

Biodegradation of 3,5,6-trichloro-2-pyridinol by *Cupriavidus* sp. DT-1 in liquid and soil environments

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Abstract

The bacterial strain *Cupriavidus* sp. DT-1 can degrade 3,5,6-trichloro-2-pyridinol (TCP) and transform it into 2-hydroxypyridine (2-HP). This is a unique degradation pathway of TCP but incomplete. In the present study, strain DT-1 could degrade 2-HP at a high concentration 500 mg/L and use it as sole carbon source for growth. Three metabolites (nicotine blue, maleamic acid and fumaric acid) were detected in the medium and the complete degradation pathway of TCP was derived. Inoculation of TCP-contaminated soils with strain DT-1 resulted in a degradation rate 94.4% and 86.7% as compared to 20.4% and 28.4% in uninoculated soils, respectively. Fluorescent marker gene *gfp* was introduced into strain DT-1 and a new strain DT-1-*gfp* was created, viability test showed the strain could survive well in soils for more than 35 d. This finding suggests that strain DT-1 has potential for use in bioremediation of TCP-contaminated environments.

Introduction

TCP is a typical representative of organochlorine pollutant with a long half-life ranging from 65 to 360 days in the natural environment (Fang et al., 2019). It is the primary degradation product of chlorpyrifos but more mobile than the parent compound due to its greater water solubility (Yang et al., 2005; Kim and Ahn, 2009; Li et al., 2010). Because there are three chloride atoms in its molecular structure, TCP has high toxicity to organism and resistance to microbial degradation (Rayu et al., 2017). Due to these characteristics, TCP is classified as persistent pollutant by the U.S. EPA. It can cause more widespread contamination in the aquatic environments and soils (Dores et al., 2016; Rayu et al., 2017). Remediation the environment polluted of TCP still poses a challenge (Wang et al., 2019).

2-HP is a class of N-heterocyclic organic pollutants with the characteristics of biological toxicity and long existed in the environment (Lataye et al., 2006). It is more water soluble, more mobile, and more leachable into groundwater and soils, which causes widespread contamination and threatens to ecological environment and human health (Petkevicius et al., 2018; Chu et al., 2018). More importantly, 2-HP is a primary intermediate metabolite in the degradation of N-heterocyclic organic compounds such as TCP and nicotine (Lu et al., 2013; Yu et al., 2015). Therefore, remove the residual of 2-HP from environment has great significance for eliminating the pollution caused by these toxic compounds.

Microbial degradation with the characteristics of high efficiency, low cost and no secondary pollution is an environmentally favorable decontamination method to remove the pollution of TCP (Wang et al., 2012). To date, several microorganisms with capacity to degrade TCP have been isolated from different genera (Table 1), and the biodegradation pathway of TCP has been researched recent years. Results show the intermediate metabolite during TCP degradation most commonly detected is 3,6-dihydroxypyridine-2,5-dione (Li et al., 2010; Bhuimbar et al., 2011; Cao et al., 2012). Different from other strains, strain *Cupriavidus* sp. DT-1 has a unique metabolic pathway for degradation of TCP with the metabolite product 2-HP, and further mineralizes 2-HP completely (Lu et al., 2013). However, we have only deduced the metabolic pathway from TCP to 2-HP in previous research, the subsequent degradation

pathway has not been studied thoroughly. Therefore we have not understood the complete metabolic pathway of TCP degraded by strain DT-1.

Table 1
The reported TCP-degrading microorganisms

Genera	Degrading strains	Source	Reference
Pseudomonas	P. ATCC 700113	USA (agricultural soil which had been treated with chlorpyrifos)	Feng et al., 1997
Alcaligenes	A. faecalis DSP3	China (contaminated soils around a chemical factory)	Yang et al., 2005
Paracoccus	P. TRP	China (activated sludge from three pesticides manufacturers)	Xu et al., 2008
Bacillus	B. pumilus C2A1	Pakistan (cotton fields where pesticide was sprayed extensively)	Anwar et al., 2009
Ralstonia	R. T6	China (Active sludge collected from wastewater treatment system of a chemical factory)	Li et al., 2010
Synechocystis	S. Strain PUPCCC 64	India (a rice field of a village)	Singh et al., 2011
Cupriavidus	C. pauculus P2	China (TCP-contaminated sludge from a pesticide manufacturing company)	Cao et al., 2012
Cupriavidus	C. DT-1	China (a chlorpyrifos manufacturing site in Changzhou)	Lu et al., 2013
Sphingobacterium	S. JAS3	India (a paddy field located in the Vellore district)	Abraham et al., 2013
Ganoderma	G. JAS4	India (a paddy field soil which had been exposed to chlorpyrifos pesticide)	Silambarasan et al., 2014
Mesorhizobium	M. HN3	Pakistan (Three different agricultural soil samples from fields where CP was applied frequently)	Jabeen et al., 2015
Ochrobactrum	O. JAS2	India (paddy rhizosphere of chlorpyrifos treated soil)	Abraham et al., 2016
Xanthomonas	X. 4R ₃ -M ₁	Australia (Soils from five sugarcane farms with a history of pesticide use to control sugarcane grub)	Rayu et al., 2017
Pseudomonas	P. 4H ₁ -M ₃		

Genera	Degrading strains	Source	Reference
Rhizobium	R. 4H ₁ -M ₁		
Rhodotorula	R. glutinis	Greece (tomato plants phyllosphere and phyllosphere of strawberry plants)	Bempelou et al., 2018
Rhodotorula	R. rubra		
Bacillus	B. subtilis NJ11	Pakistan (pesticide contaminated soils of Punjab)	Bhardwaj et al., 2018
Pseudomonas	P. nitroreducens AR-3	India (soil from agricultural land that was frequently sprayed with pesticides)	Aswathi et al., 2019

In this work, degradation of TCP and 2-HP in liquid culture was further researched and characterized. The degradation metabolites were identified and the complete metabolic pathway of TCP was deduced. Additionally, the abilities of strain DT-1 for TCP degradation and survival in soil were also studied to evaluate its efficiency in bioremediation of TCP-contaminated soil.

Materials And Methods

Chemicals and media

TCP (99%, powder) and 2-HP (99%, powder), purchased from Yiji chemical company (Shanghai, China); Concentrated stock solutions of the compounds (10 g/L) was prepared in sterilized water. Methanol (HPLC-grade), obtained from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China). All other reagents (analytical-reagent grade), purchased from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China).

Cupriavidus sp. DT-1 was isolated and characterized in the previous study; E. coli SM10 (λ pir) pUT- mini-Tn5-gfp (Amp^r); E. coli HB101 pRK2013 (Km^r) and all other molecular biological reagents were obtained from Genscript Biological Science and Technology Co. Ltd. (Nanjing, China).

Medium for bacterial culture: Luria–Bertani (LB) medium contained (g/L): tryptone 10.0, yeast extract 5.0 and NaCl 10.0, pH 7.0. Medium for degradation experiment : The mineral salts medium (MSM) contained (g/L): NH₄NO₃ 1.0, K₂HPO₄ 1.5, KH₂PO₄ 0.5, NaCl 0.5, MgSO₄ 0.2, pH 7.0. All the medium were sterilized by autoclaving at 121.3°C for 30 minutes.

Inoculum preparation for degradation studies

Strain DT-1 was cultured to exponential phase in LB medium at 30°C at 180 rpm, and collected by centrifugation at 6000 g for 5 min at room temperature. The cell precipitation was washed twice with

sterilized MSM and adjusted to approximately 2×10^8 CFU/mL. For the degradation experiments in liquid culture, the cells were inoculated to approximately 1×10^7 CFU/mL.

Degradation of TCP and 2-HP in liquid culture

Degradation experiment was carried out in 100 mL MSM containing 50 mg/L TCP. Culture was incubated at 30°C at 180 rpm for 24 h after inoculation of the strain, and regularly sampled for the determination of the concentrations of TCP and 2-HP every 2 h, 3 mL each time. All samples were in triplicate. Changed the carbon source in the MSM medium from 50 mg/L TCP to 500 mg/L 2-HP and inoculated the cells. Culture was incubated and sampled for determination of the concentrations of 2-HP and the growth of DT-1 at the same conditions as above.

Growth of strain D-2 in MSM culture

Diluents ranging from 10^{-4} - 10^{-1} were obtained by tenfold gradient dilution. According to dilution plate counting method, 0.2 mL diluent was spread on LB plate and cultured at 30°C for 48 h. Plates with a colony number from 30–300 were selected for counting. All samples were in triplicate.

Extraction and Analytical methods

High-performance liquid chromatography (HPLC) was used to analyze the concentration of TCP in liquid samples. 3 mL sample was centrifuged for 10 min at 15,000 g, and the supernatant was filtrated through a 0.2 μ m fiber filter. Samples were freeze-dried, mixed with 2 mL methanol, and HPLC analysis. The liquid chromatograph was Shimadzu LC-201A with SPD-M20A UV-detector (190–800 nm), column was Agilent C-18 (250 mm \times 4.6 mm, 5 μ m), The mobile phase containing methanol/water (80:20, v/v) was delivered at a flow rate of 1.2 mL/min at 25°C. The detection wavelength was 230 nm. Concentration of TCP was determined by comparison with values in the calibration curve established by concentrations between 0.1 and 100 mg/L. The limits of detection (LOD) and quantification (LOQ) were 0.016 and 0.057 mg/L, respectively. The recovery and RSD were 96.5%-101.5% and 1.10%-2.84% at concentrations ranging from 0.1 to 100 mg/L.

Concentration of 2-HP was detected following the method above. The calibration curve established by concentrations between 0.1 and 100 mg/L. The recovery and RSD were 94.6%-103.8% and 1.37%-2.66%. The limits of detection (LOD) and quantification (LOQ) were 0.021 and 0.075 mg/L, respectively.

To identify the metabolites of 2-HP, MSM containing 500 mg/L 2-HP as the sole carbon source was inoculated, and cultured at 30 °C at 180 rpm. Samples were collected from the culture and centrifuged for 10 min at 15,000 g. The supernatant was filtered through a 0.2 μ m fiber filter, freeze-dried, and dissolved in 1 mL methanol, then identified by HPLC–MS (Finnigan TSQ Quantum Ultra AM, Thermal, USA). The

HPLC system was implemented as described above. MS was operated in the electron spray ionization mode with a negative polarity, and scanning at a mass range from 30 m/z to 400 m/z.

Degradation of TCP by strain DT-1 in soil

Soil samples used for the experiment were collected from Anhui Normal University and a farmland which had no previous exposure to TCP. Samples were air-dried, sieved to 2 mm, and homogenized immediately after collection. For analyzed the physical and chemical properties of the soil, 20 g sample was weighed, dried at 105°C, and measured its water content. Soil total nitrogen and nitrate nitrogen were determined with continuous flow analyzer by kjeldahl method (Bulluck et al., 2002). Organic matter was determined by potassium dichromate volumetric method (Ciavatta et al.,1991). Select characteristics of the soil samples were showed in Table 2.

Table 2
Select characteristics of the soil samples.

From	pH	Water (%)	Total N(%)	Organic matter (%)	Total K (cmol/kg)	Total Na(cmol/kg)
campus	6.4	14.4	0.13	2.27	0.13	0.17
farmland	6.9	13.9	0.37	4.46	0.18	0.24

For the degradation research, glass beaker (200 mL) microcosms, each containing 100 g soil, were spiked with TCP (50 mg/kg soil). For the set of inoculated beakers, MSM medium (4.0 mL) containing strain DT-1 was added at the final concentration 1×10^7 cells/g of soil. On the contrary, MSM medium (4.0 mL) without DT-1 was added for the set of non-inoculated beakers. Each soil sample was incubated at 30°C under sterile condition. During incubation, the soil microcosms were weighed regularly, the weight loss was compensated by the addition of sterile water. Soil samples (5 g) were collected for analysis of TCP concentration every 5 d for 35 d. Triplicate soil samples were prepared for two sets of treatments.

Gfp -tagged of strain DT-1

Gfp gene which can encod bioluminescent protein is one of the most useful biomarkers available for monitoring purposes (Errampalli et al., 1999; Elvang et al., 2001). Strain DT-1, E. coli SM10 (λ pir) pUT-mini-Tn5-gfp, E. coli HB101 pRK2013 was cultured in LB liquid medium for 12 h, respectively. Cells were collected by centrifugation at 6000 g for 5 min at room temperature and washed twice with sterilized water. 5ML of each three bacterial suspension was intensive mixing, centrifuged at 6000 g for 5 min. The cell precipitation was dissolved with 20 μ L LB medium and the mixed bacterial suspension was spread on the filter membrane. Then the membrane was placed on a LB plate, cultured at 30°C for 24 h. Cells were washed and dissolved in saline and spread on LB plate which contained 100 mg/L ampicillin and

kanamycin, cultured at 30°C for 24 h. Bacterial colonies on the plate were the transformants of strain DT-1 which contained gene *gfp*.

Content analysis of strain DT-1- *gfp* in soils

Soil (1 g) was dissolved in 9 mL sterile water and 1 mL soil suspension was diluted 10^4 times, 0.2 mL diluted suspension was spread on LB plate and cultured at 30°C for 48 h. Plates were placed under an ultraviolet lamp to count the colonies which emitted green fluorescence. All samples were in triplicate.

Results

Degradation of TCP and 2-HP in liquid culture

The result of degradation of TCP and its metabolite 2-HP was shown in Fig. 1. There was no significant change in the concentration of TCP and 2-HP during the first 2 h. This is attributed to the fact that the strain need adaptation to a new environment and enzymes relevant to degradation have not been synthesized. During next 2–10 h, the concentration of TCP decreased while which of 2-HP increased rapidly, indicating strain DT-1 began to show its degradation activity, and 2-HP was the major degradation product. However, the total concentration of 2-HP was less than the reduction of TCP, indicating 2-HP was degraded further due to strain DT-1 could use 2-HP as the sole carbon source for growth (Fig. 2). During 10–18 h, TCP was completely degraded, and the concentration of 2-HP was decreased rapidly until disappeared.

Strain DT-1 could degrade 2-HP at a high concentration of 500 mg/L within 10 h and use it as the sole carbon source for growth. Growth and degradation occurred mainly between 2 and 8 hours (Fig. 2). The optimal initial concentration of TCP was lower in the research due to its toxic effect to microorganism at a higher concentration. However, 2-HP had less toxicity to strain DT-1, the initial concentration in the experiment was 500 mg/L. Which made the metabolites of 2-HP easier to be detected due to their higher concentrations.

Identification of 2-HP metabolites

2-HP and its metabolites were preliminarily detected by HPLC. 2-HP was used as a standard which showed a retention time of 3.58 minutes (Fig. 3A). For the sample collected 6 h after inoculation, color of the medium turned blue (Fig. S1) and three compounds were detected with retention times of 2.22, 2.71 and 3.59 min (Fig. 3B), respectively, one was 2-HP and the other two were its metabolites. For the sample collected 10 h after inoculation, two compounds were detected, one with retention time of 2.23 min was same as which detected in the sample collected at 6 h, another compound with retention time of 3.09 min was a new metabolite (Fig. 3C). Samples were analyzed with MS, the prominent protonated molecular ion of compound with retention time of 3.58 minutes was at $m/z = 94.08$ $[M-H]^-$ and identified as 2-HP

(Fig. 4a). Compound with retention time of 2.71 minutes ($m/z = 249.10 [M-H]^-$) (Fig. 4b) was identified as nicotine blue, which turned the medium from colorless to blue (Fig. S1), indicating 2,3,6-trihydroxypyridine (2,3,6-THP) was the degradation metabolite of 2-HP because 2,3,6-THP was spontaneously oxidized to nicotine blue in aerobic environment. So the compounds with retention time of 2.22 and 3.09 minutes were produced by further degradation of 2,3,6-THP, and identified as maleamic acid ($m/z = 114.08 [M-H]^-$) (Fig. 4c) and fumaric acid ($m/z = 115.10 [M-H]^-$) (Fig. 4d), respectively. The result showed the degradation pathway of 2-HP by strain DT-1 was the same as which in *Nocardia* sp. (PNO) (Shukla and Kual, 1986). But this represented the first discovery of the degradation pathway in a Gram-negative bacterium. Combined with the previous conclusion, the complete metabolic pathway of TCP degradation by strain DT-1 was obtained (Fig. 5).

Degradation of TCP and 2-HP in different soils

As shown in Fig. 6a, the campus soil containing 50 mg/L TCP inoculated with strain DT-1 resulted in a high degradation rate. The concentration of TCP decreased from 50 to 2.8 mg/kg with the rate of 94.4% in 35 d, and most degradation occurred between 10 and 30 days. 2-HP was produced by degradation of TCP, but the concentration was very low and the highest was only 9.8 mg/kg. Which indicated 2-HP was immediately degraded since existence. In uninoculated soil, only about 20.4% of TCP was degraded naturally in 35 d, and the presence of 2-HP was almost never detected. In farmland soil, the overall degradation trend is similar to that in the campus soil. But the degradation rate 86.7% in inoculated soil was slightly lower (Fig. 6b), while in uninoculated soil the degradation rate 28.4% was higher. That was because the proliferation of indigenous microorganisms due to much abundanter nutritional materials in the farmland soil, which enhanced the self-purification ability of soil but have a competitive inhibition effect on the reproduction of DT-1. The result indicated that strain DT-1 was capable of degrading TCP in different soil environments.

Gfp -tagged and trace of strain DT-1- gfp

In order to observe the survival of strain DT-1 in the soil, a fluorescent marker gene *gfp* was introduced into strain DT-1 and a new genetic engineering bacterium named DT-1-*gfp* was constructed. Figure 7 showed the colonies of strain DT-1 and DT-1-*gfp* under the UV light. Strain DT-1 was lackluster as the control and DT-1-*gfp* emitted green fluorescence. Which indicated the *gfp* gene was highly expressed in strain DT-1-*gfp*. The engineering bacterium was continuously cultured for 20 generation on the ordinary LB plate without selective pressure, and the same effect was observed under fluorescence microscope (Fig. S2), indicating the existence and expression of *gfp* gene in strain DT-1 was stable. The result of degradation experiment showed the degradation characteristics of strain DT-1-*gfp* were consistent with those of strain DT-1 (Fig. S3). So the genetic engineering bacterium was equated with the original strain and could be used for bioremediation.

Table 3 shows the survival of strain DT-1-gfp after inoculation in the soils. The initial concentration of cells was 1×10^7 CFU/mg. In the first 7 days, the bacterial population increased slightly due to the nutrients in the soil which supported the growth of cells. During 7–21 d, the total number of bacteria decreased dramatically due to competitive inhibition effect on the reproduction of DT-1-gfp by the proliferation of indigenous microorganisms in soils. And the inhibition effect was more intense in the farmland soil than that in the campus soil. In 21–35 d, the bacterial population remained stable without significant decline, indicating the microbial community in the soil reached a state of equilibrium. The result indicated that strain DT-1-gfp could fit into the simulation soil environment very well and survive for enough time to exert its ability on the bioremediation.

Table 3
The content of DT-1-gfp in campus and farmland soils.

Soil Type	Content of DT-1-gfp ($\times 10^6$ CFU/mg)					
	0d	7d	14d	21d	28d	35d
Campus soil	10	11.91 \pm 1.43	7.13 \pm 1.92	3.68 \pm 1.38	3.19 \pm 1.82	2.89 \pm 0.98
Farmland soil	10	11.02 \pm 1.37	6.34 \pm 1.05	2.47 \pm 1.11	2.69 \pm 1.04	2.12 \pm 0.95

Discussion

The market demand of organophosphorus pesticides with low toxicity represented by chlorpyrifos is increasing due to the restrictions and prohibitions of highly toxic organophosphorus pesticides, which causes the production and residue of TCP in the environment. Accumulation of TCP has a strong inhibitory effect on the growth of microorganisms, and furthermore inhibits degradation of itself and the parent compound chlorpyrifos, as well as other organic compounds (Cao et al. 2012; Singh et al. 2004). Thus, the organic pollution caused by pesticides residue in the environment is further intensified.

Biodegradation mechanism and metabolic pathway of TCP have not been researched thoroughly to date. *Ralstonia* sp. T6, *Cupriavidus* sp. P2 and *Cupriavidus nantongensis* X1^T could degrade TCP and produce 3,6-dihydroxypyridine-2,5-dione, the intermediate metabolite could be further degraded and mineralized (Li et al., 2010; Cao et al., 2012; Fang et al., 2019). But the degradation pathway was incomplete due to the subsequent degradation products were not detected. Wang identified a novel microbial consortium capable of degrading TCP in anaerobic environment, and speculated the complete degradation pathway of TCP from dechlorination to pyridine ring cleaved (Wang et al., 2019). However, the study subject was microbial consortium and the degradation condition was anaerobic. Previous study indicated that *Cupriavidus* sp. DT-1 was capable to degrade TCP in aerobic environment, and the intermediate metabolite 2-HP was produced by three steps of dechlorination (Lu et al., 2013). This was a novel metabolic pathway but not complete because the pathway after 2-HP was not clear. The biodegradation pathway of 2-HP has been studied in detail during the past decades. The first kind, 2-HP was transformed

into dihydroxypyridine and then pyridine-ring was cleaved. Different final metabolites were produced by different microorganisms, such as fumaric acid (Zhao et al., 2019; Stankeviciute et al., 2016), 5-amino-5-oxo-2-pentenoic acid, succinic semialdehyde (Zefirov et al., 1994), respectively. The second kind, 2-HP was turned into trihydroxypyridine accompanied by the production of a blue substance. The final metabolites were fumaric acid (Shukla et al., 1986), succinic semialdehyde (Khasaeva et al., 2010), and α -ketoglutarate (Vaitekunas et al., 2016) under the action of further degradation of different microorganisms. In summary, the bacteria with the ability to convert 2-HP into blue substance were all gram-positive. In this study, the metabolite of 2-HP degraded by strain DT-1 was detected and the degradation pathway was speculated. Different from the previous conclusions, blue substance was the main intermediate metabolite produced by the gram-negative strain DT-1. This is an interesting finding, and the biodegradation mechanism of 2-HP by DT-1 remains to be studied further.

The ultimate goal of research on biodegradation is applied to bioremediation for the contaminated environment. Development of bioremediation strategies for polluted agricultural soils based on the biodegrading microorganisms, represent a growing area of research worldwide (Semple et al., 2001). As an efficient and cheap biotechnology approach, bioremediation has received increasing attention and will be used a lot to clean up polluted environments (Akbar and Sultan, 2016; Uqab et al., 2016; Shishir et al., 2019). However, there are very few reports on biodegradation of TCP in the soil recently. Because most bioremediation studies were based on chlorpyrifos-contaminated soil. For example, *Ganoderma* sp. JAS4 is capable to degrade 90 mg/kg chlorpyrifos in the soil within 24 h. The main product TCP is degraded completely after 72 h and 96 h in different soils, respectively (Sivagnanam et al., 2013). This kind of researches emphasize the degradation of chlorpyrifos, but the degradation of TCP in the soil is short of study. In this work, strain DT-1 could play a good role in degradation of TCP in soil compared with that in liquid. The metabolite 2-HP was detected and its concentration change was characterized. In addition, tracer experiment showed the strain had strong vitality in the soil, indicating strain DT-1 a promising candidate for its application in the bioremediation of TCP-contaminated environments.

Declarations

Ethics approval and consent to participate

Not applicable. This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable.

Availability of data and materials

The dataset supporting the conclusions of this article is included within the article. All data are fully available without restriction

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

P detected the metabolites of 2-hydroxypyridine, constructed the engineering bacterium, and was the major contributor in writing the manuscript. AM and JZ carried out the experiment of TCP degradation by the strain in the soil. HM researched the growth and degradation characteristics of the strain. All authors read and approved the final manuscript.

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Supplementary Information

Fig. S1 Blue substance produced from the degradation of 2-HP by strain DT-1.

Fig. S2 Strain DT-1-*gfp* under fluorescence microscope.

Fig. S3 Degradation of TCP and 2-HP by strain DT-1-*gfp*. (◻) concentration of TCP;

(▲) concentration of 2-HP.

Figures

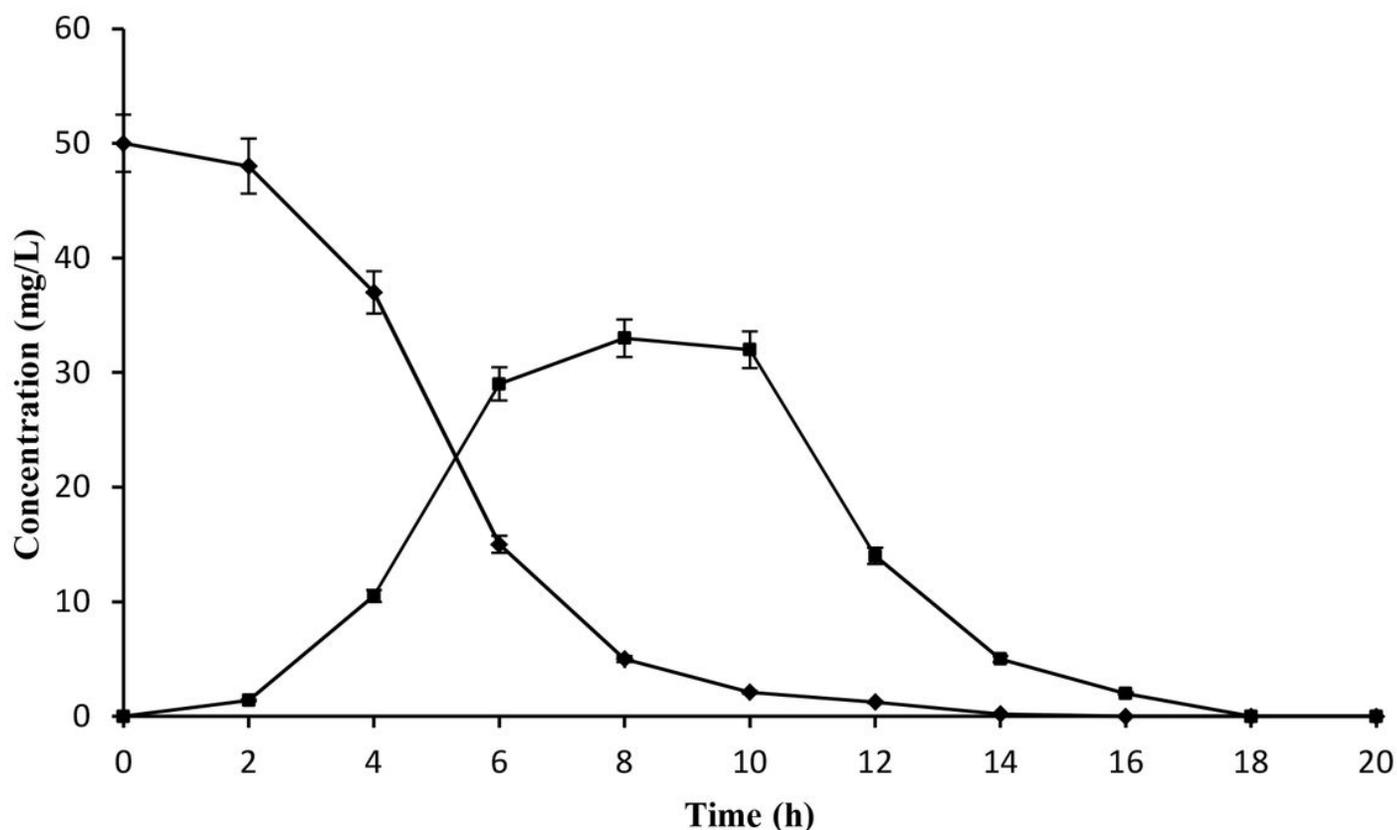


Figure 1

Degradation of TCP and 2-HP by strain DT-1. (⊠) concentration of TCP; (■) concentration of 2-HP.

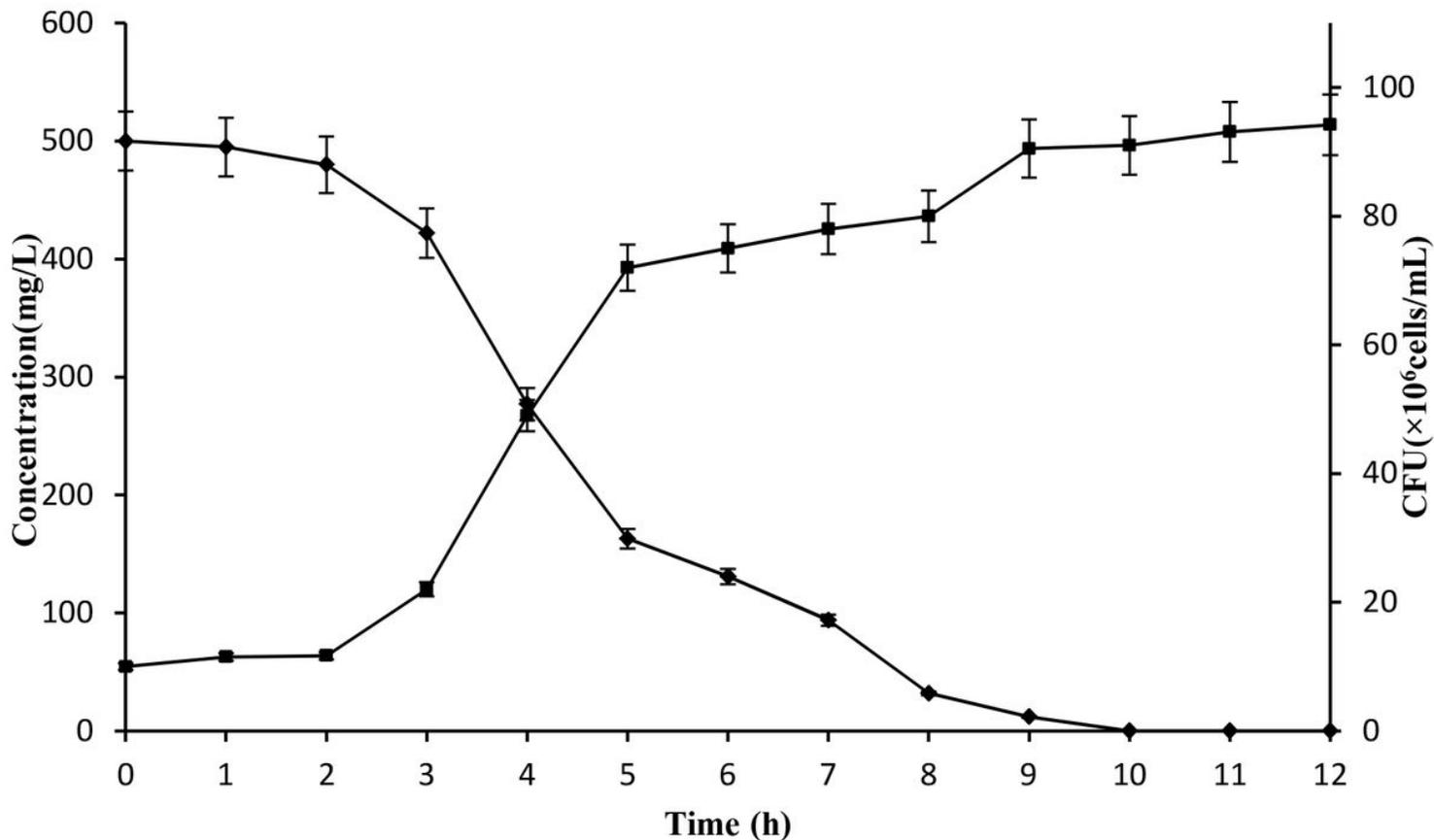


Figure 2

Utilization 2-HP as sole carbon source for growth and degradation by strain DT-1 (⊠) concentration of 2-HP; (■) cell density.

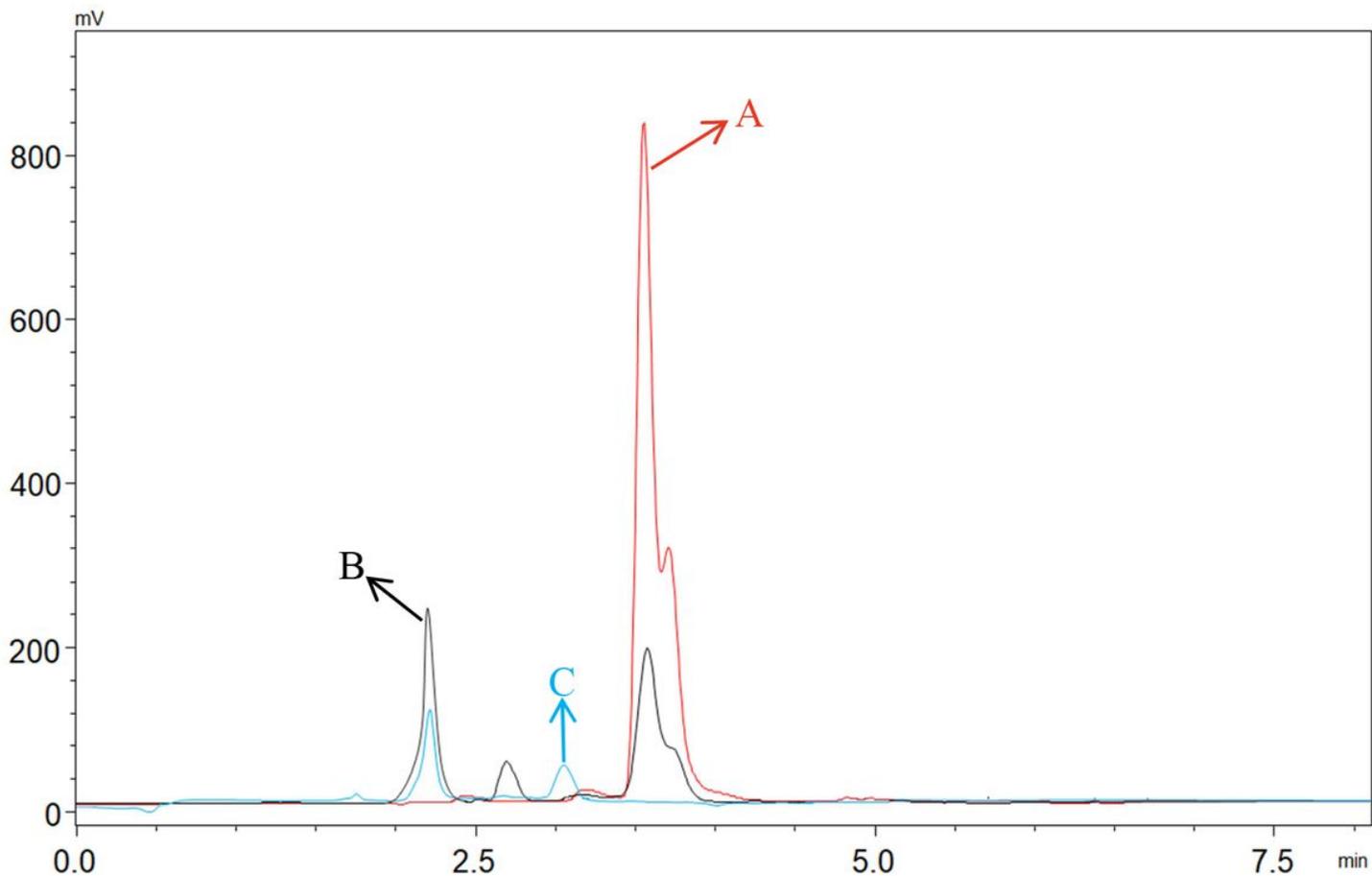


Figure 3

HPLC chromatogram of 2-HP and its metabolite after inoculation with DT-1 for 0 h (A red, RT= 3.58 minutes); 6 h (B black, RT= 2.22, 2.71 and 3.59 min); 10 h (C blue, RT= 2.23 and 3.09 min), respectively.

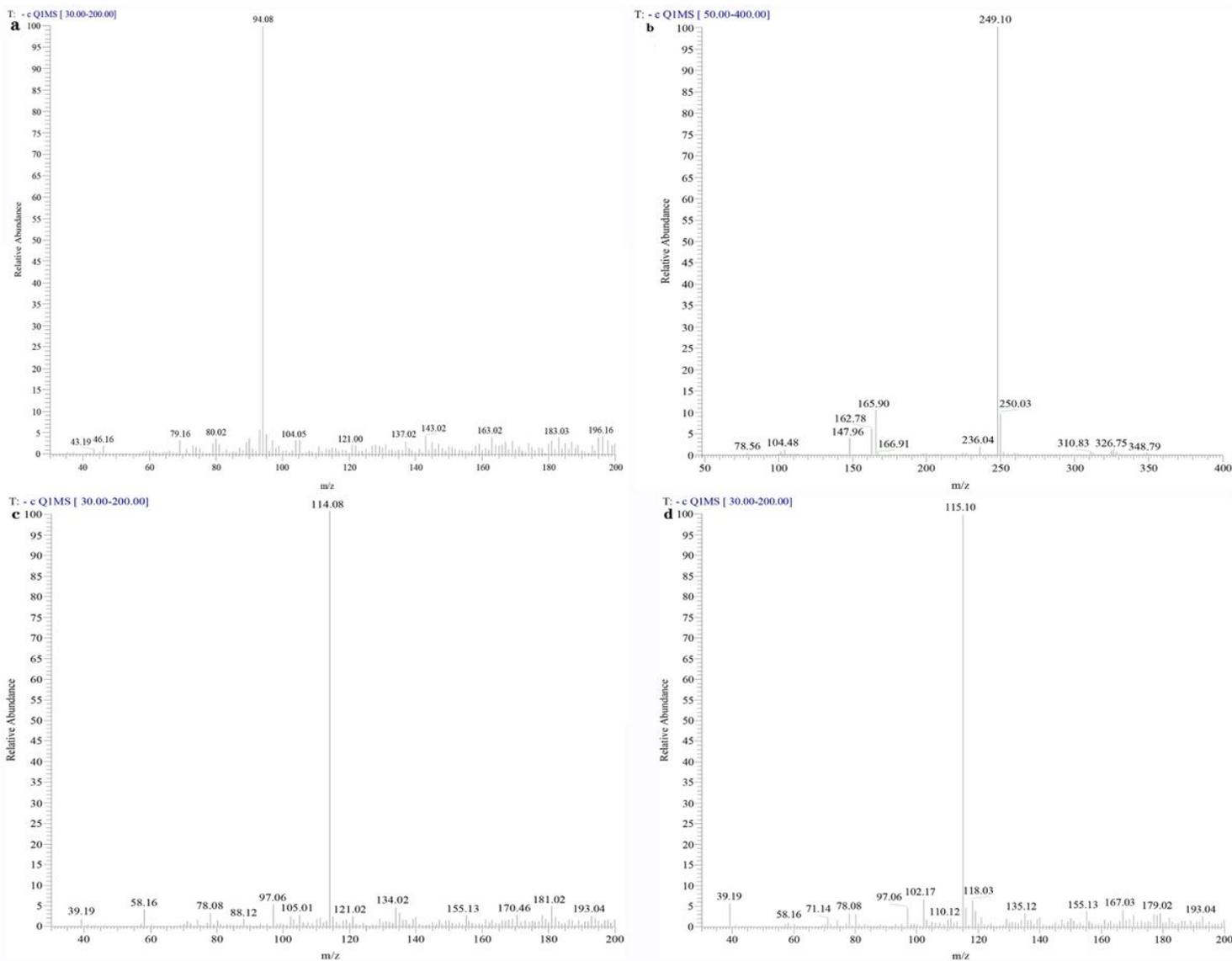


Figure 4

MS analysis of 2-hydroxypyridine degradation intermediates by strain DT-1. a. MS detection of 2-HP; b. MS detection of the blue compound; c. MS detection of the compound with retention time of 2.22 minutes; d. MS detection of the compound with retention time of 3.09 minutes.

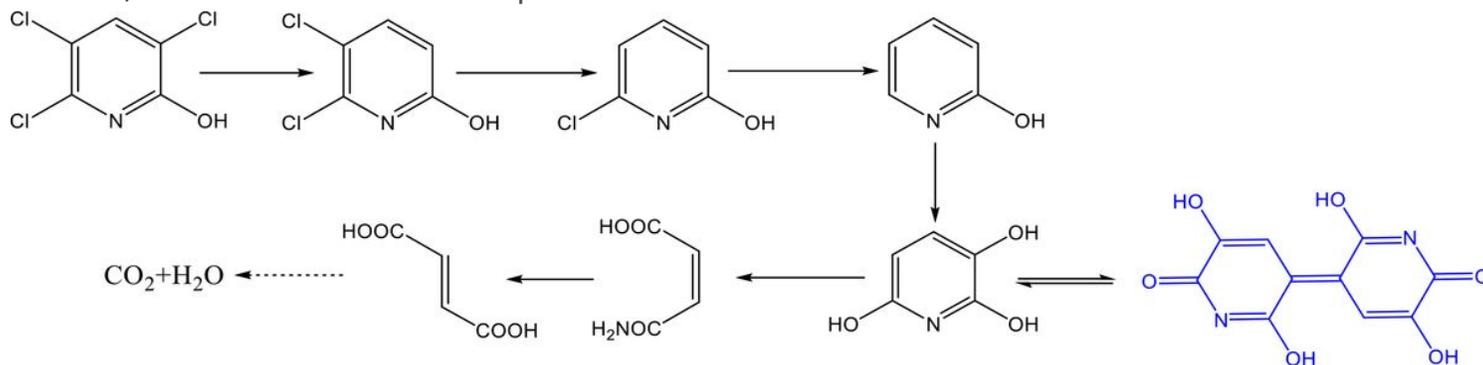


Figure 5

The complete metabolic pathway of TCP degradation by strain DT-1.

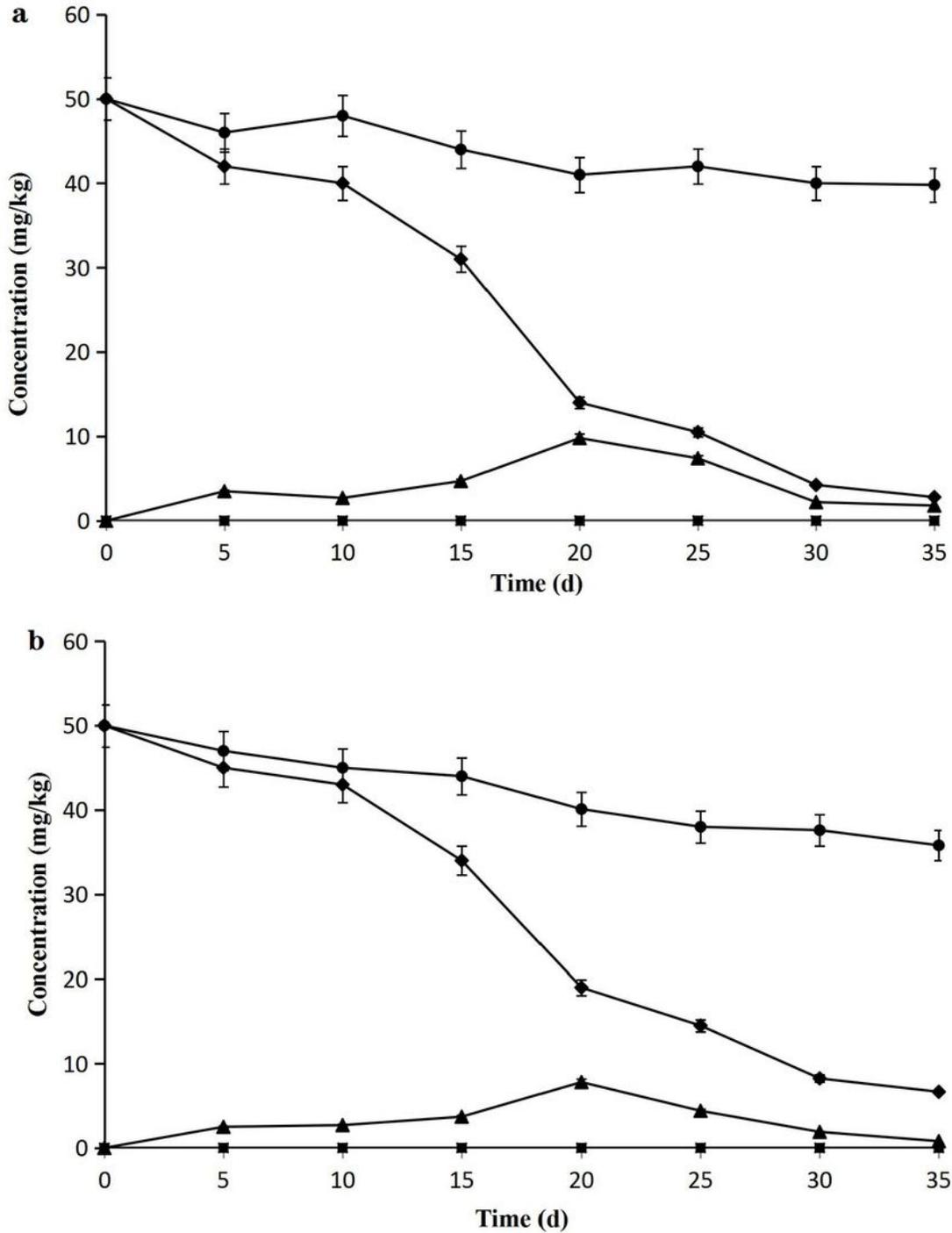


Figure 6

Degradation of TCP in campus and farmland soils by strain DT-1. a. campus soil; b. farmland soil. (●) concentration of TCP in the control soil without inoculated; (⊗) concentration of TCP in the soil inoculated with DT-1; (▲) concentration of 2-HP in the soil inoculated with DT-1; (■) concentration of 2-HP in the control soil.

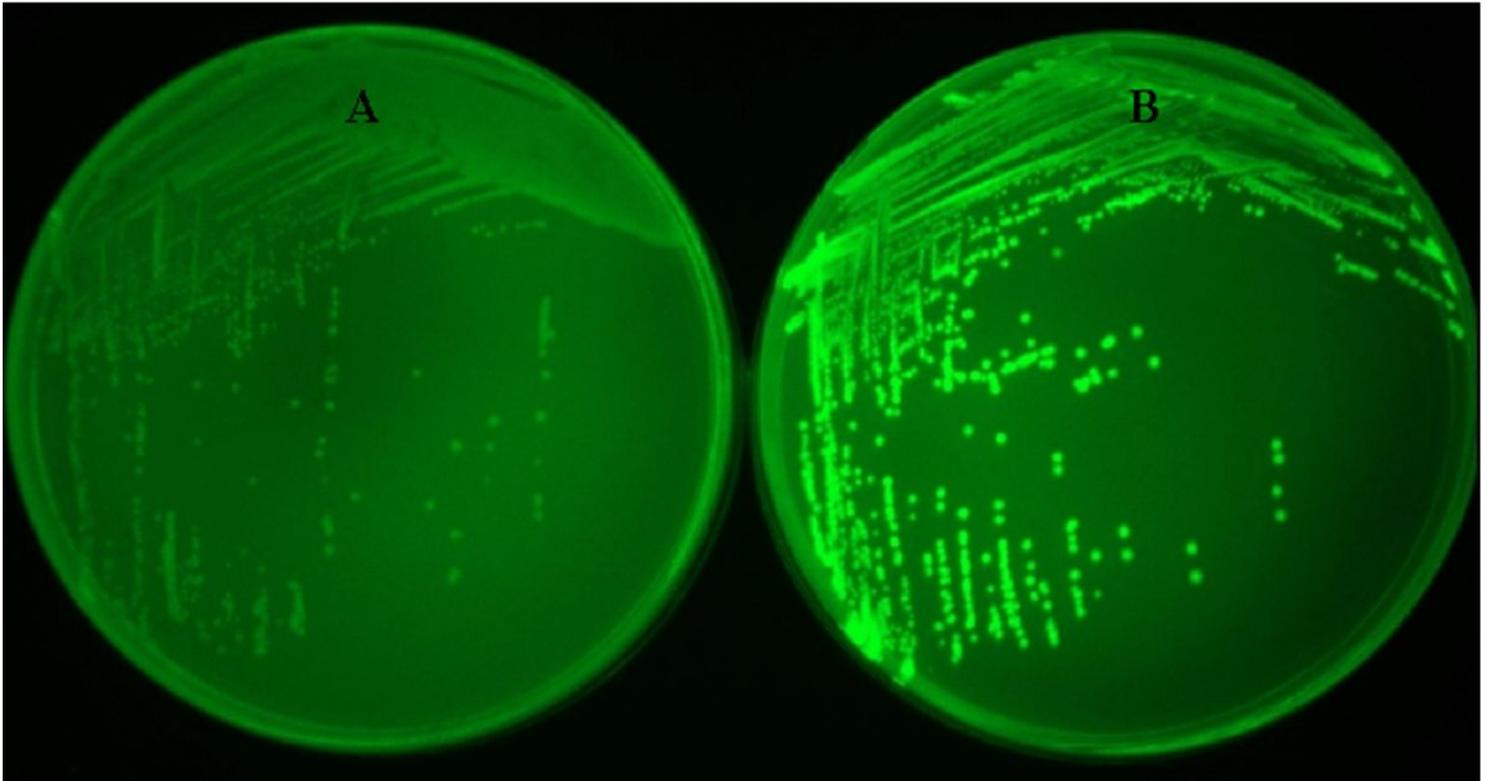


Figure 7

Strain DT-1 and DT-1-gfp under the UV light (A) DT-1; (B) DT-1-gfp

Supplementary Files

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