Heptachlor degradation characteristics of a novel strain and its application

Liping Qiu, Hu Wang and Xuntao Wang

ABSTRACT

With heptachlor as the sole carbon source, an effective heptachlor-degrading microorganism (named strain H) was isolated from the sludge of heptachlor-polluted sewage of a chemical plant, via enrichment, screening and purification. Strain H was identified as a facultative anaerobic Gram-negative bacterial strain belonging to genus Shigella based on the physiological-biochemical characteristics and the similarity analysis of its 16S rDNA gene sequence with the sequences logged in the Ribosomal Database Project and GenBank databases. When the optimal inoculation volume and the pH were 20% and 7.1–7.6, respectively, strain H was able to degrade heptachlor by more than 88.2% after 130 h, with initial concentration of heptachlor being 300 μg/L at 30 ± 0.5°C. It was also shown that strain H can grow on the degradation products of heptachlor such as 1-hydroxychlordene or heptachlor epoxide. Furthermore, additional carbon sources can accelerate the degradation rate of heptachlor because of co-metabolism. The degradation dynamics could be described by a first-order reaction model. A real-world field experiment demonstrated that strain H was effective in practical applications of heptachlor biodegradation in contaminated soil.

Key words | bacterial strain, biodegradation, heptachlor, isolation, sludge

INTRODUCTION

Persistent organic pollutants (POPs) have become a new global environmental problem due to high toxicity, bioaccumulation, semi-volatility and persistence (Maqbool et al. 2016). In 2001, the international community signed ‘The Stockholm Convention on POPs’ in the Swedish capital, in which nine of the 12 controlled substances, such as DDT (dichloro-diphenyl-trichloroethane) and heptachlor (C₁₀H₅Cl₇), are organic chlorine insecticides. China began large-scale production and the use of organochlorine pesticides including heptachlor in 1960s. Heptachlor is mainly mixed with the seed such as corn, grain, and sorghum to destroy agricultural pests such as termites and nematodes in soil (Michael 1983; Purnomo et al. 2014). Although China has banned the use of heptachlor since the 1980s, the residues of heptachlor and its conversion products could still be detected in the ecosystem due to its environmental persistence and illegal use, which causes a potential threat to the ecological environment and human health (Wei et al. 2015).

Using microbial remediation technology to control environmental pollution is a very effective method because it has the advantages of low energy consumption, high efficiency, mild reaction condition and less secondary pollution (Oliveira et al. 2015; Jardé et al. 2018). The isolation, culture and application of strains which can degrade pollutants rapidly are an important part of the bioremediation process. It has been reported that a variety of microorganisms that degrade heptachlor have been isolated. They include Bacillus, Fusarium, Micromonospora, Nocardia, Aspergillus, Rhizopus and Streptococcus (Zheng et al. 2001). However, most strains have low degradation rate and strict requirement of environmental conditions, such as temperature. Some of them need to undergo a co-metabolic process to degrade heptachlor, which cannot be directly applied to pollution remediation.

To effectively achieve the bioremediation of soil and water contaminated with heptachlor, it is very important to find bacterial species that are not only widely available but also capable of degrading heptachlor in the natural environment. In this study, a sludge sample was collected from the sludge of a sewage biological treatment pool in a chemical plant, which has been suffering from heptachlor pollution.
contamination. This sludge is thus a potential source of heptachlor-degrading bacteria with the above characteristics. We isolated a strain that can grow using heptachlor as the carbon source. Thereafter, we conducted a comprehensive study of the strain, observing its morphological characteristics, and researching its physiological-biochemical characteristics, optimal degradation conditions, substrate utilization, degradation dynamics and practical applications in the bioremediation of contaminated soil. The results of this study can be of great importance to the remediation of environments contaminated by heptachlor.

MATERIALS AND METHODS

Materials

A wet sludge sample was collected from the sludge of a sewage biological treatment pool of a chemical plant, where the sludge suffers from heptachlor pollution.

We purchased heptachlor standard (1 mg/mL in methanol) and heptachlor epoxide standard (1 mg/mL in methanol) from Aladdin Industrial Corporation (Shanghai, China), and chlordene-1-hydroxy standard (10 μg/mL in cyclohexane) from Dr. Ehrenstorfer GmbH, Germany. Chromatographically pure solvent, n-hexane, purchased from American Tedia Company was used as an extractant of heptachlor.

The mineral salt medium (MSM) included (g/L): 3 NH4Cl, 1.5 KH2PO4, 0.3 NaCl2, 1 MgSO47H2O, 3 K2HPO412H2O, 0.05 FeSO4; and 2 mL of trace elements solution which contained (g/L) 0.15 H3BO3, 0.1 ZnSO4, 0.4 MnCl24H2O, 0.04 CuSO45H2O, 0.20 CoCl26H2O, 0.1 (NH4)6Mo7O244H2O, 0.1 NiSO46H2O. Heptachlor was added as the sole carbon source, of varying concentrations for different experimental settings. Luria-Bertani (LB) liquid medium contained the following ingredients (g/L): 10.0 peptone, 5.0 sodium chloride, 10.0 yeast extract. The solid MSM was obtained by adding lowing ingredients (g/L): 10.0 peptone, 5.0 sodium chloride, 10.0 yeast extract. The solid MSM was obtained by adding 20 g/L nutrient agar to the MSM.

All other chemical reagents used in our experiments were of analytical reagent grade commercially available.

Screening and isolation of heptachlor-degrading bacterium

The goal of this study was to find the microorganisms which are able to use heptachlor as nutrient substance and multiply rapidly. In this method, sludge samples (10 g) were placed onto 500 mL sterilized Erlenmeyer flasks and then washed by sterile water. The pH was controlled to 7.0 ± 0.1 with the addition of filter-sterilized 1 mol/L HCl or NaOH on a shaking incubator (BS-1E, China) at around 120 rpm for about 60 min. After three repetitions, all the washing water was collected into a 500 mL sterilized volumetric flask to get the cell suspension, stored at 4 °C and evenly mixed for measurement before use.

Pure isolates were obtained by plate separation methods under aseptic condition. The MSM for the enrichment and domestication of the isolates from the cell suspension was sterilized for 25 min and added with heptachlor concentration gradient of 50, 100, 200, 300, 400, and 500 μg/L every 120 h for incubation of the bacteria with pH value 7.0 ± 0.1. The resulting isolates of bacteria at each concentration gradient were screened for their capability of reducing heptachlor, carried out by agar block method (Zhou & Xu 2015), on the differential solid MSMs sterilized for 25 min with pH value 7.0 ± 0.1. A few larger colonies of bacteria for heptachlor degradation were picked out and transferred into a fresh higher concentration gradient in the MSM. This process was repeated three times. Then, all the screened colonies of bacteria of the last test were transferred into fresh differential MSMs and cultured to compare the degradation capability of heptachlor at 50 °C on a rotating incubator with 120 rpm for 120 h. The strain with the highest degradation rate was then isolated and purified by plate streaking on a fresh differential solid MSM. The purification procedure was repeated three times. Finally, the pure strain (named strain H) was isolated and stored on an inclined plane in a refrigerator at 4 °C before use.

Identification of strain H

The isolated strain H was incubated on MSM containing 300 μg/L heptachlor for 5 d. Through a scanning electron microscope (S-4800, Hitachi) and a MOTIC digital biological microscope (DMBA400-P, China), the morphology and the size of the bacteria were observed. Physiological-biochemical properties of the strain were analyzed by the processes described in Bergey’s Manual of Determinative Bacteriology (Buchanan & Gibbons 1974) and Manual of Common Bacterial System Identification (Dong & Cai 2001).

Strain H was further identified by polymerase chain reaction (PCR) amplification and sequencing of 16S rDNA. The genomic DNA of strain H was obtained using a commercial genomic DNA extraction kit (SK1201-UNIQ-10, Shanghai Sangog Biotech Co., Ltd, China).The 16S rDNA gene of the strain was amplified by PCR using Taq polymerase. The following pair of universal primers
was used: forward primer 7F(5′-CAGAGTTTGATCCTGGGCT-3′) and the reverse primer 1540R (5′-AGGAGGTATCCAGCCGCA-3′) (Devereux and Willis, 1995). The PCR mixture (25 μL) contained the following ingredients: 0.5 μL genomic DNA (20–50 ng/μL), 2.5 μL 5× buffer (with 20 mmol/L Mg²⁺), 1.0 μL dNTP (2.5 mmol/L), 0.5 μL forward primer (10 μmol/L), 0.5 μL reverse primer (10 μmol/L), 0.2 μL Taq (5 U/μL), and sterile water. The PCR was performed in a PTC-100 Peltier thermal cycler (MJ Research, USA). It included an initial denaturation at 98 °C for 3 min, followed by 30 cycles of denaturation at 98 °C for 25 s, annealing at 55 °C for 25 s, and extension at 72 °C for 1 min with the last cycle followed by a 10-min extension at 72 °C. The PCR products were stored at 4 °C. The PCR products were purified and sequenced at Shanghai Sangog Biotech Co., Ltd (China) and compared with 16S rDNA sequence data from type strains available in GenBank (http://www.ncbi.nlm.nih.gov) and the Ribosomal Database Project (RDP) (http://rdp.cme.msu.edu/index.jsp) using the BLASTN and RDP sequence match routines. The sequences were aligned using multiple sequence alignment software CLUSTAL W version 1.81. Then, a phylogenetic tree was constructed by the neighbor-joining method using the MEGA software (version 4.1) according to the 16S rDNA sequences.

**Growth curve of strain H**

Strain H was inoculated to LB containing 300 μg/L heptachlor (initial strain H concentration: 1.2 × 10⁸ cells/mL) and incubated in a shaking incubator at 30 ± 0.5 °C and 120 rpm to obtain the growth curve of strain H at different phases by measuring the concentration of strain H periodically.

**The environmental factors analysis**

Strain H at the logarithmic growth phase was collected by centrifugation, and then was resuspended using physiological saline water. The resulting suspension was used as degradation inoculation solution for all the following experiments. Effects of environmental factors on the heptachlor degradation were researched in 250 mL Erlenmeyer flasks. Each flask was added with 200 mL MSM containing 300 μg/L heptachlor and 15% of inoculation solution with cell content of 1.2 × 10⁸ cells/mL, and was put in a shaking incubator at 120 rpm.

Tests on the effect of temperature were first examined at five levels, 20, 25, 30, 35 and 40 °C, at pH 7. These were followed by tests on the effect of pH at six levels from pH 5.2 to 9.3 at the identified optimal temperature. Then, we used inoculation volume of 15% at identified optimal temperature and pH value. In the first 168 h of the degradation, the concentration of heptachlor and the cell concentration of strain H were periodically measured. With the above identified optimal temperature, pH and time, the effect of different inoculation volume was investigated at six levels, 5%, 10%, 15%, 20%, 25%, and 30%. Finally, to test the ability of strain H to utilize various compounds of carbon, glucose, sucrose, lactose, protein, and starch were chosen as additional carbon sources. The isolate was grown in the medium containing one of those compounds. All MSMs contained 1.5 mg/L (Elango et al. 2011) of the given compounds. Samples from the culture, as well as from sterile controls, were collected and examined for the heptachlor degradation and bacterial growth at the identified optimal temperature, time, pH value and inoculation volume.

Degradation of different heptachlor concentrations was carried out at the optimal temperature, pH and time in 250 mL Erlenmeyer flasks at 120 rpm. Each flask contained a cells enrichment culture (1.2 × 10⁸ cells/mL) of identified optimum inoculation volume and 200 mL MSM with heptachlor concentration varying from 100 to 500 μg/L. Fermentation broths were taken at intervals of 10 h for heptachlor and biomass analyses. The experiments were conducted in triplicate, with both uninoculated flasks and flasks without heptachlor serving as controls. Uninoculated flasks with heptachlor were used for monitoring any abiotic loss of substrates during incubation.

At the above identified optimum conditions, heptachlor biodegradation was examined under aerobic and anaerobic environments. The anaerobic environment was achieved by applying a liquid seal of atoleine after all the operations (adding MSM and heptachlor solution) were completed in an anaerobic incubator (YQX-II, Shanghai, China, 99.99% nitrogen condition).

**Heptachlor degradation and the production of its metabolites at the optimal conditions**

The experiments were carried out in 250 mL Erlenmeyer flasks, each of which contained 200 mL MSM. The degradation was examined with 300 μg/L heptachlor, under the above identified optimum conditions, and a degradation time of 200 h, with no additional carbon sources.
The dynamics of heptachlor degradation

The experiments were carried out in 250 mL Erlenmeyer flasks, each of which included 200 mL MSM. The dynamics of heptachlor biodegradation were examined under the above identified optimal conditions, with the initial heptachlor concentration varying from 50 to 300 μg/L, with no additional carbon sources.

Application of strain H to the remediation of heptachlor-contaminated soil

The heptachlor-contaminated soil was collected from the soil near the storage center of an insecticide factory in June 2016. The pesticide in the storage center caused severe heptachlor pollution of the surrounding soil. The main ingredients of the soil were loessial soil, loam soil and fluvi-aquic soil. The average concentration of heptachlor was about 285.8 μg/kg.

The laboratory simulation experiment was carried out at the environmental engineering laboratory of Chang’an University, Shaanxi, China. A sample of the heptachlor-contaminated soil was collected and naturally dried with impurities removed. The sample was crushed and then sieved using a 20 mesh sieve. The laboratory simulation experiment was carried out in two 500 mL Erlenmeyer flasks, each of which contained 200 g of the soil sample. The inoculation method was carried out in two 500 mL Erlenmeyer flasks, each of which contained 200 g of the soil sample. The inoculation solution of strain H was added to the test flask until the microbial concentration reached approximately 1.2 × 10⁸ cells/g (Shreve 2017) (test experiment); the same amount of sterilized water was added to the control flask (control experiment). The moisture content of the soil sample was maintained at 40% and pH at 7.1–7.6. Both flasks were kept in an artificial climate chamber at 9–10 °C (simulation field). The heptachlor degradation was investigated after 15 days and 30 days, respectively.

The real-world field experiment was conducted in the field soil contaminated by heptachlor. Two pits of 1 m × 1 m × 0.3 m were dug and named as 1 and 2. The bottoms of the pits were treated with seepage prevention. A sample of 2 m³ heptachlor-contaminated soil was fully stirred and evenly divided into two parts and added to the two pits, respectively. The soil in the pits was humidified layer by layer to keep the water content uniform, and stirred once every 5 days to carry out intensive ventilation. The soil was ventilated under natural conditions for the rest of the time. Pit 1 was for the field test of heptachlor degradation, where the inoculation solution of strain H was added to the soil until the microbial concentration reached approximately 1.2 × 10⁸ cells/g. Pit 2 was a blank control, where the same amount of sterilized water was added to the soil. The moisture content of the soil, pH value, heptachlor concentration and the cell concentration were measured every 2 days. Experimental treatments were done in triplicate to reduce the random error caused by random sampling. The heptachlor degradation was also investigated after 15 days and 30 days, respectively. In the course of the experiment, the pH value of the soil ranged from 7.0 to 7.6. The daytime temperature was 15.5 °C on average and the nighttime temperature was 6.8 °C on average. Other physical/chemical property parameters of the soil remained stable.

Detection of heptachlor and its metabolites, and cell concentration

The concentrations of heptachlor and its metabolites were analyzed by gas chromatography–mass spectrometry (GC-MS 2010, Shimadzu Corp.). A degradation solution of a 10 mL sample obtained after the pretreatment of the bacterial cultures was placed into a 40 mL brown reagent bottle, and then 5 mL n-hexane was added (Clesceri et al. 1998; Liu & Ma 2012). The bottle was kept in a constant-temperature rotating shaker (HZQ-C, China) at 200 rpm for 20 min and then in ultrasonic waves at 28 kHz frequency for 10 min for heptachlor extraction. The extracted organic phase was separated and dried over anhydrous sodium sulfate. Finally, 1 μL of the dry organic sample was injected in the injection port of GC-MS through an organic filter of 0.22 μm pore size to analyze the concentrations of heptachlor and its metabolites. The chromatographic column of GC was a RTX-5MS from Shimadzu (30 m × 0.25 mm × 0.25 mm). The temperature of the column was maintained at 80 °C for 3 min, and then gradually increased to 260 °C at the rate of 10 °C/min, and finally kept at 260 °C for 5 min. The temperatures of the gasification and the injection port were both at 260 °C. The injection volume was 1 μL at split-less mode. The energy of MS was derived from a 70 eV electron ionization source of electron bombardment; ion source temperature was maintained at 200 °C; interface temperature was maintained at 260 °C; mass scan range was set at 40–450 m/z.

The cell concentrations of all samples were detected using visible spectrophotometry and a Petroff-Hausser counting chamber (Shen & Chen 2007).

Statistical analysis

All the experiments were carried out in triplicate. The specific degradation rate was calculated from the obtained
degradation curves by dividing the slope of the degradation curve by the associated concentration of the cells. Excel 2007 analysis software for Windows was used for the statistical tests. The standard deviation of all data points in this paper was between 0.8% and 13.6%.

RESULTS AND DISCUSSION

Identification of strain H

By a MOTIC digital biological microscope and a scanning electron microscope (S-4800, Hitachi), strain H was observed as a short rod-shaped, single-cell Gram-negative bacterium (Figure 1). The colony morphology of strain H was pale yellow and circular. The colony diameter of strain H was less than 0.3 cm, smooth on the surface and trim on the edge, and translucent. There was a slight bump in the middle. Methyl red reaction, starch hydrolysis and catalase were positive. The V-P reaction, oxidase and gelatin reaction were negative.

The bacterium was further identified by 16S rDNA analysis. After gel electrophoresis of the PCR amplification product of strain H (Figure 2), a single band of 1.5 kilobases could be seen by comparing with the DNA Ladder Mix Marker (DL2000) in the gel imager. After sequencing, the accurate length of the band was determined as 1,534 base pairs. The 16SrDNA gene sequence of strain H (accession number: BankIt2073018Seq1 MG711918) was compared with the sequences logged in the RDP and GenBank databases. The homology analysis showed that strain H had 99% homology with Shigella sonnei. Therefore, strain H was identified as genus Shigella according to the 16S rDNA analysis, as well as the phenotypic and physiochemical experiments above. The phylogenetic tree of strain H was constructed based on 16S rDNA sequences (Figure 3).

Growth curve of strain H

From the growth curve of strain H (Figure S1, available with the online version of this paper), we can see that the lag phase lasted from 0 to 30 h, the logarithmic phase lasted from 30 to 60 h, the stationary phase lasted from 60 to 120 h, and the death phase began at 120 h.

Effect of environmental factors

In the course of the whole experiment, the recovery rate of heptachlor was between 96.86% and 99.88% in the sterilization control. Since the experiment was carried out without illumination and sealing conditions, heptachlor losses were rarely caused by photolysis or volatilization.

Effect of temperature is shown in Figure S2(a) (Figure S2 is available online). It reveals that strain H was a mesophilic bacterium. The optimum growth temperature of the strain was between 30 and 35 °C. The growth rate of strain H decreased sharply when temperature was above 35 °C. The growth rate of strain H was strongly inhibited when temperature was above 40 °C.

The effect of pH on heptachlor degradation is shown in Figure S2(b). Heptachlor degradation rate and the cell
concentration increased quickly when pH increased from 5.2 to 7.6. When pH was above 7.6, the two began to decrease. The highest heptachlor degradation rate and cell concentration were observed at pH 7.1–7.6. This could be because a higher or lower pH value can cause a change in membrane charge of the microbial cells and the redox electric potential. This would in turn affect the ionization effect of heptachlor and the activity of enzymes in the process of microbial metabolism, so that the toxicity of heptachlor can be also altered (Zhou & Wang 2011; Wu et al. 2017), which would affect the bacteria’s capability to use it as a carbon source. Therefore, the optimal pH value for strain H growth was about 7.1–7.6.

Degradation time is shown in Figure S2(c). We could see that both the cell concentration and heptachlor degradation rate rapidly increased over 20–120 h, after which heptachlor degradation rate no longer increased, but the bacterial cell concentration began to decrease. Actually, as the degradation time extended, the growth of the strain had a death phase, which might have been caused by insufficient levels of carbon source, so that the growth and metabolism of strain H were restricted. Thus, the optimal heptachlor degradation time was around 120 h.

Different inoculation volumes were assessed as shown in Figure S2(d). It can be seen that both the cell concentration and heptachlor degradation rate rapidly increased within 5–20% inoculation volume but only slightly increased beyond 20%. If the inoculation volume of strain H was excessive, we reasoned, the growth and metabolism of the strain would be restricted, which might have been caused by insufficient levels of carbon source. Thus, when the inoculation volume of the strain was >20%, the degradation rate had no significant improvement, which indicated that the optimal inoculation volume was around 20%.

Different additional carbon sources were assessed as shown in Figure S2(e). Additional carbon sources can stimulate the growth of the strain. To achieve the maximal degradation rate of 96.2%, it takes 80 h with glucose as an additional carbon source. It takes 100 h with lactose or sucrose (maximal degradation rate 94.3% or 93.8% respectively), and 120–130 h with starch or protein or no additional carbon source (maximal degradation rate 88.5%, 88.6% or 88.2% respectively). In fact, small molecular saccharides can be easily used by strain H as a carbon source. Thus the degradation rate was accelerated by the co-metabolism of the difficult-to-degrade organic matter (heptachlor) with the easily degradable organic matter such as glucose (monosaccharide), lactose and sucrose (disaccharide) (Bottomley 2017). In contrast, the macromolecular compounds such as starch (polysaccharide) and protein (consisting of 20 amino acids) had less effect on the degradation rate.
Different heptachlor initial concentrations were assessed as shown in Figure S2(f) and 2(g). When the heptachlor initial concentration was ≤ 300 μg/L, strain H had strong degradation capacity for heptachlor. The largest degradation rate of 87.2% and the lag period was within 20 h when heptachlor concentration was 300 μg/L. As the heptachlor initial concentration increased above 300 μg/L, its degradation rate by strain H decreased. For instance, only 62.5% was degraded and the lag period was over 60 h when heptachlor initial concentration was 500 μg/L. The cell concentration became lower as well. Once the heptachlor initial concentration exceeded a certain value, it would inhibit the growth of strain H, thus inhibiting its degradation ability (Guttman & Rijn 2012). The optimal initial heptachlor concentration was around 300 μg/L.

Heptachlor degradation under aerobic/anaerobic environment is shown in Figure S2(h). Both the heptachlor degradation rate and the cell concentration were high under either aerobic or anaerobic environment. The degradation rate reached 87.5% and 80.4%, respectively. Thus strain H is a facultative anaerobe.

**Heptachlor degradation and the production of its metabolites at the optimal conditions**

The degradation of heptachlor is accompanied by the production of metabolites. When the degradation reaction proceeds to 130 h, the degradation of heptachlor reaches the maximum, 88.2%. At this time, mainly three metabolites were detected in the extracts from the culture medium. The metabolites were identified respectively as 1-hydroxychlordene (RT 18.869 min), chlordene epoxide (residence time (RT) 20.987 min) and heptachlor epoxide (RT 22.888 min), through GC-MS analysis, search matching with the NIST spectral library, and comparison with standard compounds and document data (Xiao et al. 2011), as shown in Figure 4.

At the same time, the concentration of the heptachlor metabolites was determined: 1-hydroxychlordene and heptachlor epoxide were 54.2 μg/L and 66.6 μg/L respectively. With the increase of degradation time, the metabolite concentrations gradually decreased. At 180 h, 1-hydroxychlordene and heptachlor epoxide reached stability, and their concentration decreased to 8.3 μg/L and 17.2 μg/L respectively, as shown in Figure 5. This can be attributed to the enzymatic activity of microorganisms, resulting in metabolites of heptachlor being further degraded. Strain H could produce intracellular enzymes such as monooxygenase and extracellular enzymes such as peroxidase (Matsuzaki & Wariishi 2004). Under the catalysis of these enzymes, heptachlor and its metabolites were transformed into less-toxic small molecular metabolites and even mineralized completely through a series of reactions such as hydroxylation, epoxidation and dechlorination, which indicates that strain H can grow on the degradation products of heptachlor, such as 1-hydroxychlordene or heptachlor epoxide. Therefore, strain H has the potential for remediation of heptachlor-contaminated soil and water.

**The dynamics of heptachlor degradation**

Analyzing the dynamics of pollutant degradation can predict the trend of microbial degradation, and optimize the conditions in the remediation system. The dynamics of heptachlor degradation are shown in Figure 6. We observed that within 20 hours, strain H was in the adaptive adjustment stage, and the degradation was not obvious, which means the toxicity of heptachlor inhibited
the growth of strain H. After 20 h, strain H was in the growth stage of rapid propagation, and the degradation rate increased because strain H became adapted to the toxicity of heptachlor. Heptachlor reached maximum degradation rate within 90 h with the initial concentration being 50 $\mu$g/L, and 120–130 h with the initial concentration being 100–300 $\mu$g/L. Obviously, a higher initial concentration of heptachlor would result in a longer degradation period. In addition, the increasing supply of heptachlor (carbon source) required a longer time for complete degradation.

The time-varying heptachlor concentration during the degradation was fit by zero-order degradation dynamics ($C = -kt + B$), first-order degradation dynamics ($\ln C = \ln C_0 + kt$), and second-order degradation dynamics ($1/C = -kt + B$), respectively ($C$, heptachlor concentration; $C_0$, heptachlor initial concentrations; $k$, reaction rate constant; $t$, reaction time; $B$, constant). The experimental results could be well described by first-order degradation dynamics when the heptachlor concentration varies from 50 $\mu$g/L to 300 $\mu$g/L. The dynamics equations, half-lives and correlation coefficients are shown in Table 1. All the dynamics equations had high correlation coefficients, which validates that the degradation process proceeded with first-order degradation dynamics equations.

**Figure 5** | Time course for degradation of heptachlor and production of some metabolites under the optimal conditions.

**Figure 6** | Heptachlor degradation dynamics at different initial concentration under the optimal conditions.
Application of strain H to the remediation of heptachlor-contaminated soil

The results of laboratory simulation experiments showed that heptachlor degradation rate by strain H was 38.6% and 66.5%, respectively, on the 15th day and the 30th day, whereas the degradation rate of the control experiment was 10.2% and 17.8%, as shown in Figure 7(a).

The results of real-world field experiments showed that heptachlor degradation rate by strain H was 28.5% and 51.2%, respectively on the 15th day and the 30th day, whereas the degradation rate of the control experiment was only 9.2% and 16.5%, respectively, as shown in Figure 7(b).

The bioremediation results of heptachlor pollution demonstrated considerable degradation capability of strain H in real soil, although the degradation performance in the laboratory simulation experiment was superior to that in the field experiment, since the remediation efficiency of strain H in practical applications could be affected by environmental factors such as temperature fluctuations, the change of pH value and uneven soil particles.

The heptachlor degradation rate after 30 days was obviously higher than that after 15 days in real soil. This was expected because the microorganisms require a period of adaptation in the soil before they initiate growth and degradation of heptachlor. In previous bioremediation studies of soil polluted by organic pollutant, it required 2 months to years to achieve reasonable bioremediation (Genovese et al. 2008). In this study, the degradation rate attained 66.5% in the laboratory simulation and 51.2% in the field after only 30 days. The test experiment was obviously better than the control experiment. This demonstrated that strain H can be effectively applied in the bioremediation of heptachlor-contaminated soil.

CONCLUSION

In this study, we isolated and screened a heptachlor-degrading microorganism named strain H, which was able to effectively degrade heptachlor at the temperature of 30 ± 0.5 °C. Experiments showed that strain H can grow using heptachlor and the degradation products of heptachlor such as 1-hydroxychlordene or heptachlor epoxide as its carbon source, and was identified as a Gram-negative, short rod-shaped, single-cell facultative anaerobe belonging to genus Shigella. After 130 h, heptachlor degradation reached more than 88%. Both laboratory simulation and real-world field experiments demonstrated that strain H was effective in practical applications of heptachlor biodegradation of contaminated soil. Thus strain H has the potential for remediation of heptachlor-contaminated soil and water.

Table 1 | Dynamics of heptachlor biodegradation

<table>
<thead>
<tr>
<th>C (μg/L)</th>
<th>In C</th>
<th>t_{1/2} (h)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>$\ln C = -0.0172t + 4.2013$</td>
<td>40.29</td>
<td>0.9105</td>
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<td>100</td>
<td>$\ln C = -0.0169t + 4.6729$</td>
<td>41.01</td>
<td>0.9618</td>
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<td>150</td>
<td>$\ln C = -0.0173t + 5.1592$</td>
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<td>0.9928</td>
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<tr>
<td>200</td>
<td>$\ln C = -0.0173t + 5.4153$</td>
<td>40.06</td>
<td>0.9902</td>
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<tr>
<td>250</td>
<td>$\ln C = -0.0171t + 5.6935$</td>
<td>40.53</td>
<td>0.9750</td>
</tr>
<tr>
<td>300</td>
<td>$\ln C = -0.0170t + 5.9130$</td>
<td>40.53</td>
<td>0.9859</td>
</tr>
</tbody>
</table>

C, heptachlor concentration (μg/L); InC, dynamics equation; t_{1/2}, half-life (h); R², correlation coefficient.
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