Journal of Experimental Biology and Agricultural Sciences, June - 2023; Volume – 11(3) page 563 – 571

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Isolation and characterization of chlorpyrifos-degrading bacteria in tea-growing soils

Lam Thanh Nguyen[1,2], Tam Bang Kieu Nguyen[2], Thanh Huu Luong[3], Huyen Thi Dam[2], Phuong Minh Nguyen[2*]

[1]Ministry of Natural Resources and Environment, 10 Ton That Thuyet, Nam Tu Liem district, Hanoi, Vietnam
[2]Faculty of Environmental Sciences, University of Science, Vietnam National University, Hanoi, 334 Nguyen Trai, Thanh Xuan, Hanoi, Vietnam
[3]Department of Environmental Biology, Institute for Agricultural Environment, Hanoi, Vietnam

Received – April 13, 2023; Revision – June 18, 2023; Accepted – June 25, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).563.571

KEYWORDS

CPF

Degradation

E. adhaerens VNN3

M. populi CNN2

Remediation

ABSTRACT

The excess use of pesticides in the agricultural sector has caused environmental pollution and affected the complete ecosystem. Among the various commonly used pesticides, chlorpyrifos (CPF) is widely used against multiple agrarian pests due to its effectiveness and higher insecticidal activities. However, along with its beneficial usage, CPF has various residual effects on the environment, causing multiple negative impacts on aquatic organisms and human health. Consequently, methods for eliminating CPF in the background are essential. Among the currently available approaches to CPF remediation, biological methods using microorganisms are eco-friendly and cost-effective. Therefore, this study was conducted to isolate and characterize chlorpyrifos-degrading bacteria from the tea-growing soil of Vietnam. For this, soil samples were collected from the 20 tea-growing areas of Vietnam. From the collected samples, three bacterial strains viz., *Methylobacterium populi* CNN2, *Ensifer adhaerens* VNN3, and *Acinetobacter pittii* CNN4 have been isolated by using streak plate method and identified based on 16S rRNA gene analysis. The study results showed that under laboratory conditions, *E. adhaerens* VNN3 had the highest CPF degradation ability and was followed by the strain *M. populi* CNN2. In liquid medium, CPF concentration (100 mg/L) was reduced by 95.2% and 81.4% by *E.adhaerens* VNN3 and *M. populi* CNN2, respectively, after 72 h. Further, under *in-vitro* conditions, the concentration of CPF was reduced from 500 mg/kg to 112 ± 1.73 (77.6%) and 197 ± 2.08 mg/kg (60.6%) by *E. adhaerens* VNN3 and *M. populi* CNN2, respectively. Based on the obtained results, it can be concluded that *E. adhaerens* VNN3 and *M. populi* CNN2 can be used for CPF-contaminated agricultural soil remediation.

* Corresponding author
E-mail: nmphuong.hn@hus.edu.vn (Phuong Minh Nguyen)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Organophosphorus (CPF) pesticides have been widely used in agriculture to control pests and insects. However, their extensive usage has caused serious environmental pollution and ecological risks (Huang et al. 2021). Chlorpyrifos (o,o-diethyl-o-3,5,6-trichloro-2-pyridinyl phosphorothionate) (CPF) is an organophosphate pesticide that has been widely used for pest control (Bose et al. 2021). It has been reported that the half-life of CPF in the soil was 60 - 120 days, depending on the soil types and environmental conditions (Elshikh et al. 2022; Huang et al. 2021). Further, 3,5,6-trichloro-2-pyridinol (TCP), a major metabolite of CPF, is also persistent in the environment with a half-life of 65 – 350 days in soil (Bose et al. 2021). Due to the wide application of CPF in agriculture, CPF and its residues have been detected in surface water, groundwater, soils, sediments, crops and even human breast milk (Huang et al. 2021; Oltramare et al. 2022; Sishu et al. 2022; Takayasu et al. 2017). The CPF concentration range from 239 to 675.4 µg/kg was determined in agricultural soils (Bhandari et al. 2020; Tan et al. 2020). The adverse effects of CPF on organisms and human health have also been well-documented (Jin et al. 2015; Huang et al. 2021; Cheng et al. 2023). Bioaccumulation of CPF affects the human endocrine and cardiovascular systems (Bose et al. 2021); furthermore, CPF and its metabolites can disrupt normal pregnancy, induce the occurrence of obesity and breast cancer (Blanco et al. 2020; Hazarika et al. 2020; Moyano et al. 2020). Underexposure to CPF, oxidative stress and disruption of neurotransmitter metabolism were observed in zebrafish (Sud et al. 2020). In addition, the genotoxicity effects of CPF have also been recorded in various aquatic organisms (Huang et al. 2020). Due to the ecological risks that CPF poses to the environment, the elimination of CPF is necessary. Current methods such as ultrasonication, photocatalytic degradation, and biochar adsorption have been used for CPF removal; however, these physicochemical methods are costly and technically challenging (Soltani-Nezhad et al. 2020; Huang et al. 2021). Biological methods using microorganisms that degrade organic compounds and complex xenobiotics are considered environmental-friendly and efficient approaches to detoxify CPF (Bose et al. 2021; Elshikh et al. 2022; Hadibarata et al. 2023). The CPF-degrading potential of various bacterial (*Pseudomonas, Stenotrophomonas, Bacillus, Klebsiella*) and fungal species (*Aspergillus terreus, Trichoderma harzianum, Ganoderma* sp. JAS4) have been well documented (Ishag et al. 2016; Khalid et al. 2018; Bose et al. 2021). Phosphoric triester hydrolases, lignolytic laccases, and carboxypeptidase (CPD) are some enzymes involved in the microbial degradation of CPF (Bose et al. 2021; Huang et al. 2021). Biodegradation of CPF is of great importance in eliminating CPF and reducing its toxicity in the environment; therefore, studies on microbial CPF degradation are highly significant. The present study aimed to isolate and characterize CPF-degrading bacteria in tea-growing soils and evaluate the potential of CPF degradation by the isolated strains.

2 Materials and methods

2.1 Isolation of CPF-degrading bacteria

The isolation of CPF-degrading bacteria was performed as per the method of Hossain et al. (2015) and Elshikh et al. (2022) with some modifications. For the bacterial isolation, 20 soil samples were collected from a depth of 20 cm of the tea-growing areas of Phu Thinh commune, Dai Tu district, Thai Nguyen province and Anh Son commune, Hung Son district, Nghe An province of Vietnam. Each soil sample was prepared by properly mixing the five diagonally taken from the selected field. The bacterial isolation was performed using the mineral salt medium (MSM) containing (g/L) K_2HPO_4 (1.8), NH_4Cl (4.0), $MgSO_4.7H_2O$ (0.5), $FeSO_4.7H_2O$ (0.1) and trace solution (1 ml/L). From this, a 5 g soil sample was added to 95 mL of the mineral salt medium (MSM) containing 50 mg/L CPF and the cultured flasks were incubated at 28 - 30^oC in an incubator for five days. After the incubation period completion, 5 mL was taken out and reinoculated in the fresh MSM medium containing CPF at 50 mg/L. The procedure was repeated three times, and the culture was diluted at a concentration of 10^{-3} to 10^{-5}. 50 µl of the diluted culture was spread on agar plates containing MSM medium supplemented with 100 mg/L CPF. The plates were incubated for five days at 28 - 30^oC, and the isolated strains were purified by streaking on the agar plates supplemented with 100 mg/L CPF.

2.2 Growth of the isolated strains and microbial density determination

The growth characteristics of the isolated strains were determined by using LB medium containing (g/L): peptone (10), yeast extract (5), NaCl (5) and 100 mg/L of CPF. After 24, 48, 72 and 96 h, the measurement of optical density (OD) at 620 nm was carried out. The bacterial density was determined by using agar plates and sample dilution at 10^{-7} - 10^{-9} concentrations.

2.3 Identification of the isolated strains using 16S rRNA analysis

According to Ausubel et al. (1994), DNA isolation was carried out. The 16S rRNA gene amplification was carried out by using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (3'-TACGGTTACCTTGTTACCGACTT-5'). The PCR was performed with a total volume of 25 µl per sample having total DNA (1 µl), Pr16F (1 µl), Pr16R (1 µl), 10 mM dNTPs (2 µl), Taq polymerase (0.25 µl), Buffer Taq polymerase 10 X (5 µl) and deionized water. The carried out thermal cycle was as follows: (1) initial denaturation at 94^oC for 3 min, (2) denaturation at 94^oC for 1 min, (3) annealing at 55^oC for 1 min, (4) extension at 72^oC for 2 min. Steps 2-4 were repeated in 30 cycles, and the final extension was conducted at 72^oC for 7 min. The PCR product was quality-checked via agarose gel electrophoresis (0.8% agarose) at 100V for

30 minutes. The 16S rRNA gene sequences were submitted to the Genbank database.

2.4 Evaluation of CPF degradation by the isolated bacterial strains

The isolates were cultured in MSM medium supplemented with 100 mg/L CPF until the OD_{620} value of 0.6 was reached. After 24, 48 and 72 h, the CPF concentration of the samples was determined, and the CPF degradation ability of the isolated strains in the cultured medium was determined.

The CPF degradation capability of the isolated strains was also evaluated under *in-vitro* conditions. For this, 20 soil substrates were collected, and 500 mg/kg CPF was added. The soil substrate was inoculated with selected isolated strains in MSM solution (OD_{620} ~ 0.6) (10 mL/kg soil sample). The samples were incubated at 30^0C for 10 days. The degradation of the residual CPF in soil samples after 3, 7 and 10 days were determined.

2.5 Determination of CPF concentration

CPF in liquid samples was extracted by using petroleum ether for 1 minute. The supernatant containing CPF was measured at 294 nm by spectrophotometric method. The CPF samples were shaken with 5 mL of acetonitrile and filtered using a 0.22 µm syringe filter. CPF concentration was measured using HPLC at 300 nm (Elshikh et al. 2022). The column used was the XDB-C18 (5 Mm, 4.6x250 mm). The mobile phase was a mixture of acetonitrile and buffer containing acetic acid and water. The retention time of CPF was 9.5 min. The residual CPF concentration in soil samples was determined by GC/MS (EPA 1996).

2.6 Statistical analysis

Data were presented as the mean values of three replicates ± standard deviation using Microsoft Excel version 2108.

3 Results and discussion

3.1 Isolation of CPF-degrading bacteria

From the collected 20 soil samples, a total of 51 bacterial strains were isolated, and among these, four CPF-degrading bacterial strains were isolated, namely CNN1, CNN2, VNN3 and CNN4. Figure 1 shows the colonies of the four isolated strains. Figure 2 shows the morphologies of the bacterial cells under an optical microscope with 100x magnification, respectively. All four strains had rod shape, single stand cells. CNN1 and CNN4 were approximately 0.5 x 1.0 µm, whereas CNN2 and VNN3 were 0.8 x 1.0 µm and 1.0 x 1.5 µm in size, respectively. The colonies of CNN1 were round, glossy, ivory-yellow, and non-pigmented (diameter: 1 - 3 mm). The colonies of CNN2 were pale pink, uniformly round, smooth, round, convex, glossy and non-pigmented (diameter: 0.5 mm). The colonies of VNN3 were white, round, small, regular, smooth surfaces, and non-pigmented

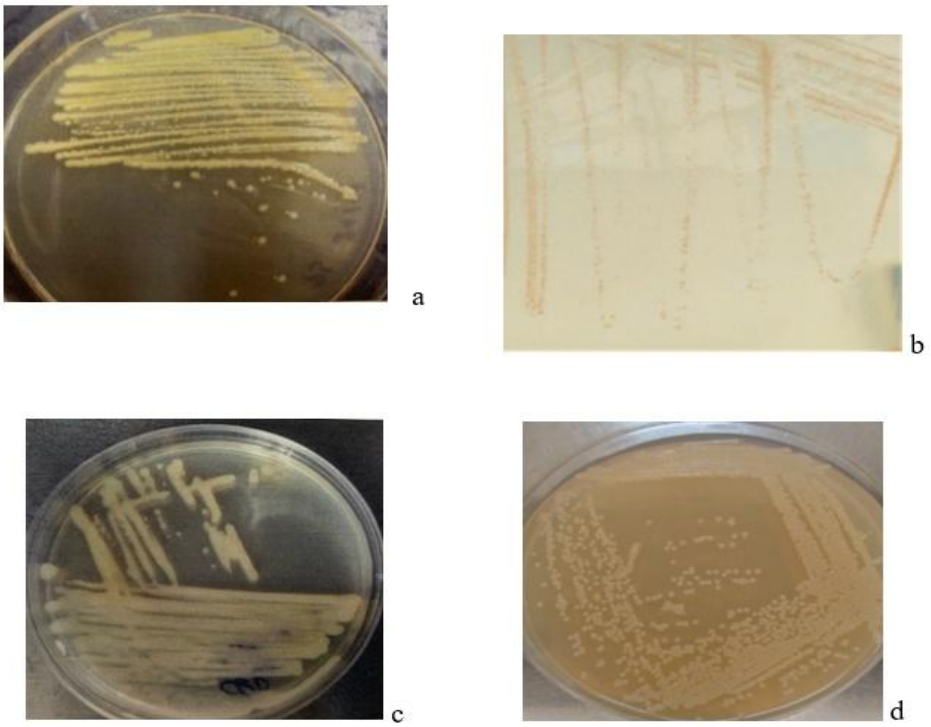

Figure 1 Colonies of the strains (a) CNN1, (b) CNN2, (c) VNN3 and (d) CNN4

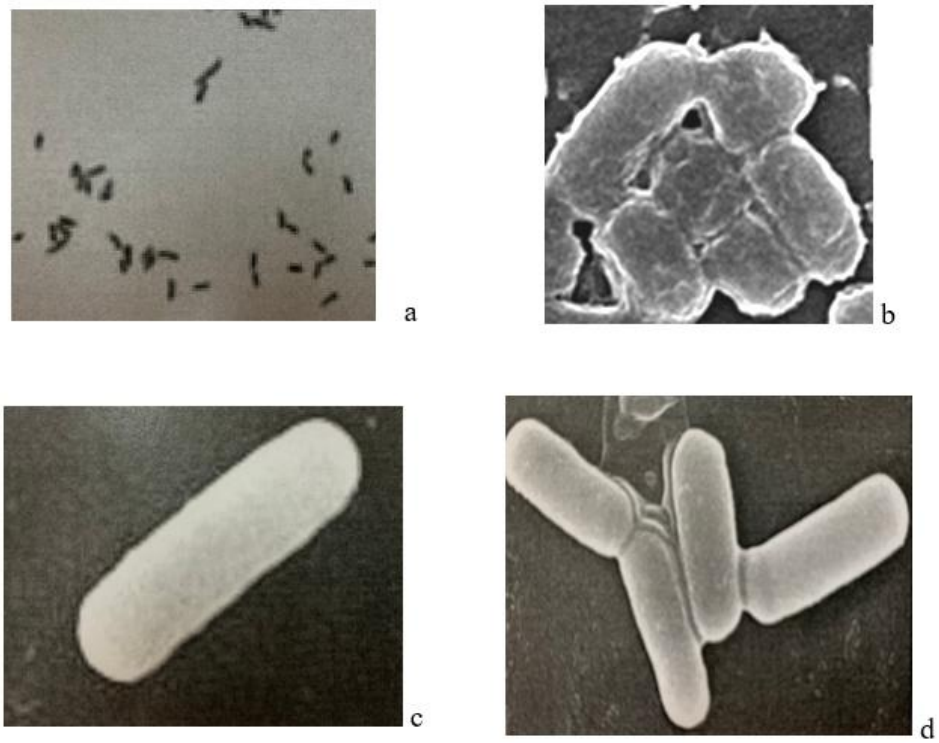

Figure 2 Morphological characteristics of the strains (a) CNN1,(b) CNN2,(c) VNN3 and(d) CNN4

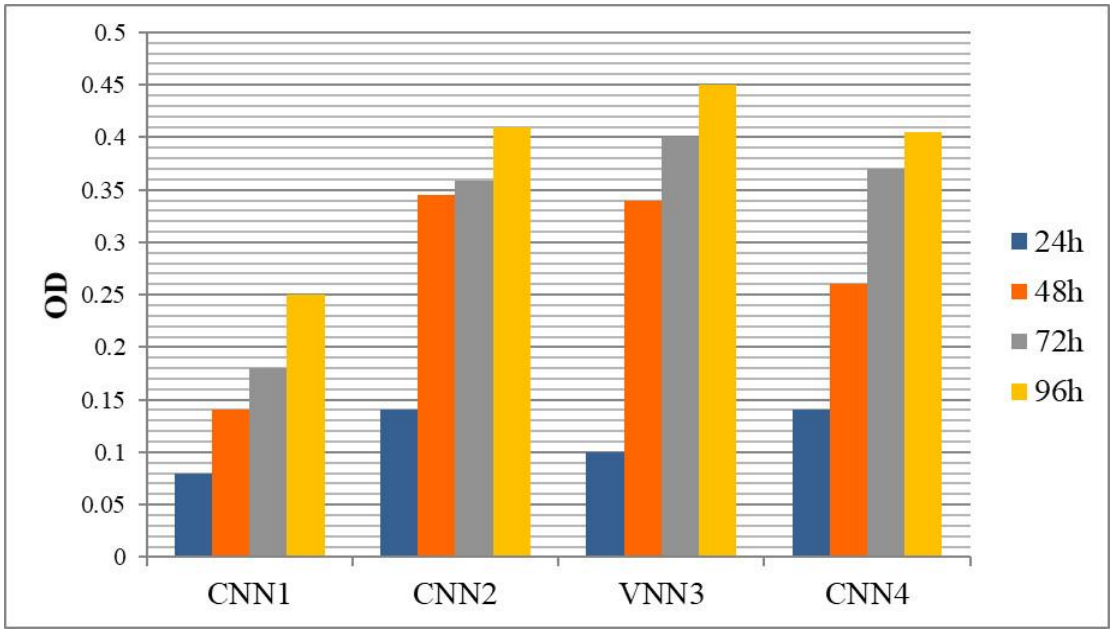

Figure 3 OD_{620} of the four isolated strains growing in CPF-supplemented LB medium

(diameter: 1.5 mm). The colonies of CNN4 were ivory white, round and small, smooth surface and non-pigmentated (diameter: 1.5 mm). In a previous study, Asamba et al. (2022) found that colonies of six CPF-degrading bacterial strains isolated from contaminated soils in Kenya were rod-shaped and pigmented.

The isolates were cultured on LB medium supplemented with the corresponding CPF substrate (100 mg/L) to determine the best growth time. The results from the measurement of OD_{620} of the four isolates are shown in Figure 3. The bacterial density of the four strains is shown in Table 1.

Table 1 Bacterial density of the four strains (CFU/mL)

Strains	Bacterial density (CFU/mL)			
	24 h	48 h	72 h	96 h
CNN1	$3.5x10^7$	$2.1x10^8$	$3.8x10^8$	$2.3x10^7$
CNN2	$2.6x10^8$	$3.2x10^9$	$8.8x10^9$	$2.8x10^8$
VNN3	$3.9x10^8$	$4.8x10^9$	$9.2x10^9$	$4.4x10^8$
CNN4	$4.3x10^8$	$5.8x10^9$	$9.0x10^9$	$5.7x10^8$

Table 2 Similarities of the 16S rRNA genes of CNN2, VNN3 and CNN4 with sequences published in GenBank

Strain	Code	Name	Similarity (%)
CNN2	AP014809.1	*Methylobacterium populi*	99.80
	MF171057.1	*Methylobacterium populi* H1.3	99.80
	MK850375.1	*Methylorubrum thiocyanatum* TA1	99.80
	AB900974.1	*Methylobacterium rhodesianum* P-15S	99.80
	GU294335.1	*Methylobacterium zatmanii* 6012	99.80
	GQ281065.1	*Methylobacterium zatmanii* NBCS25	99.80
	CP039546.1	*Methylorubrum populi* YC-XJ5	99.71
	MN094857.1	*Methylorubrum* sp.TH1	99.71
	MG190781.1	*Methylorubrum pseudosasae* IMB16-188	99.71
VNN3	KX507144.1	*Ensifer adhaerens*JS1020	98.11
	KY992904.1	*Ensifer* sp.PZS_S05	98.11
	KY660614.1	*Ensifer adhaerens* PZG_S19	98.11
	KY660602.1	*Ensifer adhaerens* PZG_S11	98.11
	KY660583.1	*Ensifer adhaerens* PZS_S05	98.11
	KX673850.1	*Ensifer* sp.P16P1	98.01
CNN4	CP033530.1	*Acinetobacter pittii* 2014S07-126	99.24
	CP040903.1	*Acinetobacter pittii* AP007	99.15
	MN049561.1	*Acinetobacter calcoaceticus* GCPIR7	99.15
	LC485224.1	*Acinetobacter pittii* SN6-2	99.15
	MK954115.1	*Acinetobacter* sp. A4	99.15
	MK834827.1	*Acinetobacter calcoaceticus* Bi	99.15
	MH890513.1	*Acinetobacter* sp. Ap6	99.15

The results showed that after 96 h, the OD_{620} of the strain VNN3 was highest (0.45), followed by CNN2 and CNN4 (0.41). The bacterial density after 96 h for these three strains was also high ($2.8 – 5.6x10^8$ CFU/mL). Among the four strains, CNN1 had the lowest OD_{620} value, and it was reported at 0.14 after 48 h and 0.25 after 96 h. Based on the results, the strains CNN2, VNN3 and CNN4 were selected for further experiment and analysis. The results based on the OD_{620} value are comparable to those recorded by Asamba et al. (2022) who reported that OD_{600} value of the six isolated CPF degrading bacterial strains was from 0.05 to 0.25 after five days. The values of bacterial density in the present study were lower than the previous research, which reported that the density of the bacterial CPF-degrading strain *Hortaea* sp. B15 was from $0.5x10^{16}$ CFU/mL to $4.5x10^{16}$ CFU/mL after 30h of incubation (Hadibarata et al. 2023). These authors found that the highest bacterial count of 3.8×10^{16} CFU/mL after 20 h enhanced the 91% CPF degradation (Hadibarata et al. 2023).

3.2 Identification of selected strains using 16S rRNA gene analysis

Isolated strains CNN2, VNN3, and CNN4 were identified using 16S rRNA gene analysis. The similarities of the 16S rRNA genes of the strains CNN2, VNN3 and CNN4 with sequences published on GenBank are shown in Table 2.

From the obtained results, it can be concluded that the three isolated strains were identified as *Methylobacterium populi*(CNN2), *Ensifer adhaerens*(VNN3) and *Acinetobacter pittii*(CNN4). Bacterial members of the genera *Methylobacterium*, *Ensifer* and *Acinetobacter* have been reported to be able to degrade CPF (Zhao et al. 2014; Li et al. 2020; McDonald et al. 2021). The strain *Acinetobacter* sp. $MemCl_4$ isolated from agricultural soil in India has been demonstrated to degrade 98% of CPF within 144 h of incubation (Pailan et al. 2016). The removal rate of CPF was found to be 89.5% to 91.1% by the bacterium *Hortaea* sp. B15 within 30 h of incubation (Hadibarata et al. 2023). In another study, 23% of CPF can be removed by *Enterobacter* sp. SWLC2 after 30 minutes (Jha et al. 2022). It has been reported that *M. populi* YC-XJ1 possesses important genes that encode enzymes responsible for degrading various exogenous compounds, including CPF (Li et al. 2020).

3.3 CPF degradation in liquid medium by the isolated bacterial strains

The isolated strains *M. populi* CNN2, *E. adhaerens* VNN3, and *A. pittii* CNN4 were selected to test their CPF degradation ability by culturing the strains in MSM medium supplemented with 100 mg/L CPF. The CPF concentration in control (without inoculation) and inoculated sample with the strain *M. populi* CNN2, *E. adhaerens* VNN3 and *A. pittii* CNN4 after 24, 48 and 72h are shown in Figure 4. The study results showed that the strain *M. populi* CNN2, *E. adhaerens* VNN3 and *A. pittii* CNN4 could degrade CPF. *E. adhaerens* VNN3 showed the highest CPF degradation rate among the three strains, followed by *M. populi* CNN2 and *A. pittii* CNN4. After 24 hrs, the CPF concentration decreased by 41.1% and 34.2% by the strain *E. adhaerens* VNN3 and *M. populi* CNN2, respectively. After 72 hrs, the CPF concentrations in the samples inoculated with the *E. adhaerens* VNN3, *M. populi* CNN2, and *A. pittii* CNN4 were 4.95 ± 0.02, 19.06 ± 0.03 and 39.96 ± 0.01 mg/L, respectively; whereas the CPF concentration in the control sample was 94.34 ± 0.98 mg/L. The decrease of CPF concentration by the strain *E. adhaerens* VNN3 and *M. populi* CNN2 after 72 hrs reached about 95.2% and 81.4%, respectively.

The ability of CPF degrading by the selected three strains in this study was higher than reported in the previous studies. Zhao et al. (2014) reported a 60.2% CPF degradation ability of the bacterial strain *Acinetobacter calcoaceticus* D10 isolated from the chives' rhizosphere after 18 days. In another study, the strain *Methylobacterium populi* YC-XJ1 isolated from desert soil has a capacity of CPF degrading by 27.3% after 72 h incubation (Li et al. 2020). Furthermore, Elshikh et al. (2022) reported that the strains *Bacillus cereus* CP6 and *Klebsiella pneumoniae* CP19 degraded about 70% CPF at 200 – 300 mg/L concentrations after 10 days.

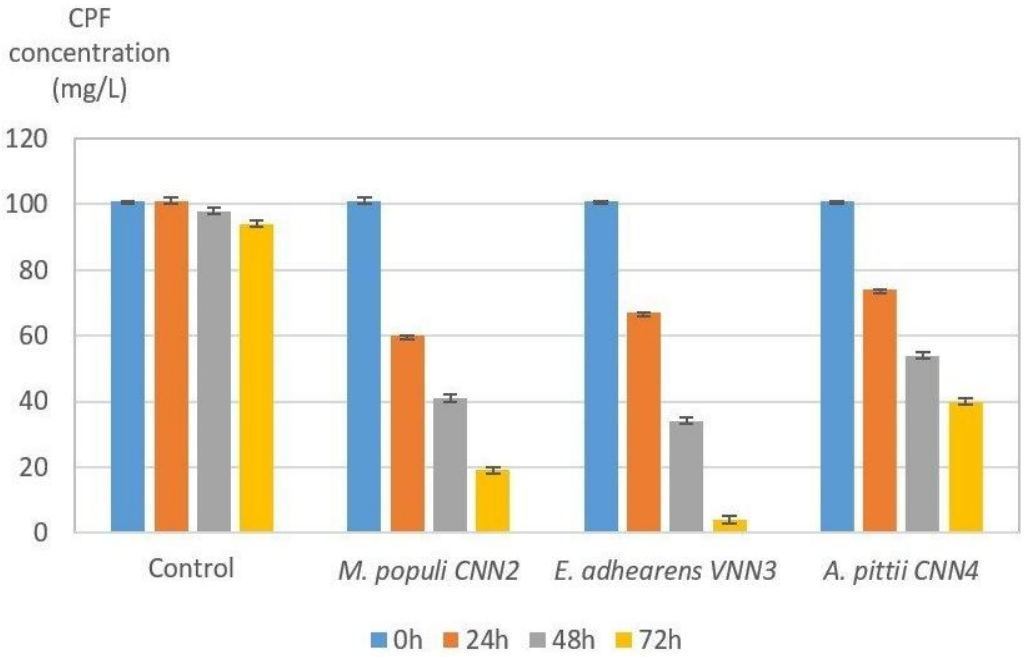

Figure 4 Changes in the CPF concentrations with different time duration by the selected strains

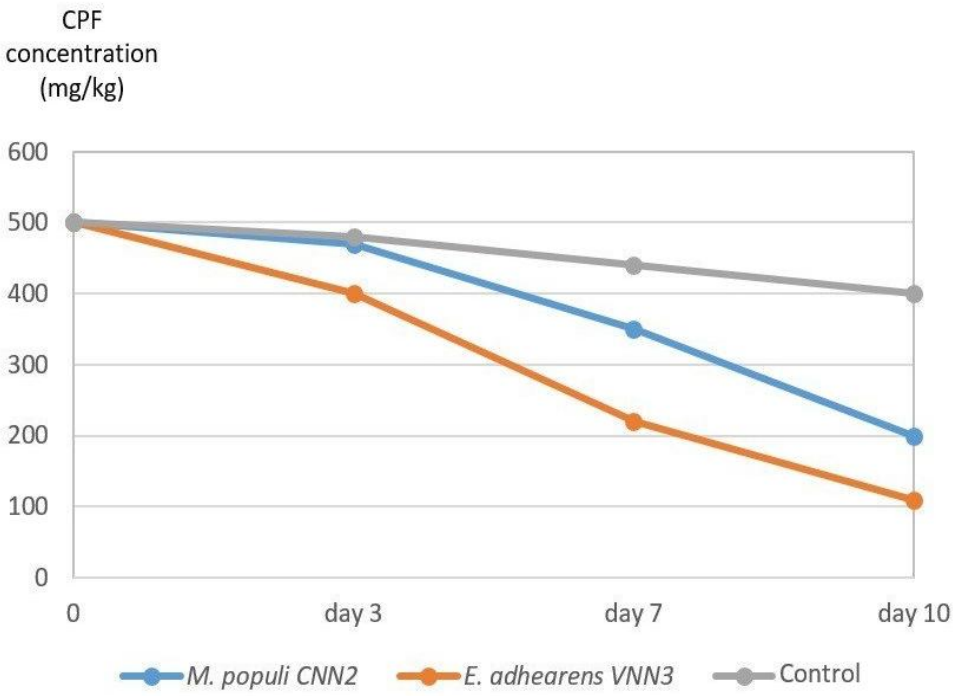

Figure 5 CPF concentration in the soil samples after inoculation of the strain *M. populi* CNN2 and *E. adhaerens* VNN3

3.4 CPF degradation ability in soil under in-vitro conditions by selected isolated strains

The changes in CPF concentration in the soil samples inoculated with the best results given bacterial strain *M. populi* CNN2 and *E. adhaerens* VNN3 after 3, 7 and 10 days of incubation are shown in Figure 5.

The results showed that the CPF concentration gradually decreased up to 3 days of incubation and sharply decreased after 10 days. The strain *E. adhaerens* VNN3 exhibited more vital CPF degradation ability than the strain *M. populi* CNN2. After 7 days, the CPF concentration was reduced from 500 mg/kg to 225 ± 1.73 and 349 ± 0.59 mg/kg by the strain *E. adhaerens* VNN3 and *M. populi* CNN2, respectively, whereas in the control sample, this reduction in the CPF concentration was reported only 438 ± 2.65 mg/kg. After 10 days, the CPF concentration were reduced by 77.6 and 60.6% (equivalent to 112 ± 1.73 and 197 ± 2.08 mg/kg, respectively) by the strain *E. adhaerens* VNN3 and *M. populi* CNN2, respectively. The results of the current study are superior to the findings of Li et al. (2020) those, who reported only a 27.3% reduction in initial CPF concentration after 3 days by the strain *M. populi* YC-XJ1 isolated from desert soil. In another study, the strains *Achromobacter xylosoxidans* (JCp4) and *Ochrobactrum* sp. (FCp1) isolated from pesticide-contaminated soil could degrade CPF concentration by 40 and 60% after 7 and 10 days of incubation, respectively (initial CPF concentration of 200 mg/kg) (Akbar and Sultan 2016). As a result, compared to other reports, the results in this study indicate that the strain *E. adhaerens* VNN3 and *M. populi* CNN2 has a higher potential of CPF-bioremediation from the CPF contaminant agricultural sites.

Conclusion

Among 20 soil samples from tea-growing sites in Vietnam, four bacterial strains that could degrade CPF were isolated. Among them, three potential strains *M. populi* CNN2, *E. adhaerens* VNN3, and *A. pittii* CNN4, have been selected for mass culturing and to identify their bioremediation potential. Among the selected bacterial strains, *E. adhaerens* VNN3 showed the highest CPF degradation ability in both cultured MSM medium and in-vitro soil experiments, followed by the strain *M. populi* CNN2. The study highlights the potential application of the isolated strains *E. adhaerens* VNN3 and *M. populi* CNN2 in CPF treatment and soil bioremediation in agricultural areas, contributing to pollution elimination and sustainable development.

Conflict of interest

The authors of this article declare no competing financial and personal interest.

References

Akbar, S., & Sultan, S. (2016). Soil bacteria showing a potential of chlorpyrifos degradation and plant growth enhancement. *Brazilian*

Journal of Microbiology,47, 563-570. https://doi.org/10.1016/j.bjm.2016.04.009

Asamba, M. N., Mugendi, E. N., Oshule, P. S., Essuman, S., Chimbevo, L. M., & Atego, N. A. (2022). Molecular characterization of chlorpyrifos degrading bacteria isolated from contaminated dairy farm soils in Nakuru County, Kenya. *Heliyon, 8*, e09176. https://doi.org./10.1016/j.heliyon.2022.e09176

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., & Struhl, K. (1994). *Current Protocols in Molecular Biology*. New York: Wiley.

Bhandari, G., Atreya, K., Scheepers, P. T. J., & Geissen, V. (2020). Concentration and distribution of pesticide residues in soil: Non-dietary human health risk assessment. *Chemosphere,253*, 126594. https://doi.org/10.1016/j.chemosphere.2020.126594

Blanco, J., Guardia-Escote, L., Mulero, M., Basaure, P., Biosca-Brull, J., Cabré, M., Colomina, M. T., Domingo, J. L., & Sánchez, D. J. (2020). Obesogenic effects of chlorpyrifos and its metabolites during the differentiation of 3T3-L1 preadipocytes. *Food and Chemical Toxicology, 137*, 111171. https://doi.org/10.1016/j.fct.2020.111171

Bose, S., Kumar, P. S., & Vo, D.V. N. (2021). A review on the microbial degradation of chlorpyrifos and its metabolite TCP. *Chemosphere, 283*, 131447. https://doi.org/10.1016/j.chemosphere.2021.131447

Cheng, C., Liu, W., Hou, K., Zhang, J., Du, Z., Li, B., & Zhu, L. (2023). Ecological safety evaluation of chlorpyrifos on agricultural soil: Effects on soil microbes. *Applied Soil Ecology, 189*, 104954.https://doi.org/10.1016/j.apsoil.2023.104954

Elshikh, M. S., Alarjani, K. M., Huessien, D. S., Elnahas, H. A. M., & Esther, A. R. (2022). Enhanced Biodegradation of Chlorpyrifos by *Bacillus cereus* CP6 and *Klebsiella pneumoniae* CP19 from municipal waste water. *Environmental Research, 205*, 112438. https://doi.org/10.1016/j.envres.2021.112438

EPA. (1996). SW-846 Test Method 3540C: Soxhlet Extraction. *Part of Test methods for Evaluating Solid Waste*. Retrieved from https://www.epa.gov/sites/default/files/2015-12/documents/3540c.pdf.

Hadibarata, T., Kristanti, R. A., Bilal, M., Yilmaz, M. , & Sathishkumar, P. (2023). Biodegradation mechanism of chlorpyrifos by halophilic bacterium *Hortaea* sp. B15. *Chemosphere, 312*, 137260. https://doi.org/10.1016/j.chemosphere.2022.137260

Hazarika, J., Ganguly, M., & Mahanta, R. (2020). A computational insight into the molecular interactions of chlorpyrifos and its degradation products with the human progesterone receptor leading to endocrine disruption. *Journal of Applied Toxicology, 40*, 434-443.https://doi.org/10.1002/jat.3916

Hossain, M. S., Chowdhury, M. A. Z., Pramanik, M. K., Rahman, M. A., Fakhruddin, A. N. M., & Alam, M. K. (2015). Determination of selected pesticides in water samples adjacent to agricultural fields and removal of organophosphorus insecticide chlorpyrifos using soil bacterial isolates. *Applied Water Science,5*, 171-179.https://doi.org/10.1007/s13201-014-0178-6

Huang, X., Cui, H., & Duan, W. (2020). Ecotoxicity of chlorpyrifos to aquatic organisms: A review. *Ecotoxicology and Environmental Safety, 200*, 110731. https://doi.org/10.1016/j.ecoenv.2020.110731

Huang, Y., Zhang, W., Pang, S., Chen, J., Bhatt, P., Mishra, S., & Chen, S. (2021). Insights into the microbial degradation and catalytic mechanisms of chlorpyrifos. *Environmental Research, 194*, 110660. https://doi.org/10.1016/j.envres.2020.110660

Ishag, S. A., Abdelbagi, A. O., Hammad, A. M. A., Elsheikh, E. A. E., Elsaid, O. E., Hur, J. H., & Laing, M. D. (2016). Biodegradation of Chlorpyrifos, Malathion, and Dimethoate by Three Strains of Bacteria Isolated from Pesticide-Polluted Soils in Sudan. *Journal of Agricultural and Food Chemistry, 64*, 8491-8498. https://doi.org/10.1021/acs.jafc.6b03334

Jha, S. K., Chishti, Z., Ahmad, Z., & Arshad, K. R. (2022). *Enterobacter* sp. SWLC2 for biodegradation of chlorpyrifos in the aqueous medium: Modeling of the process using artificial neural network approaches. *Computers and Electronics in Agriculture, 193*, 106680. https://doi.org/10.1016/j.compag.2021.106680

Jin, Y., Liu, Z., Peng, T., & Fu, Z. (2015). The toxicity of chlorpyrifos on the early life stage of zebrafish: A survey on the endpoints at development, locomotor behavior, oxidative stress and immunotoxicity. *Fish & Shellfish Immunology, 43*, 405-414. https://doi.org/10.1016/j.cbpc.2018.12.004

Khalid, S., Han, J. I., Hashmi, I., Hasnain, G., Ahmed, M. A., Khan, S. J., & Arshad, M. (2018). Strengthening calcium alginate microspheres using polysulfone and its performance evaluation: Preparation, characterization and application for enhanced biodegradation of chlorpyrifos. *Science of the Total Environment, 631-632*, 1046-1058. https://doi.org/10.1016/j.scitotenv.2018.03.101

Li, X., Wang, J., Jia, Y., Reheman, A., & Yan, Y. (2020). The Genome Analysis of *Methylobacterium populi* YC-XJ1 with Diverse Xenobiotics Biodegrading Capacity and Degradation Characteristics of Related Hydrolase. *International Journal of Molecular Sciences, 21*. https://doi.org/10.3390/ijms21124436

McDonald, N. D., Love, C. E., & Gibbons, H. S. (2021). The ChpR transcriptional regulator of Sinorhizobium meliloti senses 3,5,6-trichloropyridinol, a degradation product of the organophosphate pesticide chlorpyrifos. *Access Microbiology*, *3*, 000297-000297. https://doi.org/10.1099/acmi.0.000297

Moyano, P., García, J., García, J. M., Pelayo, A., Muñoz-Calero, P., Frejo, M. T., Anadon, M. J., Lobo, M., & Del Pino, J. (2020). Chlorpyrifos-induced cell proliferation in human breast cancer cell lines differentially mediated by estrogen and aryl hydrocarbon receptors and KIAA1363 enzyme after 24 h and 14 days exposure. *Chemosphere*, *251*, 126426. https://doi.org/10.1016/j.chemosphere.2020.126426

Oltramare, C., Weiss, F. T., Staudacher, P., Kibirango, O., Atuhaire, A., & Stamm, C. (2022). Pesticides monitoring in surface water of a subsistence agricultural catchment in Uganda using passive samplers. *Environmental Science and Pollution Research*, *30*, 10312–10328. https://doi.org/10.1007/s11356-022-22717-2

Pailan, S., Sengupta, K., Ganguly, U., & Saha, P. (2016). Evidence of biodegradation of chlorpyrifos by a newly isolated heavy metal-tolerant bacterium *Acinetobacter* sp. strain MemCl4. *Environmental Earth Sciences*,*75*, 1019. https://doi.org/10.1007/s12665-016-5834-8

Sishu, F. K., Tilahun, S. A., Schmitter, P., Assefa, G., & Steenhuis, T. S. (2022). Pesticide Contamination of Surface and Groundwater in an Ethiopian Highlands' Watershed. *Water*,*14*, 3446.https://doi.org/10.3390/w14213446

Soltani-Nezhad, F., Saljooqi, A., Mostafavi, A., & Shamspur, T. (2020). Synthesis of Fe(3)O(4)/CdS-ZnS nanostructure and its application for photocatalytic degradation of chlorpyrifos pesticide and brilliant green dye from aqueous solutions. *Ecotoxicology and Environmental Safety*,*189*, 109886.https://doi.org/10.1016/j.ecoenv.2019.109886

Sud, D., Kumar, J. V., Kaur, P., & Bansal, P. (2020). Toxicity, natural and induced degradation of chlorpyrifos. *Journal of The Chilean Chemical Society*, *65*, 4807-4816. http://dx.doi.org/10.4067/S0717-97072020000204807

Takayasu, T., Yamamoto, H., Ishida, Y., Nosaka, M., Kawaguchi, M., Kuninaka, Y., Kimura, A., & Kondo, T. (2017). Postmortem distribution of chlorpyrifos-methyl, fenitrothion, and their metabolites in body fluids and organ tissues of an intoxication case. *Legal medicine(Tokyo)*,*29*, 44-50.https://doi.org/10.1016/j.legalmed.2017.10.002

Tan, H., Li, Q., Zhang, H., Wu, C., Zhao, S., Deng, X., & Li, Y. (2020). Pesticide residues in agricultural topsoil from the Hainan tropical riverside basin: Determination, distribution, and relationships with planting patterns and surface water. *Science of the Total Environment*, *722*, 137856.https://doi.org/10.1016/j.scitotenv.2020.137856

Zhao, L., Wang, F., & Zhao, J. (2014). Identification and functional characteristics of chlorpyrifos-degrading and plant growth promoting bacterium *Acinetobacter calcoaceticus*. *Journal of Basic Microbiology*, *54*, 457-63. https://doi.org/10.1002/jobm.201200639