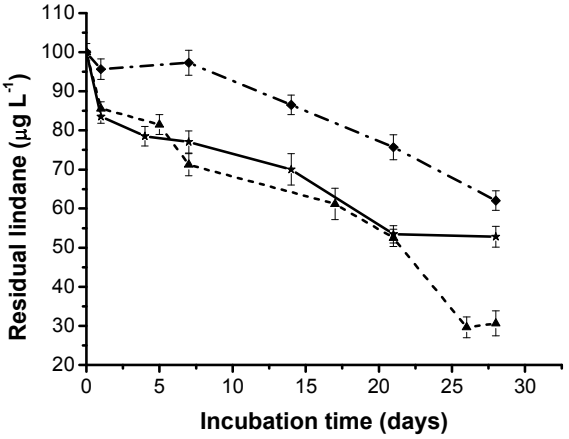

Figure 4. Effect of pH on the lindane removal by *Streptomyces* sp. M7 in SE amended with lindane 100 μg L^{-1}. pH 5 (★), pH 7 (▲), pH 9 (◆). Error bars represent standard deviations. Benimeli et al. [21]

In previous studies, Arisoy and Kolankaya [24] reported that pH 5 was the optimum for both growth and degradation activity of lindane by the fungus *Pleurotus sajor-caju*. Manonmani et al. [25] examined the influence of pH on the degradation of the α-HCH isomer in a basal mineral medium by an acclimated consortium of microorganisms. They found that a pH range of 6-8 was most favourable for growth and degradation of the pesticide. Siddique et al. [26] reported that *Pandoreae* species shown the highest degradation of α-and γ-HCH at an initial pH of 8 in liquid medium.

3. Lindane removal by *Streptomyces* sp. M7 in sterile soil samples

Benimeli et al. [27] studied the growth of *Streptomyces* sp. M7 in sterile soil samples added of different lindane concentrations during 4 weeks; simultaneously, lindane removal by *Streptomyces* sp. M7 was determined by gas chromatography; similar experiments were carried out without lindane as controls. As shown in Figure 5, the cell concentration increased during incubation and significant differences in the growth were not observed at different lindane concentrations added, as in the control without lindane. These results would indicate that the growth of *Streptomyces* sp. M7 in soil was not affected by the lindane concentrations assayed, suggesting that the microorganism could tolerate or maybe degrade the pesticide by producing the necessary dehalogenase enzymes as was demonstrated by Nagata et al. [28] for *Sphingomonas paucimobilis* UT26.

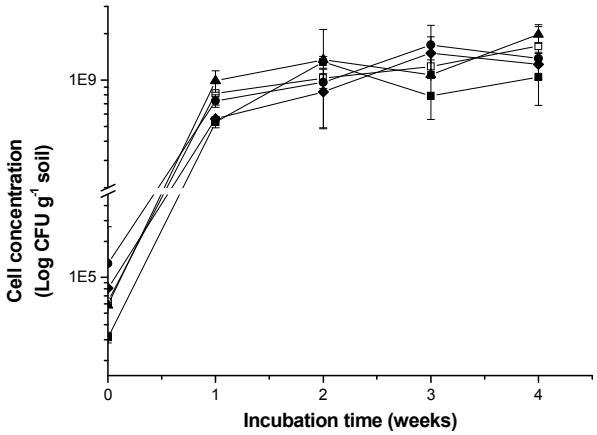

Figure 5. *Streptomyces* sp. M7 growth in sterile soil samples, during 4 weeks of incubation, without (□) and with 100 (■), 150 (●), 200 (▲) and 300 (◆) μg Kg^{-1} of lindane. Error bars represent standard deviations. Benimeli et al. [27].

Influence of different initial concentrations of lindane on pesticide removal was evaluated by determining residual lindane in the soil samples. At initial lindane concentrations of 100, 150, 200 and 300 μg Kg^{-1}, removal of pesticide after four-week incubation was 29.1, 78.0, 38.8 and 14.4%, respectively. Lindane concentrations in uninoculated

control soils were unchanged after four weeks of incubation. This inhibition in the removal ability of *Streptomyces* sp. M7 could be due to the toxicity of metabolites, which might have former but not detected in this study by the methods employed. For demonstrating that, it could be necessary to measure the intermediary metabolites of lindane degradation as was determined by Nagata et al. [28] for *Sphingomonas paucimobilis* UT26.

Awasthi et al. [29] found that at low initial concentrations of endosulfan in soil (50 and 100 μg g^{-1} soil), the degradation was very rapid in inoculated soils; at higher concentrations, the degradation rates were slower, leading to a total inhibition of the degradation activity at the initial concentration of 10 mg g^{-1}.

Similar findings were reported by Okeke et al. [30], who studied the ability of a *Pandoreae* sp. strain to remove lindane in liquid and soil slurry cultures. Their results indicated that the rates and extent of lindane removal increased with increasing concentrations up to 150 mg L^{-1} but declined at 200 mg L^{-1}, after 4-6 weeks of incubation.

However, in a similar study, Liu et al. [15] found opposite results when they inoculated a *Streptomyces* sp. strain in sterile soil samples spiked with metolachlor and observed that the amount of residual metolachlor was higher at lower herbicide concentrations, after one-week incubation.

Our results indicate that *Streptomyces* sp. M7 could remove lindane from soil but at limited pesticide concentration and the viable bacterial count of the soil culture indicated growth and survival of *Streptomyces* sp. M7. This is not surprising because the soil contains available organic nutrients that the bacterium may prefer for growing. Then, lindane could be used as a secondary substrate source as it was demonstrated previously by Benimeli et al. [22] in culture medium where glucose at limited concentration was added.

To arrive at the optimal number of bacterial cells for effective removal of lindane, the influence of different inoculum sizes (0.5 to 4.0 g kg^{-1} ww soil) was studied in sterile soil samples with lindane 100 μg kg^{-1} ww soil (Figure 6). 28 days after inoculation with different *Streptomyces* sp. M7 concentrations into lindane-amended autoclaved soil, the cell population increased rapidly in 2 weeks and was followed by a stationary phase from 2 to 4 weeks. This growth profile was observed for all assayed bacterial concentrations and was similar in contaminated as well as non-contaminated soil samples. These results reinforce the hypothesis according to which lindane concentrations present in soil were not toxic for *Streptomyces* sp. M7. The inoculum 2 g cells Kg^{-1} soil (ww) significantly led the maximum bacterial count (9.0 x 10^{12} CFU Kg^{-1} ww of soil).

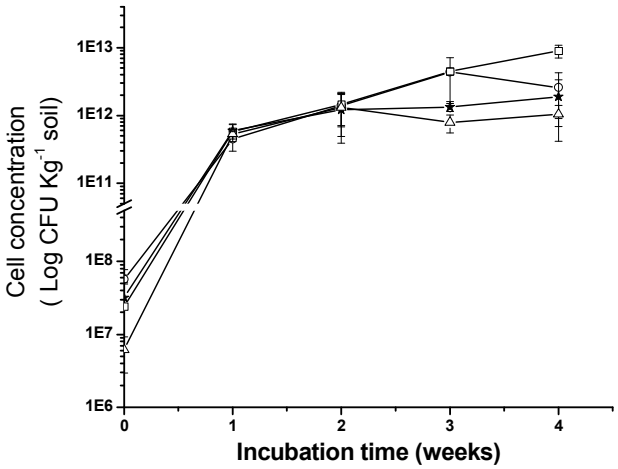

Figure 6. Effect of inoculum size on the growth of *Streptomyces* sp. M7 in sterile soil samples, during 4 weeks of incubation. Symbols used: (△) 0.5 g Kg^{-1} soil, (★) 1.0 g Kg^{-1} soil, (□) 2.0 g Kg^{-1} soil, (○) 4.0 g Kg^{-1} soil. Benimeli et al. [27].

A substantial decline of the residual lindane at different inoculum concentrations was observed within 0-2 weeks of incubation whereas the compound did not disappear from the uninoculated sterile control. However, the percentage of lindane removal was not entirely proportional to the amount of *Streptomyces* sp. M7 initially added to the soils, indicating that the two parameters are not in direct proportion. Maximal pesticide depletion (56.0%) was observed at 2 g cells Kg^{-1} soil (ww) and thereafter decreased at 4 g cells Kg^{-1} soil (Table 1).

Table 1. Effect of inoculum size on the lindane removal by *Streptomyces* sp. M7 in sterile soil samples, after 4 weeks of incubation. Lindane initial concentration: 100 μg Kg^{-1} ww soil. Benimeli et al. [27]

Inoculum size g Kg^{-1} soil (ww)	Lindane removal (%)
0.5	**24.4**
1.0	**30.8**
2.0	**56.0**
4.0	**53.6**

In a similar work, Liu et al. [15] found that approximately 80% of the pesticide metolachlor was transformed in a sterile soil inoculated with a *Streptomyces* sp. strain after one week of incubation; however, the rate of metolachlor transformation was not proportional to the inoculum size. Ajithkumar et al. [31] studied the inoculation of 3-chloro and 4-chlorobenzoate-treated sterile soil with a chlorobenzoate-degrading *Pseudomonas aeruginosa* 3mT; they probed that three inoculum sizes (1, 2 and 4 mg cells Kg^{-1} soil) were effective in degrading the chemical in the soil, but the degradation was faster with a larger inoculum. On the other hand, Johri et al. [32] found that an increase in the inoculum size of *Sphingomonas paucimobilis* resulted in an increase in the degradation rate of the HCH isomers in the culture medium, indicating that the two parameters are in direct proportion.

4. Bioremediation of lindane contaminated soil samples by *Streptomyces* sp. M7

Non-sterile soil spiked with lindane 100 μg Kg^{-1} was inoculated with 2 g Kg^{-1} *Streptomyces* sp. M7 and after 14 days incubation *Zea mays* seeds were grown in this soil. Concentrations of residual lindane in soil were monitored periodically to assess the effectiveness of the inoculated strain in the bioremediation of the soil. On the other hand, it was investigated the pesticide effects on maize plants seeded in lindane-contaminated soil previously inoculated with *Streptomyces* sp. M7 [27].

A strong decrease in the residual concentration of added lindane was observed in strain *Streptomyces* sp. M7-inoculated soil, after 14 days of incubation 68% of lindane was removed (Figure 7). There were no evident changes in the concentration of the pesticide in the uninoculated soil, indicating that the native microorganisms were not involved into the pesticide removal.

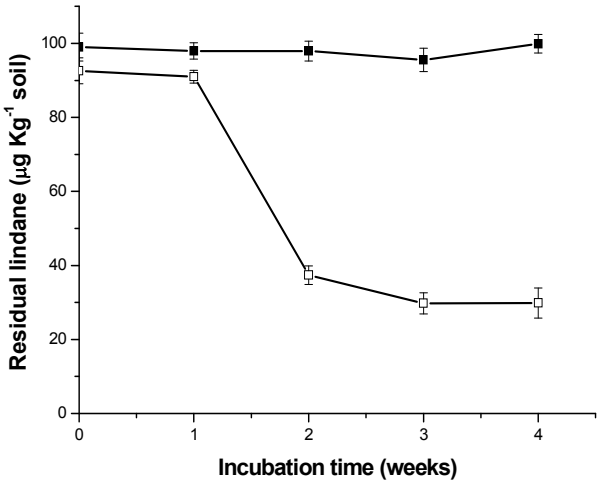

Figure 7. Removal of 100 μg Kg^{-1} of lindane by *Streptomyces* sp. M7 in non-sterile soil samples, during 4 weeks of incubation. 2.0 g cells (ww) Kg^{-1} soil was added as inoculum. Symbols used: (□) residual lindane in uninoculated control soil, (■) residual lindane in inoculated soil. Benimeli et al. [27].

Table 2. Growth, seed germination and seedling vigour of maize seeds in lindane bioremediated soil by *Streptomyces* sp. M7. Benimeli et al. [27].

	Control	Treated[a]	Bioremediated[b]
Root (cm)	14.2 ± 4.7	15.4 ± 3.9	16,0 ± 2.8
Shoot (cm)	5.3 ± 1.1	5.1 ± 1.1	5.3 ± 1.0
Leaves (cm)	13.7 ± 2.1	14.7 ± 2.1	14.0 ± 1.5
Germination (%)	100	84	100
Vigour index	195 ± 6	172 ± 5	213 ± 4

[a] Soil treated with γ-HCH (100 μg Kg^{-1} soil)
[b] Soil treated with γ-HCH (100 μg Kg^{-1} soil) and bioremediated with *Streptomyces* sp M7 (2 g Kg^{-1} soil)

In soil bioremediated with *Streptomyces* sp. M7, normal germination (100%) and an increased in the seedling vigour were observed, compared to the control maize seeds (Table 2). It is evident that the pesticide was removed effectively by the inoculated microorganism before seed germination began and the involvement of indigenous microflora capable of metabolizing lindane was not observed.

In a similar study by Krueger et al. [33], soybean and pea seedlings susceptible to the herbicide dicamba were protected from its deleterious effect by inoculating soils with dicamba degrading microorganisms. Ajithkumar et al. [31] demonstrated that chlorobenzoates adversely affect the seed germination and seedling vigour of tomato. However, the bioremediation of the soil with *Pseudomonas aeruginosa* 3mT protected the tomato seeds resulting in the normal germination and seedling vigour. Bidlan et al. [34] observed that the effect of tech-HCH on germinating radish and green gram seeds was nullified by treatment of contaminated soil with a HCH-degrading microbial consortium.

Although no maize plant growth promotion was observed when *Streptomyces* sp. M7 was inoculated into the contaminated lindane soil samples, the fact that this pesticide can be removed and therefore its uptake by the plant into biomass could be lower, allows the use of microorganisms for soils bioremediation, avowing the contamination through the food chain.

5. Lindane and metabolites determination in cell-free extract by GC-mass spectrometry analysis

The first signs of the aerobic lindane degradation were determined by Nagata et al. [28], who demonstrated that *Sphingobium japonicum* UT26 possesses a dechlorinase enzyme, LinA (γ-hexachlorocyclohexane dehydrochlorinase, EC 4.5.1), encoded by the linA gene that catalyses two dehydrochlorination steps: γ-HCH to 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN) via γ-pentachlorocyclohexene (γ-PCCH). In addition to γ-HCH and γ-PCCH, α- and δ-isomers of HCH were also dehydrochlorinated by LinA, whereas β-HCH was not [35]. Furthermore, it was experimentally confirmed that dehydrochlorination of γ-HCH proceeds by a 1,2-ante dehydrochlorination reaction [36].
Regarding the environmental problems caused by lindane and the current lack of information about the presence of dechlorinase activity in *Streptomyces*, the aim of this point was to demonstrate, for the first time, a specific dechlorinase activity in *Streptomyces* using lindane as substrate. In order to determine lindane and metabolites in cell-free extract of *Streptomyces* sp. M7, the strain was grown in flasks with 250 ml of MM containing γ-HCH 100 μg ml^{-1} and incubated at 30 ºC at 100 rpm for 48 and 96 hours. At the beginning of the experiment, the inoculum was 150 μl of concentrated spore suspension (10^9CFU ml^{-1}). The lindane and its metabolites were extracted by solid phase extraction (SPE) using C18 columns, evaporated to dryness under reduced pressure and the residue was re-suspended in hexane. Routine quantitative determinations of lindane (γ-HCH) γ-pentachlorocyclohexene (γ-PCCH) and 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN) were carried out with gas chromatograph-micro electron capture detection (GC-μECD) [37-38].

The Gas chromatography results of the cell-free extracts obtained at 48 and 96 of growth of *Streptomyces* sp. M7 revealed the appearance of γ-PCCH (Rt 6.26 min) and 1,4-TCDN,(Rt 5.29 min), the first and second product of the lindane catabolism by the specific dechlorinase in the catabolic way proposed by Nagata et al. [36]. The relative abundance of γ-PCCH and the 1,4-TCDN increased one and half times, at 96 h compared to 48 h of growth (Table 3).

Table 3. Relative abundance analysis by GC-Mass of γ-HCH and intermediates γ-PCCH, and 1,4-TCDN during aerobic degradation of γ-HCH by *Streptomyces* sp. M7. Cuozzo et al.[38]

	Lindane and metabolites	Retention time (min)	Relative abundance*
Cell-free extract at 0 h	γ-HCH	7.78	732750.96 ± 1041.37
Cell-free extract at 48 h	γ-HCH	7.78	674895.41 ± 1961.84
	γ-PCCH	6.26	38270.56 ± 650.79
	1,4-TCDN	5.29	14795.47 ± 451.97
Cell-free extract at 96 h	γ-HCH	7.78	561952.29 ± 2310.10
	γ-PCCH	6.26	70372.05 ± 3268.05
	1,4-TCDN	5.29	25988.30 ± 992.09

*The relative abundance is referred to the ions of different atomic or molecular mass (mass-to-charge ratio) within a sample. It frequently refers to the measured relative abundances of isotopes of a given element.

However, these results indirectly demonstrated the presence of one specific enzyme in the lindane degradation way from *Streptomyces* sp. M7. This is the first report on dehalogenase activity in actinomycetes with lindane as specific substrate. It has only been reported in *Sphingomonas* [36] and a putative 2,5-dichloro- 2,5cyclohexadiene-1,4-diol dehydrogenase (2,5-DDOL dehydrogenase) was reported in *Frankia* [39] .Genetic studies of this strain are necessary for a proper understanding of the principle of its ability to degrade different chlorinated hydrocarbon compounds.

6. Lindane removal by actinomycetes defined consortia in Minimal Medium

Actinomycete strains were isolated from soil samples from an illegal deposit of organochlorine pesticides in the state of Santiago del Estero, Argentina [40]. The lindane removal ability of isolated actinomycete, individually and as a mixed culture under controlled laboratory conditions was evaluated. Thus, lindane microbial degradation has been studied using pure and mixed microbial cultures. Mixed cultures have shown to be more suitable for bioremediation compared with pure cultures because their biodiversity can enhance environmental survival and increase the catabolic pathways available for contaminant biodegradation [41].

Streptomyces sp. M7 (M7), *Streptomyces coelicolor* A3 (ScA3) and four actinomycete isolates (A2, A5, A8 and A11) were cultivated, as pure and mixed cultures, in Minimal Medium with lindane (1.66 mg L^{-1}). Microbial cells were used to obtain cell-free extracts for dechlorinase activity assays and the supernatants from these cultures were used to determine residual lindane by gas chromatography. The actinomycetes isolated were characterized by 16S rDNA amplifications, sequenced and were mostly identified as members of *Streptomyces* genus.

Enzyme activities ranged between 5.14 to 143.10 μmolCl^-/h/mg protein and percentage of lindane removal ranged between 11 to 62%. The mixed culture A2-A5-M7-ScA3 showed the best activity but consortia A2-A5-ScA3, A8-A11-M7 and A2-A5-A11-M7 showed the minimal residual lindane value. Because the no linear relationship between dechlorinase specific activities (DEA) and residual lindane concentrations (RL) was observed, we analyzed the ratio between these two parameters and the consortium A2-A5-M7-A11 was selected as the most efficient for lindane biodegradation.

These native streptomycetes present ability to grow as microbial consortium and to remove lindane. These actinomycete consortia can be potentially used as a practically convenient technology for the biological removal of lindane.

7. Conclusions

The present results clearly suggest that *Streptomyces* sp. M7 has the capacity to growth in a soil extract broth, a nutritionally poor medium, in the presence of lindane and to remove the pesticide. Since streptomycetes are metabolically diverse and relatively resistant to adverse conditions that may occur in the soil environment, *Streptomyces* sp. M7, could be considered as attractive targets for lindane degradation *in situ*. In addition, the development of bioremediation processes using indigenous microorganisms is advantageous. To work with isolated strains indicate that they are already adapted to the substrate, local soil and climatic conditions. On the other hand regulatory and legislation

issues are simpler, compared to the introduction, release of exogenous and genetically modified organisms into the environment.

Streptomyces sp. M7 bioremediation activity is not inhibited by the natural soil microbial flora. Moreover, *Streptomyces* sp. M7 growth was not inhibited by 300 μg kg^{-1} of lindane. For the first time a dehalogenase activity in a *Streptomyces* strain with lindane as specific substrate was determined.. Our results demonstrate that synthesis of the dechlorinase enzyme was induced by the presence of lindane and this activity was optimal in the culture medium at pH 7-9.

However, our data pertain to laboratory studies and field trials are promising to further develop for bioremediation under natural conditions using *Streptomyces* sp. M7.

8. References

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