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Isolation of bacteria capable of degrading organophosphate flame retardants

Thi Lan Anh Nguyen[1, 2, *], Thi Phuong Pham[2], Thi Thu Hien Tran[2], Khac Hieu Phung[3], Thi Thu Lan Tran[2], Hai Yen Dao[2]

[1]*Faculty of Biotechnology, Hanoi University of Pharmacy, 13-15 Le Thanh Tong Street, Hoan Kiem District, Ha Noi, Viet Nam*

[2]*Institute of Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Street, Cau Giay District, Ha Noi, Viet Nam*

[3]*Institute of Technological Science and Environment, 149 Giang Vo Street, Dong Da District, Ha Noi, Viet Nam*

[*]Emails: *lananhdn86@gmail.com*

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Abstract. Organophosphate flame retardants (OPFRs) are increasingly being used in industrial applications and are often released into the environment, causing potential risks to human health. Therefore, in this study, wastewater samples from To Lich River contaminated with OPFRs were collected and enriched in a medium supplemented with OPFRs to isolate bacterial strains capable of degrading OPFRs. As a result, 10 bacterial strains were isolated and identified based on 16S rRNA gene sequence analysis. These bacterial strains belong to the genera *Achromobacter*, *Pseudomonas*, *Bordetella*, and *Rhizobium*. All 10 bacterial strains exhibited the ability to degrade some OPFRs, but only two strains, *Achromobacter* sp. BWTL6 and *Rhizobium* sp. BWTL7, effectively degraded all 5 tested OPFRs. These two bacterial strains demonstrated the capability to degrade more than 70 % of tris(1,3-dichloro-2-propyl)phosphate (TDCPP) (at 10 mg/L concentration) within 7 days. Furthermore, both *Achromobacter* sp. BWTL6 and *Rhizobium* sp. BWTL7 were able to utilize tris(2-chloroethyl) phosphate (TCEP), triethyl phosphate (TEP), tris(2-butoxyethyl) phosphate (TBEP), and trimethyl phosphate (TMP) for bacterial growth, and OPFR removal rates ranging from 40 to 58 %. In addition, bacterial strain *Bordetella* sp. BWTL3 degraded 61.5 % of TDCPP and 97.7 % of TBEP after 7 days of culture. In this study, all two bacterial strains belonging to the *Achromobacter* and *Rhizobium* are potential candidates for remediation of environments contaminated with OPFRs.

Keywords: Achromobacter, *Rhizobium*, tris(1,3-dichloro-2-propyl)phosphate (TDCPP), tris(2-butoxyethyl) phosphate (TBEP), tris(2-chloroethyl) phosphate (TCEP).

Classification numbers: 3.1.1.

1. INTRODUCTION

Organophosphorus Flame Retardants (OPFRs) are known as additives in manufacture of plastics and flame retardants, commonly applied in construction materials, household products, and different industries [1, 2]. Due to their excellent fire-retardant properties, good physical and

chemical properties, OPFRs are incorporated into flammable materials to prevent combustion and slow down the spread of flames after ignition [3]. OPFRs are commonly used extensively in the production of flame retardants, plasticizers, antifoaming agents, lubricants, and hydraulic fluids in various industries, including plastics, furniture and decorative materials, building materials, textiles, and electronic equipment [4, 5]. OPFRs are considered emerging pollutants due to their increasing widespread use as flame retardants in recent years, followingthe phase-out of certain brominated flame retardants (polybrominated diphenyl ethers - PBDEs) due to their environmental persistence, biotoxicity, and bioaccumulation [4]. Based on their different functional groups, organophosphate esters (OPEs), which are chemical components of OPFRs, can be classified into halogenated and non-halogenated OPEs. Halogenated OPEs, such as tris(2-chloro-1-methyl ethyl) phosphate (TCPP), tris(1,3-dichloro-2-propyl)phosphate (TDCPP), and tris(2-chloroethyl) phosphate (TCEP), have been banned or restricted in products for children and residential upholstered furniture in the United States [6]. In Europe, restrictions on the use of TDCPP and TCPP have also been enacted due to their their carcinogenic potential [7]. The restrictions on halogenated OPEs have promoted the development of non-halogenated OPEs, including tris(2-butoxyethyl) phosphate (TBEP), triisobutyl phosphate (TiBP), tris(n-butyl) phosphate (TnBP), and trisphenyl phosphate (TPhP) [8, 9].

OPEs are incorporated into polymer materials through physical blending rather than chemical bonding, which makes them easily released into the surrounding environment through evaporation, abrasion, and dissolution [10]. Indeed, OPEs have been detected in a variety of environments, including air, water, sediment, dust, and soil [11]. In addition, OPEs have been detected in animals, plants, and even in hair and nails, urine, and breast milk [12, 13]. Prolonged exposure and accumulation of OPEs including TBEP, TnBP, TPhP, and other OPEs in the human body can cause various adverse effects, including nephrotoxicity, neurotoxicity, reproductive toxicity, carcinogenicity, and endocrine disruption [14, 15]. In the natural environment (water, air, and soil), chlorinated OPEs exhibit high persistence and low degradation capability [8, 16-18]. Biological treatment is considered a suitable approach for effectively removing undesired OPEs, being cost-effective and environmentally friendly [19]. Several bacterial species, including *Citrobacter* sp., *Serratia odorifera*, *Rhodopseudomonas palustris*, *Providencia* sp., *Delftia* sp., *Klebsiella pneumoniae*, and *Sphingobium* sp., have been studied for their ability to degrade TnBP [20-27]. *Brevibacillus brevis* is known to have the capability to degrade TPhP and tricresyl phosphates [28].

In recent years, the rapid pace of urbanization and industrialization, coupled with a lack of effective waste control and treatment measures, especially in wastewater treatment plants, has resulted in water pollution in Hanoi, particularly an increasing contamination of surface water with OPFRs [29]. Truong et al. (2013) have determined the pollution of OPFRs such as TCEP, TCPP, TDCPP, TnBP, TBEP, TPhP and diphenyl phosphate (DPhP) in surface water samples in To Lich River, with concentrations ranging from 24 to 1950 ng/L. Therefore, the main objective of this study is to isolate effective microbes for bioremediation of OPFRs. This study aims to provide new insights into the degradation efficiency of OPFRs by potential microbial strains.

2. MATERIALS AND METHODS

2.1. Materials

Two surface water samples (L1: 21°02'04" N, 105°48'21" E and L2: 21°02'26" N, 105°48'16" E) were collected from To Lich River within the urban area of Hanoi to isolate bacterial strains capable of degrading OPFRs.

Seven standard OPFRs, including trimethyl phosphate (TMP), triethyl phosphate (TEP), tris(2-ethylhexyl) phosphate (TEHP), TBEP, TPhP, TCEP, and tris(1,3-dichloro-2-propyl)phosphate (TDCPP), were supplied by Dr. Ehrenstorfer (LCG, Germany). Surrogate standard (TEP-d15, phosphoric acid tripropyl ester-d21, phosphoric acid tributyl ester-d27, triphenyl phosphate-d15, and tris(2-ethylhexyl) phosphate-d51) were provided by Toronto Research Chemicals Inc. (Canada). In this study, analytical grade methanol was supplied by Merck (Germany). All glassware was cleaned and rinsed with solvent before use. Methanol was used to prepare the mixed stock solution (containing all analytes at a concentration of 4000 mg/L) and standard curve solution (1–100 μg/L) used for analyzing the concentrations of the OPFRs. The prepared mixed stock solution was stored at -20°C.

2.2. Methods

2.2.1. Sampling method

Water samples were collected into dark glass bottles. All sampling equipment was pre-rinsed with ethanol, distilled water, and acetone before use and then rinsed three times with water samples before collection. Single-use gloves were used during the sampling process. The collected samples were stored at 4 °C for further studies.

2.2.2. Isolation of bacteria capable of degrading OPFRs from water and sediment samples collected from To Lich River

Ten grams of wastewater samples collected from To Lich River were transferred into 250 ml Erlenmeyer flasks containing 90 ml of modified A-Cl medium. The medium consisted of glucose 10 g/L, $(NH_4)_2SO_4$ 1 g/L, $MgSO_4.7H_2O$ 0.2 g/L, $Ca(NO_3)_2.4H_2O$ 0.032 g/L, yeast extract 0.5 g/L, and 1 ml/L of trace element solution, with a pH of 7. The trace element solution consists of 500 mg $FeSO_4.7H_2O$, 143 mg $MnSO_4.2H_2O$, 22 mg $ZnSO_4.7H_2O$, 12 mg $CoSO_4.7H_2O$, 3 mg $CuSO_4.5H_2O$, 2.3 mg $Na_2WO_4.2H_2O$, and 2 mg $Na_2MoO_4.2H_2O$ dissolved in 1 liter of distilled water [30]. The enrichment culture samples were simultaneously supplemented with seven OPFRs including TMP, TEP, TEHP, TBEP, TPhP, TCEP, and TDCPP (10 mg/L of each OPFRs). These compounds were added as a source of carbon and phosphorus in the culture flasks, which were then shaken at 150 rpm at 30°C for 10 days. After 10 days, the 1st enriched culture samples (10% v/v) were transferred to Erlenmeyer flasks containing 90 ml of fresh modified A-Cl medium supplemented with the OPFRs. The cultures were incubated under the same conditions as mentioned above. After another 10 days, 10 ml of these cultures (2nd enrichments) were then transferred into 90 ml fresh modified A-Cl medium added with OPFRs described above. The 3rd enrichments steps were carried out the same way as was done for the 2nd enrichments. Finally, the 3rd enriched culture was spread onto A-Cl agar plates containing 2 mg/L of each OPFRs as the sole phosphorus source. The A-Cl agar plates were then incubated at 30°C until colonies had formed. After pure-colony isolation, these isolated bacteria were stored at −80°C in 30% glycerol.

2.2.3. Bacterial identification

The total DNA of the bacterial strains was extracted using the ABT kit (ABT Solutions LLC, Vietnam) following the manufacturer's instructions. A partial sequencing of the 16S rRNA gene with a size of approximately 1500 bp was amplified using the primer pair 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1512R (5′-ACGGYTACCTTGTTACGACTT-3′) [31]

as described previously. Reactions were performed in a PCR G-STORM (England). PCR products were purified and sequenced by Macrogen Europe. These sequences were compared to known 16S rRNA gene sequences available on the GenBank database using BLAST searches at the National Center for Biotechnology Information (https://blast.ncbi. nlm.nih.gov/Blast.cgi). The sequences were aligned and a phylogenetic tree was visualized using the MEGA version 7 software by the neighbor-joining method [32]. The nucleotide sequences of the isolated bacterial strains were registered on the NCBI gene bank. The nucleotide sequences of the partial 16S rRNA gene for the 10 bacterial strains BWTL1, BWTL2, BWTL3, BWTL4, BWTL5, BWTL6, BWTL7, BWTL8, BWTL9, and BWTL10 have been deposited in GenBank under accession numbers ranging from OR481672 to OR481681.

2.2.4. Degradation of OPFRs by isolated bacterial strains

Isolated bacterial strains were inoculated into A-Cl medium and shaken at 150 rpm, 30 ^{o}C for 24 hours to increase biomass growth. Subsequently, the cell culture broth was centrifuged (6000 rpm for 3 min), and washed three times with A-Cl medium. The bacterial culture supernatant (OD600 ~ 0.2) was added to a 100 ml Erlenmeyer flask containing 40 ml of A-Cl medium supplemented with 10 mg/L of each OPFRs as the sole phosphorus source to evaluate the degradation of OPFRs in 7 days of cultivation. Control sample contains only medium and OPFRs at the same concentrations. The experiments were conducted in triplicate. Culture samples were taken at day 0, 1, 3, 5 and 7 to determine the concentration of OPFRs and cell growth rates at OD 600 nm. The optical density at a wavelength of 600 nm (OD600) of the culture medium was measured using a Hitachi UV-2900 spectrophotometer (Hitachi, Japan) to determine bacterial density.

2.2.5. Quantification of OPFRs

An UHPLC system (UltiMate 3000+, Thermo Scientific, MA, USA) in combination with a MS/MS spectrometer (API 3200, Applied Bio-System/MDS SCIEX, USA) were applied to analyze OPFRs group in this study. Acclaim Mixed-Mode HILIC column (2.1 mm × 150 mm, 5 μm, Thermo Fisher) was selected to separate the target analytes. The column chamber temperature was maintained at 35 °C. The mobile phase consisted of two channels, channel A (ultrapure water) and channel B (Acetonitrile). The mobile phase flow rate was 0.3 mL/min. The solvent gradient program was illustrated as follows: hold 30 % channel B for the first 5 min, increase linearly to 70 % channel B at 8 min, increase rapidly to 100 % channel B at 13 min, then gradually reduce to only 30 % channel B at 15 min, maintain up to 20 min before the next sample injection. In MS/MS detector, the positive electrospray ionization [33] mode and multiple reaction monitoring (MRM) mode were employed. The optimal key parameters include ion spray voltage of 5000 V, collision gas pressure of 0.02 MPa, sheath gas pressure of 0.18 MPa and auxiliary gas pressure of 0.22 MPa. The source temperature was set at 400 °C. TraceFinder 4.0 software was applied to process the data set.

2.2.6. Statistical analysis

Data and statistical analyses were performed using Excel 2013 and GraphPad Prism 8.0.2. All of the experiments were performed in triplicate, and the data are expressed as means ± standard deviation. One-way analysis of variance (ANOVA) was used to determine the

significant differences between values, followed by Dunnett's T3 multiple comparison test. A probability of $p \leq 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

3.1. Isolation and selection of bacteria capable of degrading OPFRs

From collected wastewater samples of To Lich River, bacterial strains capable of utilizing OPFRs were isolated by the enrichment method on A-Cl medium supplemented with OPFRs. Microbial consortia growth on agar plates were then separated into pure strains, and bacterial morphology was observed.

Ten microbial strains isolated on agar plates have different colony morphologies. The majority of colonies were morphologically similar and could be divided into four main groups according to diameter: groups 1 - 1.5 mm in diameter has a round shape, smooth border, slightly convex surface, and creamy-white color (denoted as BWTL1, BWTL3); group 2 - 4 mm in diameter has the round shape, smooth surface, slight convexity and milky to creamy yellow color (denoted as BWTL4, BWTL6, BWTL7, BWTL8); the 2 - 5 mm diameter group has the round shape, flat and matte surface with irregular, creamy-yellow edges (denoted as BWTL2, BWTL10) and the 2-6 mm diameter group has the round shape, slightly rough, flat, sparkling surface, and a cloudy creamy-white color (denoted as BWTL5, BWTL9).

3.2. Classification of isolated bacterial strains based on 16S rRNA gene sequence

In Figure 1, it can be observed that bacterial strains BWTL2, BWTL4, BWTL5, BWTL9, and BWTL10 are clustered together on a common branch, indicating their close genetic relationship with the genus *Pseudomonas*. Further comparison of GenBank data reveals that the 16S rRNA sequences of these strains exhibit a 99 % similarity to the following *Pseudomonas* species: *Pseudomonas* sp. RKKPA 5 (OR268212.1), *Pseudomonas resinovorans* S12 (KT380602.1), *Pseudomonas nitroreducens* D-3 (KC625329.1), *Pseudomonas* sp. BZ19 (MW578879.1), and *Pseudomonas aeruginosa* PaSa1910 (OR261093.1). Consequently, these bacterial strains are named as follows: *Pseudomonas* sp. BWTL2, *Pseudomonas* sp. BWTL4, *Pseudomonas* sp. BWTL5, *Pseudomonas* sp. BWTL9, and *Pseudomonas* sp. BWTL10. The BLAST results indicated that bacterial strain BWTL3 has a 98% similarity to *Bordetella petrii* BjF2 (KP259605.1) and *Bordetella muralis* T6220-3-2b (NR 145920.1).Therefore, the bacterial strain BWTL3 is named *Bordetella* sp. BWTL3. The comparison of 16S rRNA gene sequences also reveals that two bacterial strains, BWTL1 and BWTL6, have high similarities of 99% and 98%, respectively, with *Achromobacter xylosoxidans* SH29B (KT337529.1) and *Achromobacter xylosoxidans* (MW177949.1). Accordingly, these bacterial strains are named *Achromobacter* sp. BWTL1 and *Achromobacter* sp. BWTL6, respectively. The phylogenetic tree (Figure 1) indicates that bacterial strains BWTL7 and BWTL8 have 99 % similarity with *Rhizobium* sp. FPK12 (MT949891.1) and *Rhizobium* sp. R3-74 (JQ659640.1). Based on the BLAST results and the close relationship shown on the phylogenetic tree, it can be concluded that bacterial strains BWTL7 and BWTL8 belong to the genus *Rhizobium* and are named *Rhizobium* sp. BWTL7 and *Rhizobium* sp. BWTL8, respectively.

Microbial biodegradation are often employed to remediate environments contaminated with OPFRs. Bacterial strains belonging to genera such as *Brevibacillus*, *Sphingomonas*,

Sphingopyxis, *Rhodococcus* can degrade some OPFRs through biodegradation processes. Specifically, the genus *Pseudomonas* has been studied and confirmed to be able to utilize TnBP as a phosphorus source for its growth and the degradation of OPFRs [34, 35]. In addition, Chaudhari *et al* (2012) studied the degradation of TnBP compound using free and immobilized cells of *Pseudomonas pseudoalcaligenes* MHF ENV. Immobilization of *Pseudomonas pseudoalcaligenes* MHF ENV onto agarose increased the degradation rate of TnBP by 2.8 times compared to non-immobilized cells. The degradation efficiency reached 96%, and the degradation rate was 100.8 mg/L.h at a flow rate of 21 mL/ h [34]. The identification of the 10 bacterial strains, isolated from water samples contaminated with OPFRs from To Lich River in Hanoi, suggests their potential utility in the remediation of environments polluted with OPFRs.

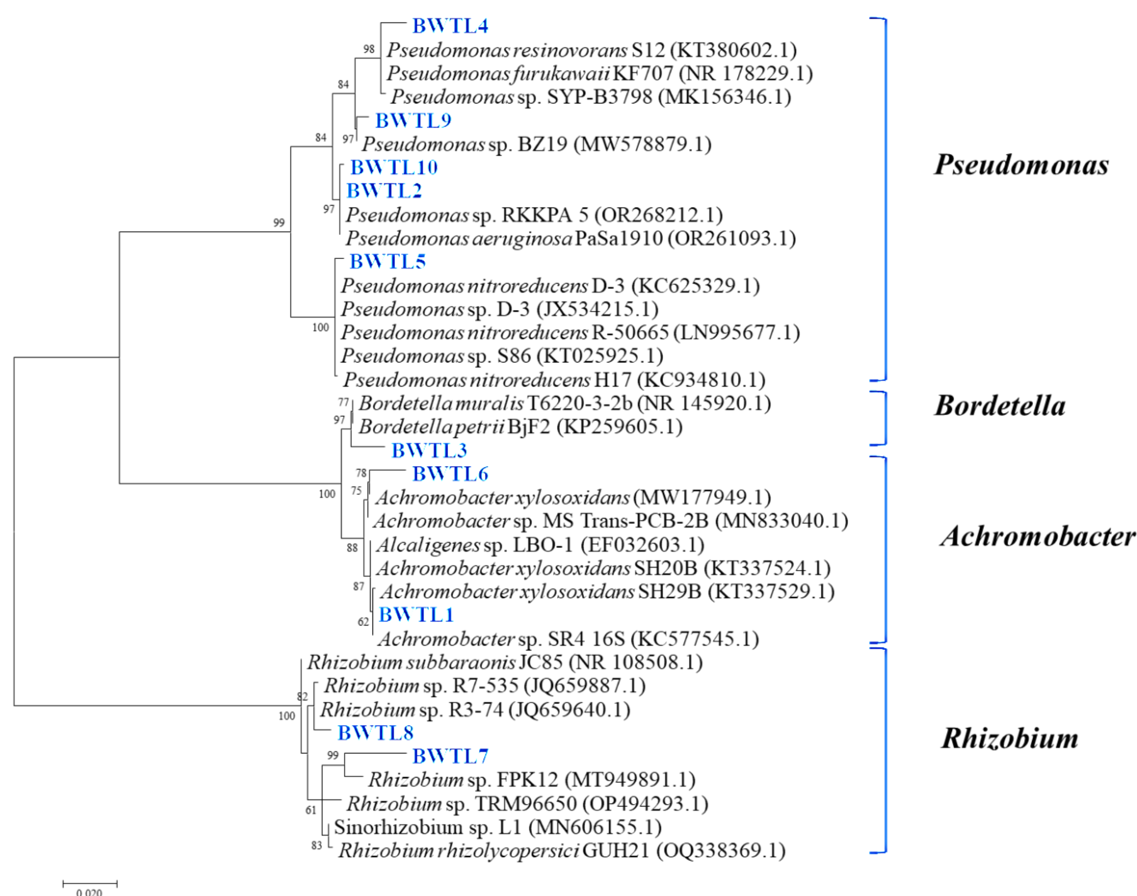

Figure 1. Phylogenetic tree of isolated bacterial strains. Bootstrap values greater than 50% are displayed at the nodes.

3.3. Degradation of OPFRs by isolated bacterial strains

The growth of the 10 isolated bacterial strains on A-Cl medium supplemented with 10 mg/L of each OPFRs is presented in Figure 2. In general, all 10 bacterial strains exhibited increased cell biomass after 7 days of cultivation, with optical densities at 600 nm ranging from 0.432 to 1.718. The bacterial strain *Achromobacter* sp. BWTL6 achieved the highest cell biomass with an OD 600 nm value of 1.718. Following that, the bacterial strains *Bordetella* sp. BWTL3 and

Rhizobium sp. BWTL7 reached OD 600 nm values more than 1. In contrast, the two bacterial strains, *Pseudomonas* sp. BWTL4 and *Pseudomonas* sp. BWTL9, showed poor growth with a small increase in biomass after 7 days of cultivation. These results indicate that some of the above mentioned bacterial strains have the ability to grow robustly in an environment with OPFRs as the carbon and sole phosphorus source, suggesting their significant potential for future applications.

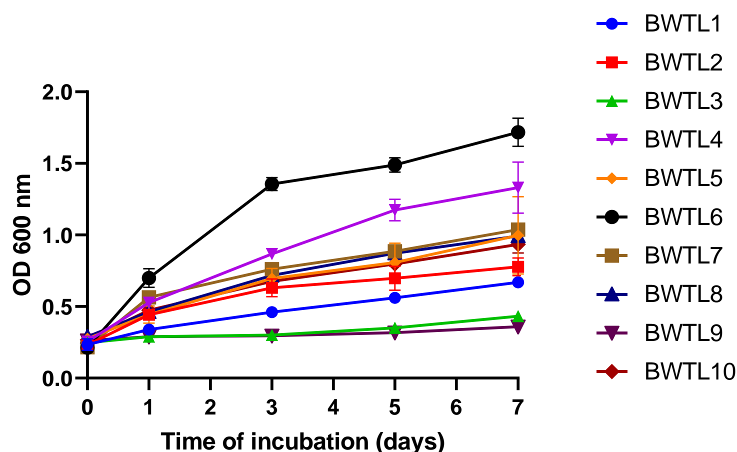

Figure 2. Growth of isolated bacterial strains on A-Cl medium supplemented with OPFRs.

In the degradation process of individual OPFRs, Hou et al. (2021) observed a significant increase in microbial biomass with optical density measured at 600 nm gradually increasing from 0.1 to 2.5 [36]. The results of our study are similar to this study, showing the growth and development of bacterial strains on A-Cl medium supplemented with OPFRs after 7 days of cultivation. During the microbial growth, microorganisms gradually adapt and utilize OPFRs as carbon and phosphorus sources for their survival. In the metabolic system, easily degradable substrates such as glucose are preferentially used as energy and carbon sources for microbial growth before OPFRs provide sufficient carbon. Consequently, co-metabolism of nonhalogenated OPFRs and external organic sources may facilitate the degradation of these pollutants.

A total of 10 strains were isolated from wastewater samples of To Lich River enriched on A-Cl medium supplemented with OPEs. The degradation efficiency of 7 different OPFRs (10 mg/L each compound, cultured for 7 days) by these isolated strains is presented in Figure 3 and Table 1. Figure 4 shows mass spectra obtained by UHPLC-MS/MS analysis for control sample and bacterial treatment (*Bordetella* sp. BWTL3, *Achromobacter* sp. BWTL6 and *Rhizobium* sp. BWTL7) after 7 days of cultivation. The results in Figure 3 and Table 1 showed that in the control sample, degradation of two OPFRs including TPhP and TEHP, was observed with degradation percentages of 40.3 % and 59 %, respectively, on the day 7 of cultivation, indicating that these compounds are not stable over time. The degradation efficiency of most OPFRs increase over time. After 1 and 3 days of cultivation, the degradation efficiency of OPFRs in the culture samples were still low. Subsequently, the degradation efficiency gradually increased on the day 7 of cultivation. Bacterial strains such as *Bordetella* sp. BWTL3, *Pseudomonas* sp. BWTL4, *Pseudomonas* sp. BWTL5, *Pseudomonas* sp. BWTL9, and *Pseudomonas* sp. BWTL10, either could not degrade or show very low degradation of TMP and TEP on the day 7 of

cultivation. The degradation efficiency of TCEP and TBEP was very low in culture samples with bacterial strains *Pseudomonas* sp. BWTL2, *Pseudomonas* sp. BWTL4, *Pseudomonas* sp. BWTL5, *Pseudomonas* sp. BWTL9, and *Pseudomonas* sp. BWTL10. Meanwhile, the degradation efficiency of TDCPP ranges from 30 - 74 % in all culture samples on the day 7 of cultivation. Among the ten isolates, two bacterial strains, *Achromobacter* sp. BWTL6 and *Rhizobium* sp. BWTL7, exhibited clear degradation of the OPFRs. The bacterial strains *Achromobacter* sp. BWTL6 and *Rhizobium* sp. BWTL7 degraded > 70 % of TDCPP (at 10 mg/L concentration) within 7 days. Furthermore, significant degradation of TCEP, TEP, TBEP, and TMP was observed in both bacterial strain cultures compared with the uninoculated control (One-way ANOVA, $p \leq 0.0001$). It's indicated that *Achromobacter* sp. BWTL6 and *Rhizobium* sp. BWTL7 could effectively degrade and mineralize five OPFRs with high degradation rate and cell growth. In addition, *Bordetella* sp. BWTL3 strain was capable of degrading 61.5 % of TDCPP and 97.7 % of TBEP on the day 7 of cultivation (One-way ANOVA, $p \leq 0.0001$). Meanwhile, the results in Table 1 show that the two bacterial strains, *Pseudomonas* sp. BWTL4 and *Pseudomonas* sp. BWTL9, had the lowest capability to degrade OPFRs, and cell growth was poor in both strains. This result may be due to OPFRs being toxic to the cells of these bacterial strains, leading to reduced growth in the cultures. The degradation of TMP, TEP, TCEP, and TBEP by the remaining isolates were all below 20 % (Table 1).

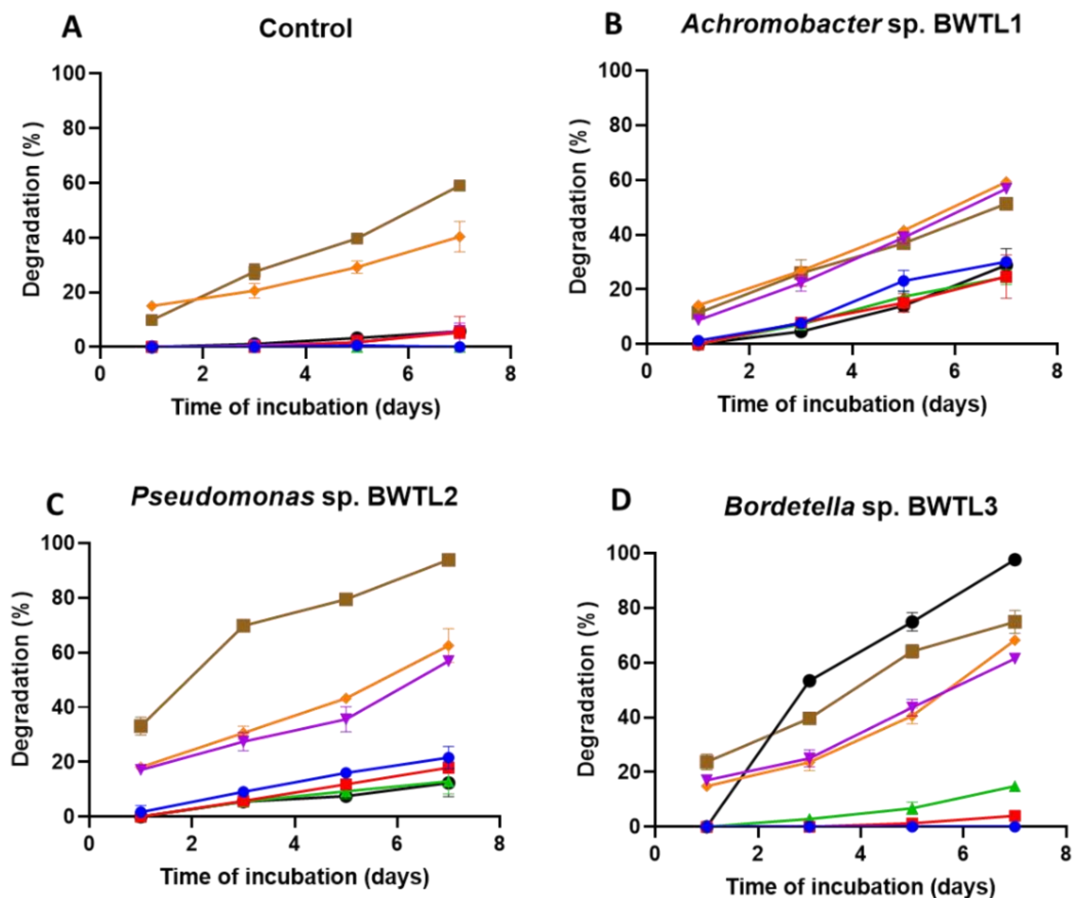

Figure 3. Degradation of OPFRs by isolated bacterial strains (A-D) after 7 days of cultivation.

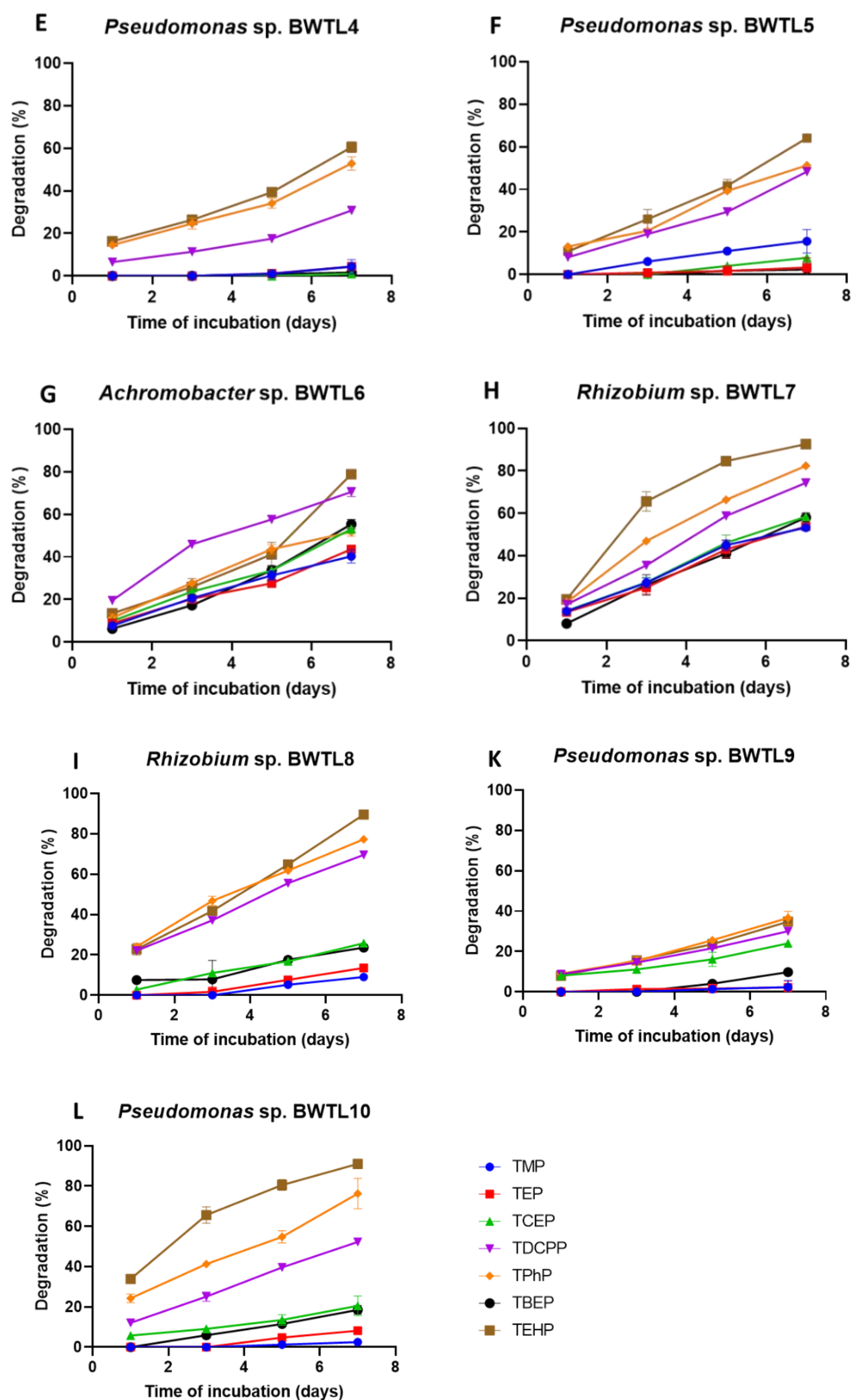

Figure 3 (continue). Degradation of OPFRs by isolated bacterial strains (E-L) after 7 days of cultivation.

Table 1. Degradation efficiency of OPFRs by isolated bacterial strains after 7 days of cultivation.

Sample	Degradation efficiency of OPFRs (%)						
	TMP	TEP	TCEP	TDCPP	TPhP	TBEP	TEHP
Control	0	5.3	0	5.8	40.3	5.8	59.0
Achromobacter sp. BWTL1	30.1[c]	24.7[c]	24.5[c]	56.8[c]	59.3[c]	28.8[c]	51.3
Pseudomonas sp. BWTL2	21.6[c]	18.0[a]	12.9[b]	56.9[c]	62.5[c]	12.3	93.9[c]
Bordetella sp. BWTL3	0	4.0	14.8[c]	61.5[c]	68.2[c]	97.7[c]	74.9[c]
Pseudomonas sp. BWTL4	4.3	4.5	1.0	30.7[c]	52.8[a]	1.6	60.5
Pseudomonas sp. BWTL5	15.6[c]	3.3	7.9	48.4[c]	51.3[a]	2.4	64.2
Achromobacter sp. BWTL6	40.3[c]	43.6[c]	53.1[c]	70.6[c]	51.7[a]	55.4[c]	78.8[c]
Rhizobium sp. BWTL7	53.3[c]	53.8[c]	58.5[c]	74.4[c]	82.4[c]	58.1[c]	92.6[c]
Rhizobium sp. BWTL8	9.1	13.5	25.8[c]	69.5[c]	77.3[c]	23.6[c]	89.5[c]
Pseudomonas sp. BWTL9	2.4	2.2	24.0[c]	30.1[c]	36.6	9.8	34.8[c]
Pseudomonas sp. BWTL10	2.6	8.2	20.5[c]	52.3[c]	76.3[c]	18.6[b]	91.1[c]

The letter a, b, c indicate significant difference in degradation efficiency of OPFRs compared with uninoculated control (One-way ANOVA, $p \leq 0.05$, 0.001, 0.0001), respectively.

Table 2 shows specific MS/MS parameters obtained in the positive electrospray ionization mode. The result was found that retention time of OPFRs range from 2.97 to 21.47 minutes.

Table 2. Analyte specific MS/MS parameters obtained in the positive electrospray ionization mode.

Analyte	**Precursor Ion (m/z)**	**Product Ion (m/z)**	**Collusion Energy (eV)**	**Retention Time (min)**
TMP	141	79, 109	22, 16	2.97
TEP	183	99, 127	18, 20	4.74
TCEP	285	63, 99	22, 20	5.63
TDCPP	431	99, 209	26, 13	12.12
TPhP	327	77, 152	40, 40	12.78
TBEP	399	199, 299	17, 15	14.58
TEHP	435	99, 113	20, 13	21.47

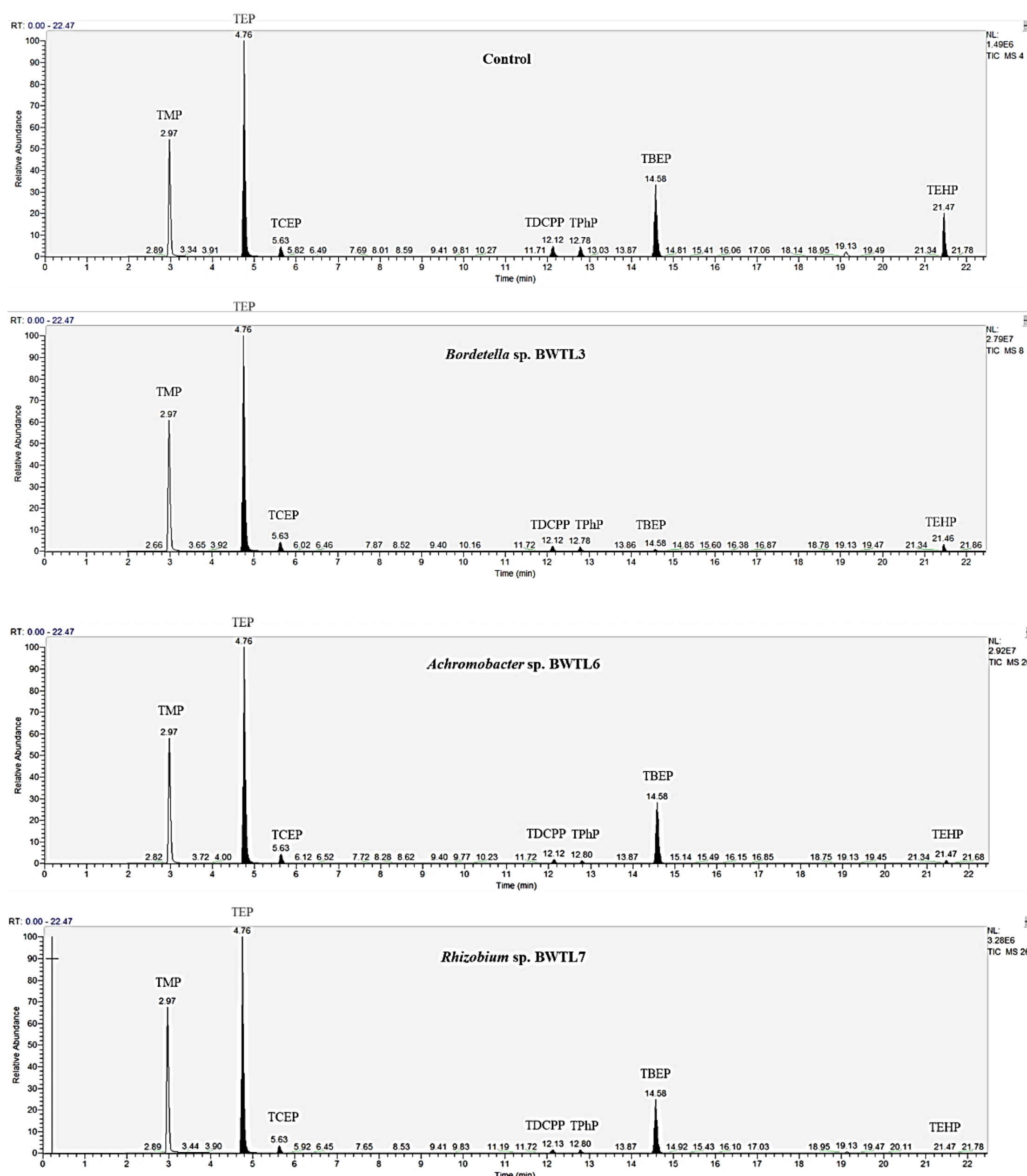

Figure 4. Mass spectra obtained by UHPLC-MS/MS analysis for control sample and bacterial treatment (*Bordetella* sp. BWTL3, *Achromobacter* sp. BWTL6 and *Rhizobium* sp. BWTL7) after 7 days of cultivation

Currently, there are limited studies on microorganisms capable of degrading OPFRs. Some bacterial strains belonging to the genera *Roseobacter*, *Sphingomonas*, *Sphingobium*, *Brevibacillus*, *Rhodococcus*, and *Sphingopyxis* have been reported to degrade OPFRs [30, 37, 38]. According to the study by Wang et al (2019), *Rhodococcus* sp. YC-JH2 and *Sphingopyxis* sp. YC-JH3 able to degrade 37.36 % and 96.2 % of TPhP (50 mg/L), respectively, within 7 days [37]. Kawagoshi et al (2004) reported that the *Roseobacter* strain YS-57 could degrade more

than 99 % of 0.5 mg/L TPhP within 3 days in the absence of glucose [39]. *Brevibacillus brevis* was found to be capable of degrading 92.1 % of TPhP at a concentration of 3 μM and the author demonstrated the role of the enzyme cytochrome P450 monooxygenase in the pathway of degrading TPhP [38]. The bacterial strain *Ochrobactrum tritici* WX3-8 is capable of degrading 75 % of TEHP as the sole carbon source after 104 hours [40]. However, in our study, the results show that these two compounds TPhP and TEHP are easily degraded over time in the absence of microorganisms, with 40.3 % and 59 % of degradation after 168 hours (Table 1). Strain *Sphingomonas* sp. TDK1 and strain *Sphingobium* sp. TCM1 were capable of degrading 100 % of TCEP and TDCPP at a concentration of 20 μM within 6 hours, resulting in the formation of 2-chloroethanol and 1,3-dichloro-2-propanol as metabolic products from TCEP and TDCPP [30]. In our study, bacterial strains *Bordetella* sp. BWTL3, *Achromobacter* sp. BWTL6 and *Rhizobium* sp. BWTL7 demonstrated biodegradation capabilities for several persistent OPFRs. To the best of our knowledge, this is the first study on the biodegradation of all five OPFRs by bacterial strains belonging to the genera *Bordetella*, *Achromobacter* and *Rhizobium*. This study opens up prospects for the application of potential bacterial strains capable of degrading OPFRs in different environments in Vietnam.

4. CONCLUSIONS

Ten bacterial strains belonging to four genera *Pseudomonas*, *Bordetella*, *Achromobacter* and *Rhizobium* were isolated from enrichment cultures of wastewater samples from To Lich River, Ha Noi, capable of degrading OPFRs with different degradation efficiency. Two bacterial strains, *Achromobacter* sp. BWTL6 and *Rhizobium* sp. BWTL7, demonstrated efficient biodegradation of all five OPFRs including TDCPP, TCEP, TEP, TBEP, and TMP. Therefore, two strains of *Achromobacter* sp. BWTL6 and *Rhizobium* sp. BWTL7 has great potential for bioremediation in OPFRs contaminated environments. Among the four genera known to degrade OPFRs, *Achromobacter*, *Bordetella*, and *Rhizobium* have not been previously reported to exhibit OPFR degradation capabilities.

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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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