Evidence for Enhanced Dissipation of Chlorpyrifos in an Agricultural Soil Inoculated with Serratia Rubidaea Strain ABS 10

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Abstract

Important mineralization of ^{14}C-chlorpyrifos was found in a Tunisian soil exposed repeatedly to this insecticide. A bacterial strain able to grow in minimal salt medium (MSM) supplemented with 25 mg L^{-1} of chlorpyrifos was isolated from this soil. It was characterized as *Serratia rubidaea* strain *ABS 10* using morphological and biochemical analyses, as well as 16S rRNA sequencing. In liquid culture *S. rubidaea* stain *ABS 10* was able to almost entirely dissipate chlorpyrifos within 48 hours of incubation. Although, *S. rubidaea* strain *ABS 10* was able to grow on MSM supplemented with chlorpyrifos and to dissipate it in liquid culture, it was not able to mineralize ^{14}C-chlorpyrifos. Therefore, one can conclude that the dissipation capability of this bacteria might be attributed to its capacity to adsorb CHL. In both non-sterile and sterile soil inoculated with *S. rubidaea* strain *ABS 10*, chlorpyrifos was more rapidly dissipated than in respective controls.

Introduction

After the ban of organochlorines (such as DDT, dieldrin or heptachor) and carbamates (such as carbofuran), organophosphorus insecticides (OPs) have been extensively used in agriculture as a substitute insecticides because of their high efficiency and their supposed relatively low persistence and low effect on the environment (Yang et al. 2005). However, the widespread use of OPs has led to severe environmental issues because these compounds are often transported away from the sites where they have been applied. For instance, OPs may enter aquatic environments *via* soil percolation, air drift or surface runoff (Liang et al. 2011). Numerous reports indicate that OPs are toxic to humans causing metabolic disorders and neuropathy in response to both acute and chronic exposure (Iyer and Makris, 2010). Among OPs, chlorpyriphos (CHL) with a broad-spectrum activity is one of the most frequently used. CHL has relatively low water solubility (2 $mg.L^{-1}$) and is spontaneously hydrolyzed to 3,5,6-trichloro-2-pyridinol(TCP) (Li et al. 2008). Although being applied on crop cover, a large proportion of this insecticide reaches the soil, where both abiotic (i.e. sorption chemical degradation) and biotic (biodegradation) processes control the fate and the activity of this compound. Particularly, the half-life (DT_{50}) of chlorpyrifos in soil is usually between 10 and 120 days, depending on the soil type, climate and other environmental conditions such as composition of microbial communities (Abraham and Silambarasan, 2016). Indeed, microbial activity has been deemed the most influential and significant cause of the OPs pesticides removal (Li et al. 2005). The ability of microorganisms to degrade OPs is viewed as the primary means of removing these agrochemicals from soils (Cycon et al. 2013). A range of species of bacteria capable of degrading OPs especially CHL by co-metabolism or by using pesticides as a source of carbon and phosphorous (Cycon et al. 2013) has been reported by several researchers, *Enterobacter* stain B-14, *Stenotrophomonas* sp. Strain YC-, *Sphingomonas* sp. Strain, Dsp-2, *Paracoccus* sp. strain TRP, *Bacillus pumilus* strain C2A1, *Cupriavidus* sp. DT-1, *Alcaligenes faecalis*, *Flavobacterium* sp, *Klebsiella* sp, *Serratia* sp, *Pseudomonas* sp (Silambarasan and Abraham 2013, Chisti et al. 2013). Furthermore, it has been observed that repeated application of OPs lead to the enhancement of their biodegradation in reason of the selection of degrading microbial populations (Singh et al. 2003). More

investigation on microbial degradation of the OPs are required in order to not only understand processes involved in their degradation but also to be able to develop bioremediation strategies to clean contaminated soils (Cycon et al. 2013). Indeed, microbe based remediation relying on biosorption, bioaccumulation, biotransformation or biomineralisation processes (Ayangbenro and Babalola 2017), has received an increasing attention as it seems an applicable and cost-effective biotechnology to clean up soils polluted with OPs (Singh et al. 2006; Chen et al. 2011). On the one hand, biotransformation and biomineralization both contribute to the transformation of the pollutant, the last being viewed as the gold standard as it leads to the complete transformation of the pollutant. Moreover, biosorption and bioaccumulation contribute to the stabilization of pollutant in the environmental matrice by forming non-extractable residues that are almost not-transferable to other compartment of the environment. From this point of view, these last two processes are of interest to clean contaminated water by using microbial biomass as a low-cost biobased adsorbent (Khadivinia et al. 2014) or to stabilize pollutant in a given contaminated matrices' in order to avoid further dispersion in the environment.

Hence, the present study aims not only to estimate the adaptation of microbial community of an agricultural soil regularly exposed to CHL to its enhanced mineralization, but also to isolate and characterize bacterial isolates able to grow on CHL as sole carbon source. The ability of one bacterial isolate to dissipate CHL was estimated in liquid culture and in soil microcosms incubated under controlled conditions in the laboratory in order to estimate its interest for bioremediation purposes.

Materials And Methods

Chemicals and culture medium

The tested compounds CHL (99.5% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents (dimethyl sulfoxide DMSO and dichloromethane) used in this study were of high purity and analytical grade. Organic free water was prepared with a Milli-Q/Milli-Ro system (Millipore Corp., Bedford, MA, USA). Stock solutions of chlorpyrifos were prepared at 5000 mg L^{-1} in dimethyl sulfoxide (DMSO). For each experiment, 1 mL of CHL was added in the medium. For the microbial assays, mineral salt medium (MSM) and Luria-liquid broth (LB) were used. The medium (MSM) contained 20 mL of K_2HPO_4, 10 mL of $MgSO_4$ $7H_2O$, 10 mL of NaCl, 1 mL of $CaCl_2$, 2 g of boric acid, 0,2 g of $ZnSO_4$, 0,8g of $CuSO_4$, 0,25 g of NaNO, and 0,05g of $CO(NO_2)_2$, 1mL of $FeSO_4$ $6H_2O$ was added per liter of distilled water. The medium (LB) contained 10 g of peptone, 5 g of yeast extract and 10 g of NaCl per 1 liter of distilled water (pH 7.0). After autoclaving (121°C, 20 min) and cooling the medium was supplemented with a suitable CHL solution prepared as described above.

Sampling

The soil sample used in this study was collected from field site located in Mornag approximately 20 km away from Tunis (36° 67' 70.39 N, 10° 27' 53.78 E), Tunisia. The sampling site has been in use for

intensive agricultural practices since long and this soil has received leachates after application of chlorpyrifos for several years. Soil sample was collected from a depth of 20 cm. Soil was mixed thoroughly and plant debris were removed. Then soil was sieved at 2mm and kept at 4°C until its use (for less than 3 weeks).

Mineralization of ^{14}C-CHL in soil microcosms

The potential of the soil microbial community to mineralize chlorpyriphos (CHL) was evaluated using ^{14}C-labelled CHL (Izotop, specific activity = 118 µCi/mg) as described by El-Sebai et al. (2007). Four individual replicates of 20 g soil microcosms were studied. For each replicate 1 mL of a methanol solution comprising 60 mg.L^{-1} of ^{14}C-CHL and 0.068 µ Ci of ^{14}C-CHL was applied on an aliquot of 1g of dried soil. After evaporation CHL contaminated soil was thoroughly mixed with the rest of soil. Soil humidity was then adjusted to 40 % of the water holding capacity (WHC) and kept constant all along the 70 days of incubation in the radiorespirometer. $^{14}CO_2$ evolved from ^{14}C-CHL was quantified by liquid scintillation counting (LS 6500 Multi-Purpose Scintillation Counter, Beckman) using ACSII scintillation fluid (Amersham) (Storck et al. 2017).

Enrichment procedure, isolation and characterization of chlorpyrifos degrading strain

Preliminary screening experiments were performed to obtain strains that were tolerant to CHL. The study was conducted as described previously by Ben Salem et al. 2016.

Only one soil with three replicates was used in this study. Fresh soil sample was divided in six subsamples of 50 g dry weight equivalent. Aqueous CHL solution were prepared at 4.8g. L^{-1} (which corresponds to 10 times the recommended dose agronomic purpose) the day of its application starting from Robust ® formulated solution. The duration of experiment was two weeks.

Soil samples were incubated at room temperature under laboratory conditions. They were watered every two days to keep soil humidity at 40% of water holding capacity (WHC). Every two days, they were irrigated with 10mL of commercial chlorpyrifos solutions as 10X concentrated solutions of pesticides to exert a selective pressure favorable to the emergence of degrading bacterial populations. Three samples were not treated with CHL but with equivalent amount of pure water (control). Two-days after CHL application, soil was sampled to immediately carry bacterial isolation. Briefly, one g of soil was added to 10 mL of physiological water (NaCl 9‰) and serially ten-times diluted. 100 µL of 10, 10^{-2} and 10^{-4} dilutions were streaked on PCA plates that were incubated for 16 hours at 37°C. Each colony growing on the plate was purified using the Z streak technique.

Primary distinction between all the isolates was based on the size, color and morphology of their colonies on the PCA plates. Bacterial colonies showing different morphologies were selected and further characterized using API 20E (Biomérieux, Lyon, France) following the recommendations of the Bergey's manual of systematic bacteriology (Krieg et al. 1984). In addition, 16S rRNA amplicon generated by PCR using the 27f and 1492r universal primer pair (Gürtler and Stanisich 1996) from DNA extracted from the bacterial isolate was sequenced as previously described (Devers et al. 2008). 16S rRNA sequence was compared to other sequences available in GenBank database (http: //www. ncbi.nlm.nih.gov/genbank) using the BlastN search analysis (http://www.blast.ncbi.nlm.nih.gov/). Sequence was deposited in the Genbank database (SUB8916609 S10 MW494965).

Bacterial growth kinetics in different liquid media

To investigate the growth of *Serratia rubidaea* strain *ABS 10* with chlorpyrifos, 200 µl of strain (OD 600 = 0.8) were inoculated into 20 mL of either MSM or nutrient broth medium added with CHL at a final concentration of 25 $mg.L^{-1}$. The culture was incubated at 30±2°C on a rotary shaker at 120 rpm. The bacterial growth was regularly monitored for 5 days by measuring the turbidity of the culture using a spectrophotometer at 600 nm.

Inoculum preparation

A bacterial colony was inoculated in LB medium and grown to reach the exponential phase. It was then collected by centrifugation at 5000 g for 5 min. The cell pellet was washed twice with 0.9% of sterile NaCl and then re-suspended in NaCl to obtain the bacterial suspension at a concentration of approximately 3.10^6 CFU /mL. The cell density (OD 600 nm) was measured using UV-Visible spectrophotometer (Lu et al. 2013)

Dissipation of chlorpyrifos by *S. rubidaea* strain ABS 10 in aqueous medium

CHL dissipation studies were performed in 250 mL Erlenmeyer flasks containing 100 mL of sterile MSM supplemented with CHL at 25 mg L^1 and inoculated with 1 mL of bacterial strain (approximately 3×10^6 $cells.mL^{-1}$). Un-inoculated media comprising with the same concentration of CHL were used as control. All the samples were incubated at 30 ± 2°C on a rotary shaker at 120 rpm for 5 days. Samples were periodically taken from the culture under aseptically conditions to measure the remaining pesticide concentration the culture medium.

Dissipation of chlorpyrifos by *S.rubidaea* strain *ABS 10* in soil

To study the dissipation of chlorpyrifos in sterilized (SS) or natural (NS) soil inoculated with *S .rubidaea* strain *ABS 10* (B) or not *S. rubidaea* strain *ABS 10.* Briefly, 100 g of sterilized soil (SS) or natural soil (NS) was placed in a 250 ml Erlenmeyer flask, inoculated or not with 30 ml of a *S. rubidaea* strain *ABS 10* suspension (B) containing 3×10^6 cells.mL^{-1} and treated with CHL at 25 mg kg^{-1}. The amount of carbon, nitrogen and phosphorous were calculated using the relationship C/N/P 100:10:1. The sources of carbon, nitrogen and phosphorous were glucose, $(NH_4)_2SO_4$ and K_2HPO_4 respectively (Martin et al. 2007). Sterilized soil not inoculated with *S. rubidaea* strain *ABS 10* was used to estimate abiotic dissipation of CHL (Pino et al. 2011). All flasks were incubated in an incubator at 30 ± 2 °C. Samples were periodically removed aseptically to determine the pesticide concentration. Each treatment was performed in triplicate (n_{tot}=12).

Analytical methods

At regular intervals, 5–10 mL cultures were withdrawn from aqueous medium and centrifuged at 7200 × g for 10 min to obtain a cell-free medium. CHL was extracted twice from the supernatant with an equal volume of dichloromethane (DCM). Organic layers of DCM were pooled and evaporated at 28 ± 2 °C. For the analysis of CHL in soil, 5 g of soil samples was mixed with 10 ml of dichloromethane. The samples were ultra-sonicated for 30 min at 30°C. After that, the mixture was centrifuged for 30 min on a rotary shaker at 120 rpm. Then the samples were allowed to stand until the soil had settled, and the clear supernatant was used to determine the pesticide concentration by GC-MS. Levels of CHL were measured by GC-MS using an Agilent 6850N gas chromatograph (Agilent Technologies, USA), equipped with an Agilent6973 MS detector. A capillary column HP-5MS (30 m, 0.25 mm, 0.50 mm) was used while chromatographic separation was achieved with the following method: the GC oven temperature was initially set at 70°C, for 2 min, and raised to 270°C at a rate of 20°C/min and held for 10 min. The injector and detector were set at 250 and 280°C, respectively. The carrier gas Helium was used as at a constant flow rate of 1 mL/min. Electron impact (70ev) mass spectra were recorded from 100 to 550 amu (atomic mass unit).

Data analysis

The four kinetic models proposed by the FOCUS working group on pesticide degradation kinetics (FOCUS, 2006) were used to calculate pesticide dissipation kinetic parameters. The four kinetic models proposed by the FOCUS working group on pesticide degradation kinetics (FOCUS, 2006) were used to calculate pesticide dissipation kinetic parameters: the single first order kinetic model (SFO), and the biphasic models hockey stick (HS), first order multi-compartment model (FOMC) and double first order in parallel model (DFOP).

Data obtained from the dissipation experiments were fit to the exponential decay model. The first-order model and the DT_{50} was calculated as follows:

$$C_t = C_0.e^{-kt} \qquad (1)$$

$$t_{1/2} = DT_{50} = Ln\,(2)/k \qquad (2)$$

Where C_t is the concentration of pesticide remaining in MSM or soil after t days, C_0 is the initial concentration of pesticide in MSM or soil. k and t are the rate constant (d^{-1}) and degradation time in days respectively (Focus, 2006)

Statistical analysis

All the experiments were performed in triplicates. The data were statistically analyzed using two-way analysis of variance (ANOVA). When significant differences test ($P \leq 0.05$) were observed, the means were separated using Graphpad Prism, v7.00.

Results And Discussion

Mineralization of ^{14}C- CHL in soil microcosms

After a ten days lag-phase, the soil microbial community efficiently mineralized ^{14}C-CHL reaching up to 70% of mineralization of initial radioactivity applied (Fig. 1). During the exponential phase, the rate of mineralization was 1.3 % ^{14}C-CO_2 per day. The mineralization curve had sigmoid shape characteristics from microbial community adapted to enhanced degradation of pesticides. One could hypothesize that in response to the repeated CHL treatments applied to this arable soil the microbial community adapted to its enhanced biodegradation that provides nutrient and energy sources for the growth CHL-degrading community.

Indeed, the emergence of degrading microorganisms, among which CHL-degraders, has been observed in soils regularly exposed to different pesticides (Singh, 2009). The use of pesticides as nutrient and energy sources provides a selective advantage over other microorganisms (Copley, 2009). Adaptation to enhanced degradation seems to be a common trait to soil microorganisms in response to repeated pesticide treatments (Crouzet et al. 2010; De Andrea et al. 2003; Hussain et al. 2011; Vischetti et al. 2008; Weaver et al. 2007). This is an environmental-friendly functional trait because it decreases the persistence of pesticide residues in the soil as well as their dispersion in the environment and their ecotoxicological impact to non-target organisms and supported ecosystem services (Topp et al. 2004).

Isolation and characterization of bacterial strain growing on CHL medium

In order to isolate bacterial strain able to transform CHL, enrichment cultures on MSM medium added with CHL were conducted. In total we have been able to isolate four bacterial isolates able to grow on MSM-CHL medium. Among these isolates, only one has the capability to grow on liquid MSM-CHL. This isolate is aerobic, none spore forming, Gram negative, straight rods with rounded ends bacteria producing small circular colonies on the nutrient agar plates. Its freshly grown culture showed positive tests for oxidase, catalase, and exhibited the ability of nitrate reduction (Table 1). Its 16S rRNA gene sequence is 99% similar to *Serratia rubidaea* strain NBRC 103169 (Ac n° NR_114232), JCM1240 (Ac n° NR_024644), and DSM 4480 (Ac n° NR_114716). Consequently, we proposed to name it *Serratia rubidaea* strain ABS S10 (S10).

Table 1
Morphological and biochemical characteristics of bacterial strain *Serratia rubidaea* strain ABS 10 (+ active; - inactive)

Characteristics	***Serratia rubidaea* strain ABS 10**
Colony morphology	small circular colonies on the nutrient agar plates
Gram strain	-
Motility	+
Indole test	-
Methyl red	-
Voges-Proskauer	+
Citrate utilisation	+
Catalase	+
Oxidase	-
Urease	-
Nitrate reduction	+
H_2S production	-
Gelatin liquefaction	+
Glucose	+
Sucrose	+
Lactose	-
Arabinose	-
Sorbitol	+
Mannitol	+
Rhamnose	-

Moreover, the growth of S10 was monitored in MSM-CHL and NB-CHL media (containing 25 $mg.L^{-1}$ of CHL) (Fig. 2). As expected S10 was unable to grow on MSM not supplemented with CHL. Contrariwise it slightly grew on MSM supplemented with CHL as sole carbon source reaching 0.06 ± 2 a.u. after seventy-two hours. Likewise, the growth of *S. rubidaea* strain ABS 10 was promoted by approximately a factor two in the NB medium complemented with CHL as compared to the control. In both cases, a sharp increase in growth was observed up to one day and the maximum growth was obtained after three days. These results suggest that S10 can use CHL for its growth. However, on mineral salt medium the culture poorly grew suggesting some metabolic limitations. This was confirmed by the fact that *S. rubidaea*

strain ABS 10 was unable to mineralize ^{14}C-CHL labelled on the pyridine ring, suggesting that this strain was not able to get access to C of the ring. Our observation is in accordance with earlier studies reporting that a range of bacterial isolates such as, *Serratia sp* (Xu et al. 2007) *Sphingobacterium sp, Alcaligens sp* (Abraham et al. 2013) *Serratia marcescens* (Cycon et al. 2013) *Pseudomonas kilonensi* (Khalid et al. 2016) able to grow in minimum salt medium supplemented with CHL as sole carbon source. In addition, the supplementation of MSM-CHL medium with simple C source such as glucose or sucrose or more complex one such as NB was shown to promote the growth of *Pseudomonas kilonensi* SRK1 suggesting that easily degradable C source can fuel CHL degradation (Khalid et al. 2016). In fact, CHL has reported to be degraded by bacteria co-metabolically that required additional carbon sources (Singh et al. 2006; Xu et al. 2008 Abraham et al. 2013).

Among *Serratia* species, *S. marcescens* was shown to be able to use CHL concentration of 50 mg.L^{-1} as the only carbon source when grown in MSM (Abraham et al. 2013). Furthermore, others species of *Serratia* were characterized for their high potential to grow in MSM supplemented with another OPs such as diazinon (Abo-Amer 2011).

Dissipation of CHL by strain S. rubidaea strain ABS 10 in liquid medium

The dissipation of CHL by *S*10 culture was assessed in resting cell experiment (Fig. 3). S10 rapidly dissipated CHL with a rate of approximately 2.03 mg CHL per day. Within 24 hours, 92% of CHL initially added was dissipated by *S. rubidaea* strain ABS 10 while only 20% of dissipation was observed in the control, which consists of sterile medium not inoculated with this strain. It is noteworthy that as expected CHL was also dissipated in the control but at slower rate than in the *S. rubidaea* strain ABS 10 culture, and after 5 days of incubation 50% of the initial dose of chlorpyrifos remains. CHL half-life in S10 culture was significantly lower than in the control (1.15 vs 4.95 days, respectively) thereby confirming its ability to dissipate it. Keeping in mind that *S. rubidaea* strain ABS 10 was unable to mineralize ^{14}C-CHL, one could suggest that the rapid CHL dissipation in *S. rubidaea* strain ABS 10 culture was not due to its mineralization but probably to its adsorption on bacterial cells as it was previously shown for the herbicide 2,4-D (Benoit et al. 1998) or the insecticide chlordecone (Merlin et al. 2014) on fungal biomass. Indeed, giving the fact that CHL is highly hydrophobic, it has strong affinity to phospholipid bilayer constituting microbial cell membrane on which it can sorb. CHL was previously reported to be hydrolyzed co-metabolically by microorganisms (Chisti, et al. 2013) fueled by other C sources than CHL which, in this case, does not constitute a source of C or energy to sustain its growth (Singh et al. 2006; Xu et al. 2008; Abraham et al. 2013).

Dissipation of CHL in soil

Dissipation of CHL was monitored in sterile- (Fig. 4, panels A) and native-soil (Fig. 4, panels B) microcosms inoculated with *S*10 or not (control). Similar CHL dissipation kinetics were observed in non-inoculated sterile and native soils. These two kinetics of dissipation were biphasic and CHL DT_{50} was estimated to 2.1 days for both treatments. Despite the fact that the native soil microflora is able to mineralize this insecticides (CHL), no differences in the dissipation kinetics observed in sterile- and native-soils treated CHL). This apparent discrepancy is explained by the fact that the dissipation was monitored only during a 6 days period of time during which mineralization of ^{14}C-CHL is very low (Fig. 1). Therefore over this short period of time CHL mineralization does not contribute to observed dissipation which is most likely mainly governed by abiotic processes such as sorption on soil components.

The inoculation of *S*10 in the sterile and native soils resulted in a marked increase in the rate of CHL dissipation as compared to their respective control (Fig. 4). In the inoculated native and sterile soils the CHL DT_{50} were estimated to 1.1 days and 1.4 days, respectively (Table 2). Having in mind that the dissipation of CHL in S10 culture was controlled by its sorption to bacterial cells, the improvement of the dissipation of CHL observed in inoculated soils might be attributed to its biosorption on microbial cells. This hypothesis is supported by the hydrophobic nature of CHL which provides to it a strong lipophilicity compatible with the development of strong interaction with the cell membrane of microorganisms (Angelova and Schumauder 1999).

Table 2
Kinetic data for the dissipation of CHL in aqueous medium (Minimum Salt Medium MSM) and soils (SS: sterile soil and NS: native soil) by Serratia rubidaea strain ABS 10 (B). Controls not inoculated with Serratia rubideae strain ABS 10 were included. Data of dissipation kinetics were fitted to a decay model, where K is the rate of dissipation (day-1) and DT50 is the time required to reach 50 % of the initial pesticide dose.

Medium	Treatment	Equation	R^2	k (day^{-1})	DT50 (days)
MSM	Control	$C_t = 27.3e^{-0,1x}$	0.9	0.1	4.5
MSM	B	$Ct = 9.8e^{-0,6x}$	0.8	0.6	1.1
NS	Control	$C_t = 39.7e^{-0,3x}$	0.9	0.3	2.1
NS	B	$C_t = 7,3e^{-0,6x}$	0.7	0.6	1
SS	Control	$Ct = 39,4e^{-0,3x}$	0.9	0.3	2.1
SS	B	$Ct = 6,7e^{-0,5x}$	0.6	0.5	1.4

In this short-term experiment, the dissipation of CHL was mainly governed by abiotic processes which combined the sorption to soil components and the biosorption to *S. rubidaea* strain ABS 10 cells (3.10^6 $CFU.mL^{-1}$)

Several microorganisms including bacteria, fungi and algae have been already reported as effective biosorbents for removal of dyes, metal and even pesticides due to its low cost, non-toxic approach regeneration capability and high efficiency for pollutant uptake (Pathak and Dikshit, 2011). Particularly, bacteria have been used as biosorbents owing to their ubiquity, size, and ability to grow under controlled conditions and resilience to an extensive range of environmental conditions (Ayangbenro and Babalola 2017). Various heavy metals have been tested on bacteria species such as *Pseudomonas*, *Enterobacter*, *Bacillus* and *Micrococcus* species. Their excellent sorption capacity is due to their high surface-to-volume ratios and their numerous potential active chemosorption sites, such as the teichoic acid on the cell wall (Mosa et al. 2016). Likewise, Zakeri et al. 2010 reported also that *Serratia* sp was an efficient radium biosorbent and might be appropriate candidate for designing biosorption remediation system.

Conclusion

This study showed that in response to long term exposure to CHL, the soil microflora adapted to its enhanced mineralization. *S.rubidaea* strain ABS 10 a bacterial strain able to grow on mineral salt medium added with CHL as sole carbon source was isolated from this soil. Although this strain was able to rapidly dissipate CHL in liquid culture, it was not able to mineralize ^{14}C-CHL labelled on the pyrazine ring. In addition, S10 was shown to be a good biosorbent able to fully dissipate CHL within one day both in liquid medium and in soil microcosms. The present study offers new insight in the development of a remediation technology of CHL and other hydrophobic pollutants, based on the use of S10 as a biosorbent.

Declarations

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Authors’ contributions

All authors contributed significantly to this work. Method and optimization, analysis, and data evaluation were performed by ABS, HC, PC, VC, FML and SF. PC and V.C conducted the analytical assays. MD and JB carried out the experiments of mineralization and 16S rRNA sequencing. TG did the statistical analysis and the figures. The first draft of the manuscript was written by ABS and FML. All authors commented on previous versions of the manuscript. All authors read and approved the final submitted manuscript.

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Data availability

The datasets from which the current study was created are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

Not applicable

Consent to participate

Not applicable

Consent for publication

The manuscript has been read and approved for submission by all the named authors

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Figures

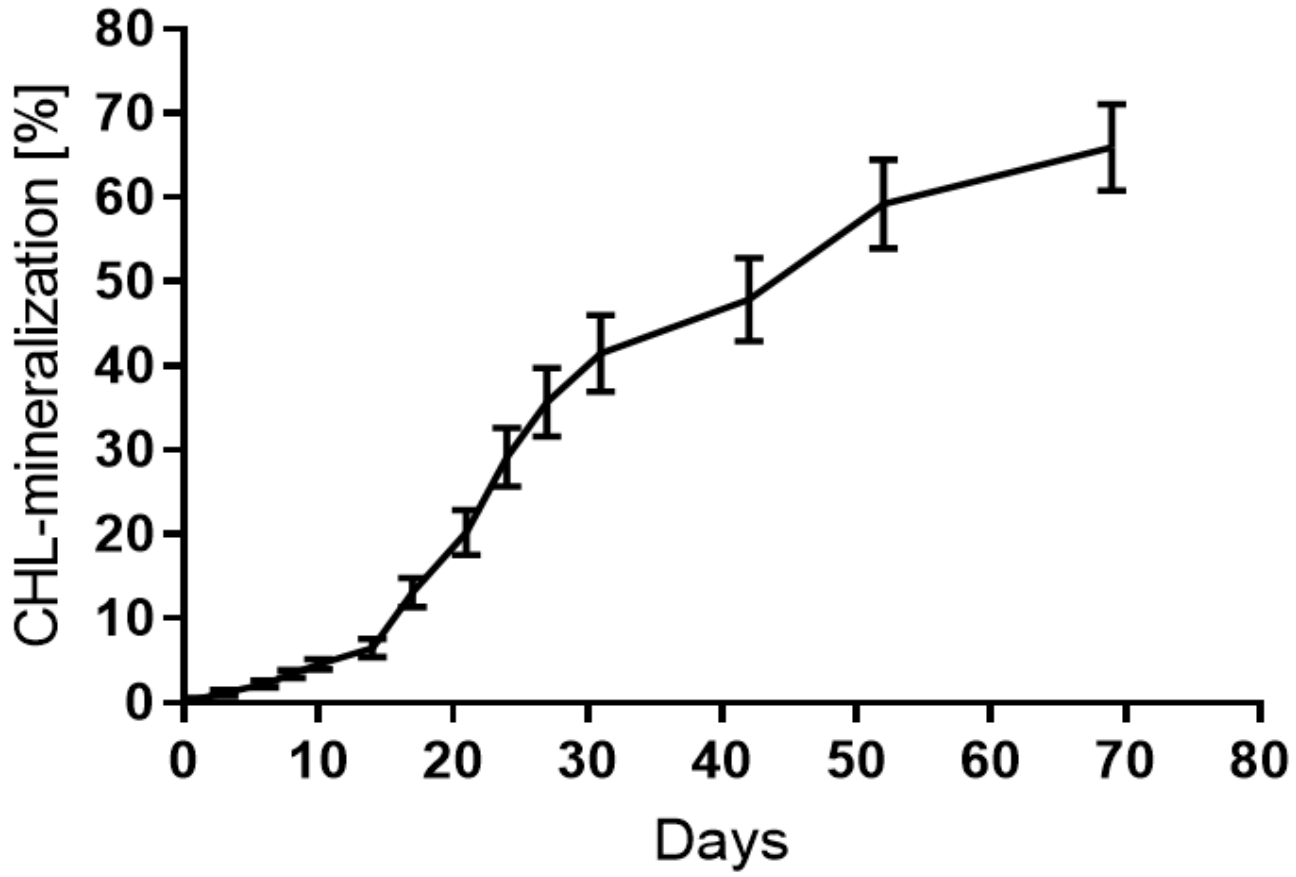

Figure 1

Kinetics of mineralization of 14C-chlorpyrifos (CHL) in the soil over an eighty days incubation period. Mean values ± standard deviation of percentage of initially added 14C-CHLare shown (n=3)

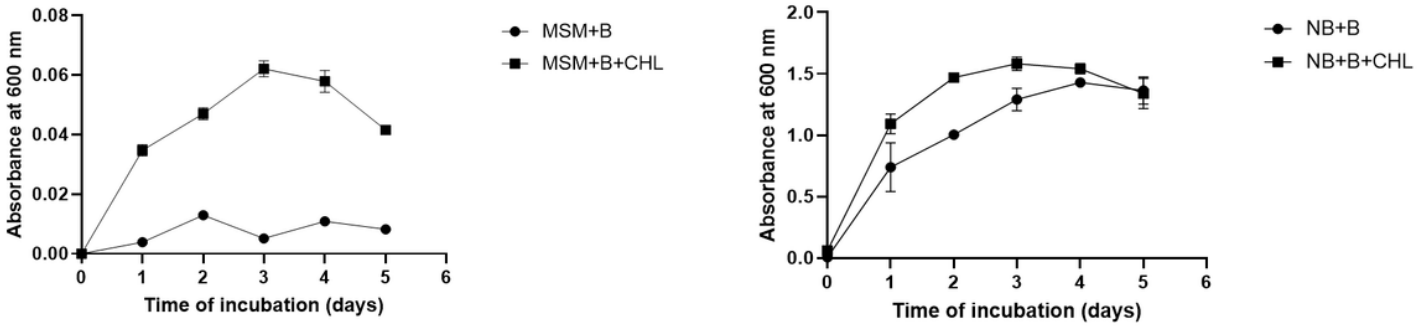

Figure 2

Growth of Serratia rubidaea strain ABS 10 (B) in mineral salt medium (MSM) and nutrient broth (NB) liquid media supplemented with chlorpyriphos (CHL) or not. Mean values (arbitrary unit, a. u., absorbance at 600 nm) ± standard deviation are shown (n=3 per treatment).

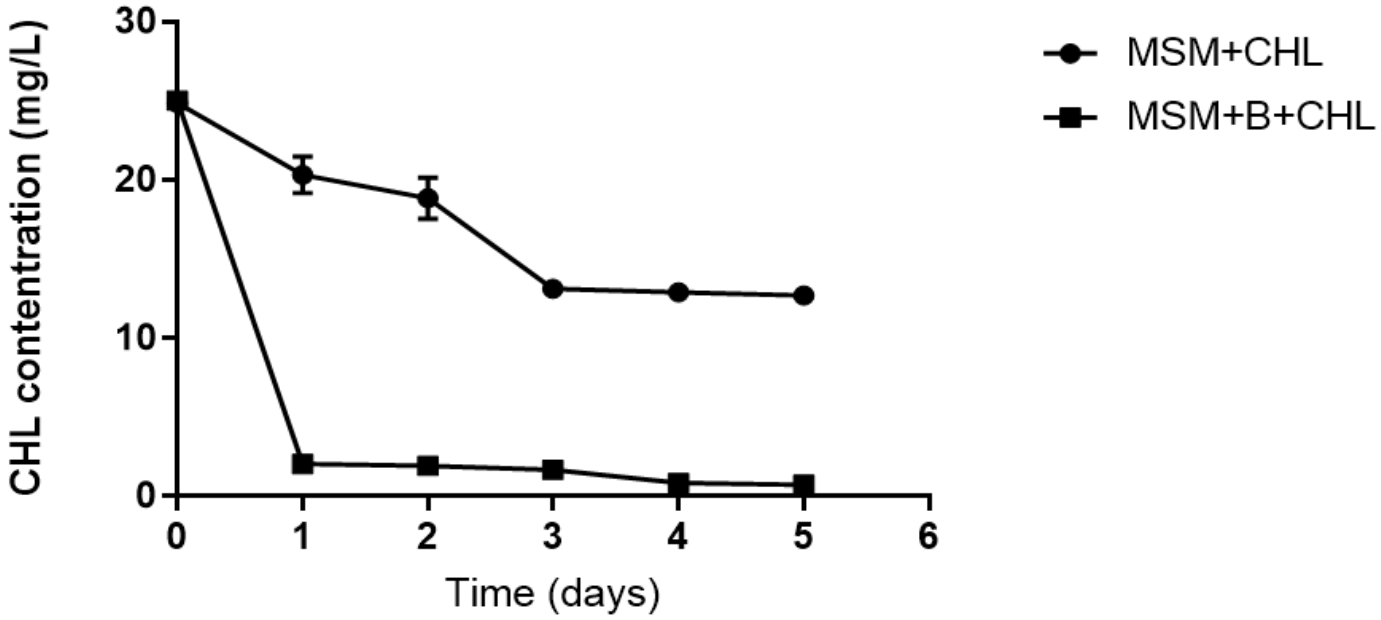

Figure 3

Dissipation of chlorpyrifos (CHL) in Serratia rubidaea strain ABS 10culture (B) and in mineral salt (MSM). Mean values ± standard deviation are shown (n=3 per treatment).

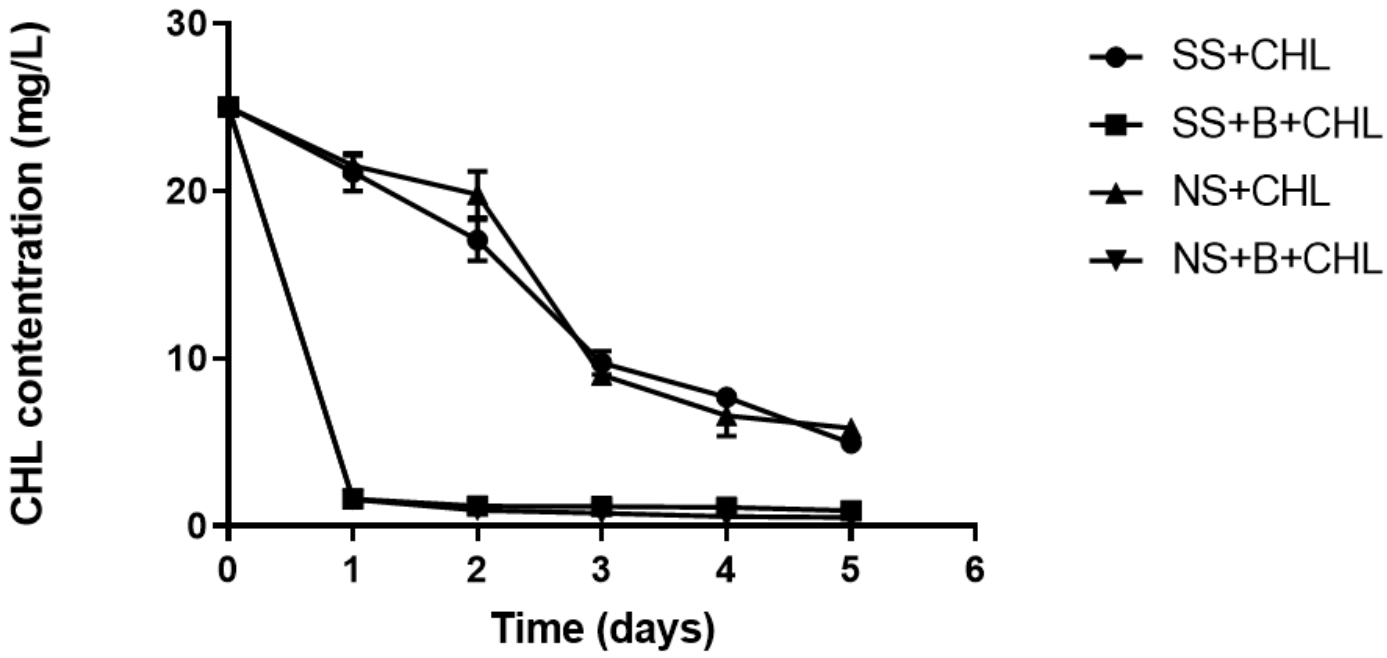

Figure 4

Dissipation of chlorpyrifos (CHL) in sterile (SS) or native (NS) soil inoculated with Serratia rubidaea strain ABS 10 (B) of not. Mean values ± standard deviation are shown (n=3 per treatment).