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ORIGINAL ARTICLE

Preliminary study on isolation and characterization of bacteria with defluorination towards perfluorooctanoic acid (PFOA)

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Abstract

Perfluorooctanoic acid (PFOA) belongs to the class of persistent organic pollutants widely distributed in the environment, and has the characteristics of high bioaccumulation and refractory degradation in organisms. To date, there are limited reports on the microbial degradation of PFOA. In this pursuit, the present study envisaged the screening of microbes capable of degrading PFOA from the soils of eight sites in Huaxi district of Guizhou province, China, using the enrichment culture. Sixteen bacterial isolates were obtained in total, and their growth curves were found to be unimodal or bimodal in PFOA-spiked MSM cultural solutions. Five isolates of them, viz. 4-a, 5-b, 6-a, 7-c and 8-a, were further selected for in-depth study of PFOA degradation, and it was found that all of isolates could increase the concentrations of fluoride ions in the cultural solutions. The five PFOA-degrading bacteria were identified as *Pseudomonas* sp. Obtained *Pseudomonas* resources would contribute to the studies on the degradation of PFOA by the facultative anaerobes.

Keywords *Pseudomonas* · Perfluorooctanoic acid · Defluorination · Fluoride ions

Introduction

Perfluorinated compounds (PFCs) are a class of persistent organic pollutants with widespread distribution in the environment. They consist of 4 to 14 carbon atoms in the form of alkyl chains as the basic skeleton, wherein all hydrogen atoms are replaced by fluorine atoms along with various functional groups (Gebbink and Letcher 2012). Due to the good characteristics on surface activity, chemical stability, hydrophobicity and oleophobicity, PFCs are widely used in the industrial production, daily necessities, food contact materials and other fields (Wu et al. 2012). However, studies have shown that PFCs have adverse effects, including liver toxicity (Sakr et al. 2007), immunotoxicity (Grandjean et al. 2012), neurotoxicity (Gallo et al. 2013), reproductive toxicity (Leter et al. 2014), developmental toxicity (Halldorsson et al. 2012), endocrine toxicity (Lin et al. 2013) and potential carcinogenicity (Barry et al. 2013). In particular, the concentrations of perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), the most widely studied members of PFCs, obtained from the serum of the U.S. and Chinese adults were 4.99, 1.94 ng/mL and 5.2, 4.1 ng/mL, respectively (Zhang et al. 2019), which indicated that the level of PFOA exposed in drinking water in Chinese population was significantly higher than that in American countries. This reveals that there are various sources of PFCs exposure in China, including surface water and soil (Liu et al. 2020; Gao et al. 2015). Currently, PFCs are considered to be a potential safety hazard that seriously threatens the ecological environment and human health due to their extreme chemical stability, widespread existence in the environment and high accumulation via food chains in organisms. Therefore, it is necessary to take measures to degrade PFCs in the environment for the prevention of harm to human beings. Currently, there are chemical (Yang et al. 2020; Deng et al. 2021), physical (Jovicic et al. 2018; Kim et al. 2020; Tang et al. 2021) and biological (Roesch et al. 2020) methods for the decontamination of PFCs. In particular, most of the research

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on PFOA removal and degradation is still in the laboratory stage, and the technologies to remove nearly 100% PFOA mainly include electrocoagulation processes that are compensated with energy consumption (Lin et al. 2015; Liu et al. 2018), adsorption/desorption combined with photocatalytic reduction processes (Ren et al. 2023), foam/ozone fractionation processes with the aid of cationic surfactant (C_8TAB) (Li et al. 2021), Hydrochar/KI/UV process (Hu et al. 2023), nano-$MgAl_2O_4$@CNTs composite materials (Yin et al. 2023), anion exchange resins (Parvin et al. 2023), ultrasonic pyrolysis (Xiong et al. 2023), MDPL(microwave discharge plasma in liquid) technique (Sun et al. 2023), UV/Fe(III)-saturated montmorillonite system (Zhang et al. 2023), EO-UV and persulfate electrolytes (Uwayezu et al. 2023), etc. Other methods developed to degrade PFOA involve bioremediation of *Ensifer adhaerens* (Chetverikov and Loginov 2019), *Acidimicrobium* sp. (Huang and Jaffé 2019), microbial consortia (Beškoski et al. 2018) and microbial laccase (Luo et al. 2018), and chemical oxidation aided by microwave irradiation (Liu et al. 2020) and heat (Lee et al. 2010), and chemical reduction with Mg-aminoclay coated nonoscale zero valent iron (Arvaniti et al. 2015) and titanium(III) citrate with vitamin B_{12} and Cu^0 (Lee et al. 2017), and the combination of oxidation and reduction (Liu et al. 2021; Han et al. 2023). However, owing to the low effectiveness of these technologies (Dombrowski et al. 2018), a number of advanced oxidation/reduction processes were designed to efficiently degrade polyfluoroalkyl substances (PFAS), especially PFOA with a 90 − 100% degradation efficiency using mechanochemical process (Zhang et al. 2013; Wang et al. 2019), 58.6% defluorination by electrochemical process (Garcia-Costa et al. 2020), 97.2 − 100% defluorination by sonochemical treatment (Lei et al. 2020; Phan Thi et al. 2014), 100% mineralization using Gamma ray with a ^{60}Co source (Zhang et al. 2014), over 93.3% defluorination using eBeam with the suitable dose of nitrate, alkalinity or fulvic acid (Wang et al. 2016), 71% and 93% − 100% degradation efficiency using pulsed corona discharge plasma (Zhan et al. 2020) and photochemical reaction (Liu et al. 2013; Phan Thi et al. 2013; Tenorio et al. 2020) respectively and a maximum degradation of 100% using photocatalysis based on different photocatalytic materials (Park et al. 2018; Wu et al. 2018; Do et al. 2020; Liu et al. 2022). Collated with chemical and physical degradation, bioremediation can be a green, low-cost and environmentally friendly method for PFOA degradation with no stringent reaction conditions and energy input (Leung et al. 2022); However, it is a time-consuming process and offers low decomposition efficiency. Most of the previous studies associated with the bioremediation of PFCs focused on the conversion of PFCs to PFOA or PFOS, and paid a less attention on the further defluorination degradation of PFOA and PFOS (Roesch et al. 2020; Dinglasan et al. 2004). Only during the past decade, some researches concentrated on the biodegradation of PFOA with the aid of specific aerobic, anaerobic microbes, and microbial consortia through different action mechanisms. Of those, the mechanism of reductive defluoriantion of PFOA by the anaerobic *Acidimicrobium* sp. A6 was found to be undergoing the coupling of the ammonium oxidation and iron reduction involving in Feammox process under the anaerobic condition (Huang and Jaffé 2019; Sima et al. 2023). However, the results of Liou et al. (Liou et al. 2010) on the biodegradation and transformation of PFOA by five different anaerobic microbial communities from the municipal wastewater treatment plant, industrial site sediment, agricultural soil, and soils from two fire training areas showed that PFOA was biologically inactive under all the examined conditions. For the aerobic microbial communities, Harris et al. (Harris et al. 2022) speculated that dehalogenase enzymes from *Delftia acidovorans* could be responsible in the degradation and defluorination of PFOA. The results of Chetverikov and Loginov (Chetverikov and Loginov 2019) indicated that, during 4 days of incubation of *Ensifer adhaerens*, the decarboxylation of PFOA occurred with the release of fluoride ions. The decrease of PFOA concentration and the appearance of new metabolites monitored by LC/MS in the media confirmed the existence of reductive defluorination for microbial consortia (Beškoski et al. 2018). In the present study, PFOA was used as the substrate to screen the microbial resources capable of degrading PFOA through enrichment culture of soil suspension. The acquisition of dominant microorganisms can open new avenues for a complete degradation of PFOA in the environment.

Materials and methods

Collection of soil samples

Sample sites have been a National Urban Wetland Park now, which were a densely populated area about 15 years ago. To verify the feasibility of isolating PFOA degradable microbial strains from the sludge soil commonly reported by previous studies (Huang and Jaffé 2018; Li et al. 2017), eight samples of soils (Table 1) were collected from sludge (#4 ~ 8) and beneath the earth surface (#1 ~ 3) from 8 sites of Huaxi district, Guiyang city, Guizhou province of China. Specifically, the samples were obtained from: #1-the meadow of Guizhou Light Industry Technical College, #2-the meadow of Guizhou Normal University, #3-the hill of Guizhou Medical University, #4-the pond of Guizhou Medical University, #5-the sludge of Ten-mile Beach, #6-the sludge of Huaxi Park, #7-the upstream sludge and #8-the downstream sludge of Huaxi River (as illustrated in

Table 1 Physico-chemical characteristics of soils obtained from different sites

Code of soil samples	Parameters					
	pH	Total nitrogen (%)	Organic matter (%)	Hydrolysable Nitrogen (mg/kg)	Available phosphorus (mg/kg)	Available potassium(mg/kg)
#1	6.49	0.23	3.81	125.31	3.25	139.80
#2	6.40	0.26	3.74	122.44	2.59	150.60
#3	6.81	0.11	2.13	112.63	1.30	94.33
#4	6.37	0.63	8.94	253.71	20.15	105.30
#5	6.02	0.85	11.26	211.37	18.82	86.20
#6	6.24	0.86	12.86	246.82	22.49	90.41
#7	6.34	0.88	14.18	281.31	25.30	80.22
#8	6.23	0.93	14.62	295.27	26.13	76.31

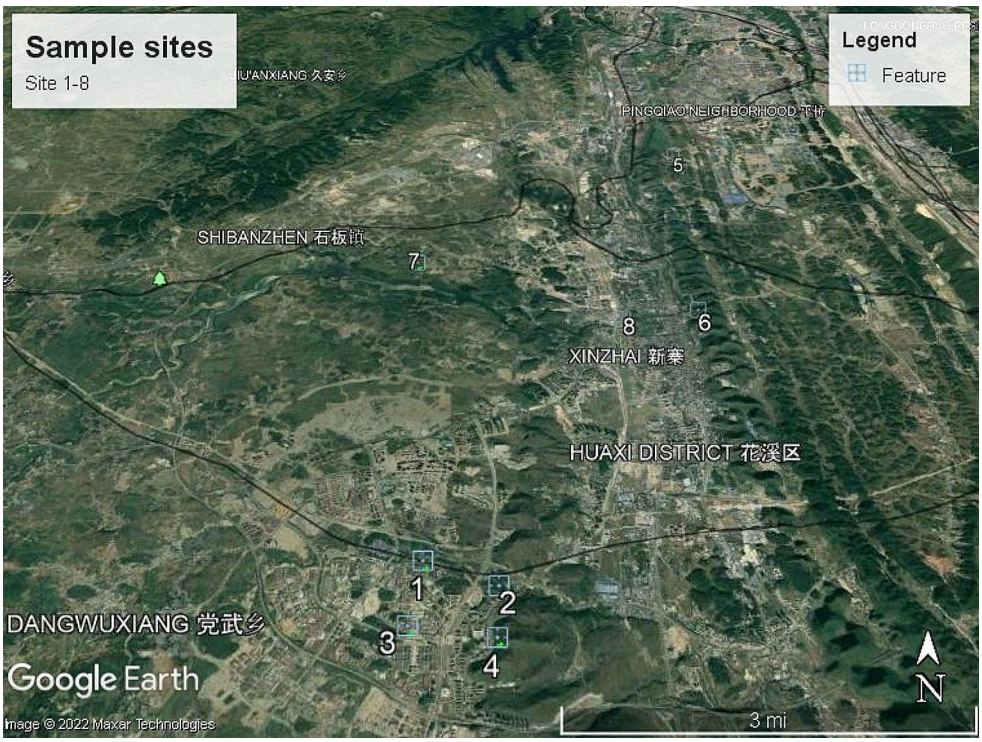

Fig. 1 Sample sites of soils from Huaxi district, Guiyang city, Guizhou province of China (Visualized by Goolge Earth Pro developed by Google)

Fig. 1). The physico-chemical characteristics of respective soils were determined by conventional laboratory methods (Bao 2008) (Table 1).

Reagents, cultural media and culture conditions

PFOA (AR, 96%) was purchased from Macklin (Shanghai, China). Fluorine ion standard solution (10,000 μg/mL) was purchased from Guangzhou Sopo Biological Technology Co., LTD. Luria-Bertani cultural medium (LB) was composed of 10.0 g sodium chloride, 10.0 g tryptone, 5.0 g yeast extract and 15.0 g agar in 1 L of purified water. Basic salt cultural medium (MSM) comprised 1.0 g NH_4NO_3, 1.5 g K_2HPO_4, 0.5 g KH_2PO_4, 0.2 g $MgSO_4$, 1.0 g NaCl and 1.0 mL trace elements solution in 1 L of purified water without or with agar (15.0 g). The composition of trace elements solution (1 L) was following: 0.13 g $MnSO_4{\cdot}H_2O$, 0.23 g $ZnCl_2$, 0.03 g $CuSO_4{\cdot}H_2O$, 0.42 g $CoCl_2{\cdot}6H_2O$, 0.15 g $Na_2MoO_4{\cdot}2H_2O$, 0.05 g $AlCl_3{\cdot}6H_2O$. All the bacterial strains isolated were cultured on LB or MSM agar on 90 mm petri dishes at 28°C. For the shaking-flask culture of bacterial isolates, rotation rate of 120 rpm and constant temperature of 28 °C was employed in the whole experiment.

Preparation of inoculum

1.0 g of fresh soil samples, respectively, from eight sites was poured into a 250 mL flask containing 100 mL sterilized water and shaked at rotation rate of 120 rpm for half an hour, and the soil suspensions obtained after the deposition of 10 min were used as the inocula for the degrading trials of PFOA.

Enrichment culture of bacteria degrading PFOA

100 µL of each soil suspension was added to a 500 mL flask containing 100 mL of MSM culture solution, and 1000 mg/L PFOA concentration. Addition of equal volume of inactivated soil suspension was regarded as the control. The shaking-flask culture was carried out according to the conditions as mentioned above for the enrichment culture of specific bacteria degrading the PFOA. The turbidity for the suspension cultures of the eight different origins of soils was observed by naked eyes for 28 consecutive days at the interval of seven days.

Purification of bacteria degrading PFOA

100 µL of each enrichment cultural solution was diluted in 1 mL of sterile water, and 10 µL of this diluted bacterial suspension was plated on MSM agar with PFOA of 1000 mg/L in each of three 90 mm petri dishes. Plates were incubated at 28 ℃ for 7 days, and every single colony appearing on PFOA-added agar medium was picked up with sterile transfer loop and transferred on LB plates. The plates were incubated for two days at 28 ℃. The morphological uniform of each bacterial colony on LB plates was checked visually for obtaining purified bacterial strains. The purified strains were preserved in a glycerol solution (20% v/v at -70 ℃) for the further analysis of PFOA degradation.

Verification of bacterial utilization of PFOA

The purified strain was activated on LB plates, and bacterial lawn was scraped off from the agar medium by a transfer loop to obtain a loopful of bacteria, which were inoculated into a 500 mL flask containing 100 mL of MSM culture solution with 1000 mg/L of PFOA assayed using previous study (Chetverikov and Loginov 2019). A loopful of inactivated bacteria was added into the flasks as control (CK). The conditions of the shaking-flask culture for bacterial strains were indicated according to the previous description in Sect. 2 of Materials and methods. Cell densities of different cultural suspensions were measured by microplate reader (Thermo Fisher Scientific Inc., 1510) at 600 nm for five consecutive days at the interval of 24 h. Each strain was triplicate.

Five strains selected were further used to test the degrading efficacies towards PFOA. After 31 days of shaking culture as mentioned above, the bacterial suspension was filtered (0.22 µm, Ybo Technologies Co., Ltd.), and the filtrate collected was used to measure the fluoride ion concentration in the cultural suspension. To expel the possible effects of the impurities of reagents on the determination of fluoride ion, the MSM culture solution of non-replenished PFOA was also prepared concurrently for the inoculation of each strain. The determination of fluoride by ion-selective electrode (ISE) method was referred from the 'National Standards of People's Republic of China' (GB/T 5750.5 – 2006) (National Standards of People's Republic of China 2007) and the method of Harris et al. (Harris et al. 2022) with some modification. Quality control of this detection method was monitored with recovery analysis. Standard fluoride solutions of 0.02, 0.05, 0.10, 0.20, 0.30 and 0.50 g/mL were prepared by dilution of a commercial 10,000 µg/mL fluoride stock solution (Fluoride Standard Cat. No. BW20081-10000-50 Tanmo Quality Inspection Technology Co., Ltd.). The mixed solutions of 45 mL of fluoride standard and 5 mL of total ionic strength adjustment buffer(sodium citrate 114 g/L, citric acid 12 g/L, pH 5.0 ~ 7.0) were used to determine electrode potentials with pH meter (Shanghai INESA Scientific Instrument Co., Ltd., China; Model: PHS-3 C) equipped with indicator electrode-fluoride ion-selective electrode (Shanghai INESA Scientific Instrument Co., Ltd., China; Model: PF-2-01) and reference electrode was a mercurous chloride electrode (Shanghai INESA Scientific Instrument Co., Ltd., China; Model:232). A calibration curve of electrode potential slope was constructed using solutions containing fluoride at 0.018, 0.045, 0.090, 0.180, 0.270, 0.450 µg/mL. For the fluoride determination of the suspension culture for each strain, the same procedure was performed on the filtrate as that of the standards.

Identification of bacteria degrading PFOA

The bacterial strains degrading PFOA were cultured on LB plates at 28 ℃ for 120 hours, and the cultural characteristics for each strain on LB were observed. Gram reactions, spore staining, oxygen requirement, catalase, oxidase, starch hydrolysis, Voges-Proskauer test, denitrification, and the utilization of carbons and etc. were done according to the conventional methods (Holt et al. 1994; Dong et al. 2001). The 16S rRNA gene was amplified from chromosomal DNA using the universal bacterial primer set, 27F (5'-AG AGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGAC TTAACCCCAATCGC-3'). The purified PCR product was sequenced by Sangon Biotech, China. A full sequence of the 16 S rRNA gene was compiled with online tools (https://novopro.cn/tools/combine_fasta.html). The 16 S rRNA gene sequences of related taxa were obtained from the GenBank. Multiple alignments were performed with the CLUSTAL X program. Evolutionary distances were calculated using the Kimura two-parameter model and the phylogenetic tree constructed via the neighbor-joining method in the MEGA 5.05 program. Bootstrap analysis with 1,000 replicates was also conducted to obtain confidence levels for the branches.

Nucleotide sequence accession number

The NCBI GenBank/EMBL/DDBJ accession numbers for 16 S rRNA gene sequences of strains 4-a, 5-b, 6-a, 7-c and 8-a were ON533612, ON533613, ON533614, ON533615 and ON533616 respectively.

Statistical analysis

Analysis of variance using one-way ANOVA was carried out, and the analyses of significance were performed by Duncan's multiple-range test at a significance level of $p = 0.05$ using SAS 8.1 for Windows 7.

Results

Enrichment culture of soil suspensions and acquisition of bacterial strains degrading PFOA

Results of shaking-flask culture showed that the bacterial strains were present that degraded PFOA in six locations, except at two sites, including the meadow of Guizhou Institute of Light Industry (site 1) and the hill of Guizhou Medical University (site 3). The results indicated the organic-matter-rich soils sustained the growth of various types of functional bacteria (Table 1). The soils of #1 and #3 did not cause a visible turbidity of MSM cultural solutions having PFOA, and were similar to that of the control after an incubation of 28 days. Although the phenomena of turbidity occurred in the cultural solutions #2 and #4 ~ 8, the degree of turbidity for them did not intensify with time (Table 2). After a continuum of selective culture on MSM agar with PFOA (1000 mg/L) and purified culture on LB in Petri dishes, a total of 16 bacterial strains were obtained from the soil samples #2 and #4 ~ 8. Among them, strains of 2-a and 2-b1 were obtained from the sample #2, 4-a and 4-b from #4, 5-b from #5, 6-a1, 6-a, 6-c2, 6-d2 and 6-d1 from #6, 7-a, 7-b2 and 7-c from #7, and 8-a, 8-b and 8-b2 from #8. Additionally, the diameter of all the colonies formed on PFOA-added MSM agar plate was nearly 2 millimeters, when incubated at 28°C for 7 days.

Table 2 Turbidity of PFOA-supplemented MSM cultural solutions of different soil origins

Code of original soil samples	Days of culture(d)			
	7	14	21	28
#1	-	-	-	-
#2	+	+	+	+
#3	-	-	-	-
#4	++	++	++	++
#5	+	+	+	+
#6	+	+	+	+
#7	+	+	+	+
#8	++	++	++	++

Noted: ++ indicates apparent turbidity observed in cultural solutions; + indicates slightly turbidity in cultural solutions; - indicates no visible turbidity in cultural solutions

Utilization and degradation of PFOA by isolated bacteria

The growth dynamics of sixteen bacterial strains for the utilization of PFOA as sole carbon and energy source showed that there existed two growth peaks of different intensities for 4-a at 24 and 72 h respectively and 6-a, 6-d2, 6-d1, 7-b2, 7-c and 6-a1 at 24 and 96 h consistently. On the other hand, only one peak was evident for 5-b and 6-c2 at 24 h, for 8-b, 8-a and 2-b1 at 48 h, for 7-a at 72 h, for 4-b and 2-a at 96 h, for 8-b2 at 120 h, respectively during the whole incubation time (Fig. 2). Additionally, all the isolated bacterial strains exhibited different dynamic tendencies of growth during the incubation, which indicated the differential modes of utilization towards PFOA. Meanwhile, the drop on the absorbance at 600 nm meant the inhibition of some toxic intermediates and fluoride ions from bacterial metabolism of PFOA towards the isolates. Considering the origin of strains and limited facilities for shaking culture, five bacterial strains (Fig. S1), viz. 4-a, 5-b, 6-a, 7-c and 8-a isolated from different sites were used to test the degrading ability towards PFOA in the present study. The limit of detection of ISE method was 0.019 μg/mL, and a recovery range of 97.8–106.4% was achieved. A calibration line for fluoride (Fig. S2) was plotted for the calculation of the concentration of fluoride ions in the culture filtrates of five bacterial strains. After 31 days incubation for five bacterial strains, the concentration of fluoride ions in culture filtrates was determined (Fig. 3), which indicated a low defluorination efficiency of PFOA (1000 mg/L) among the five isolated strains. The concentration of fluoride ions among the filtrates of different bacteria was significantly different (Fig. 3), and the order of fluoride ion was: 6-a (0.363 μg/mL) > 7-c (0.347 μg/mL) > 4-a (0.322 μg/mL) > 5-b (0.282 μg/mL) ≈ 8-a (0.282 μg/mL). The concentration of fluoride ions in the filtrates of PFOA-supplemented bacterial cultures was significantly higher than those in the control (PFOA-supplemented MSM cultures with the inoculation of inactivated bacteria) and non-replenished PFOA bacterial cultures (negligible amounts below the detection limit) in the present experiment.

Cultural, biochemical characteristics and phylogenetic analysis for five bacterial strains

The photographs of five bacterial strains incubated for 7 days at 28°C on LB plates are shown in Fig. 3. All colonies

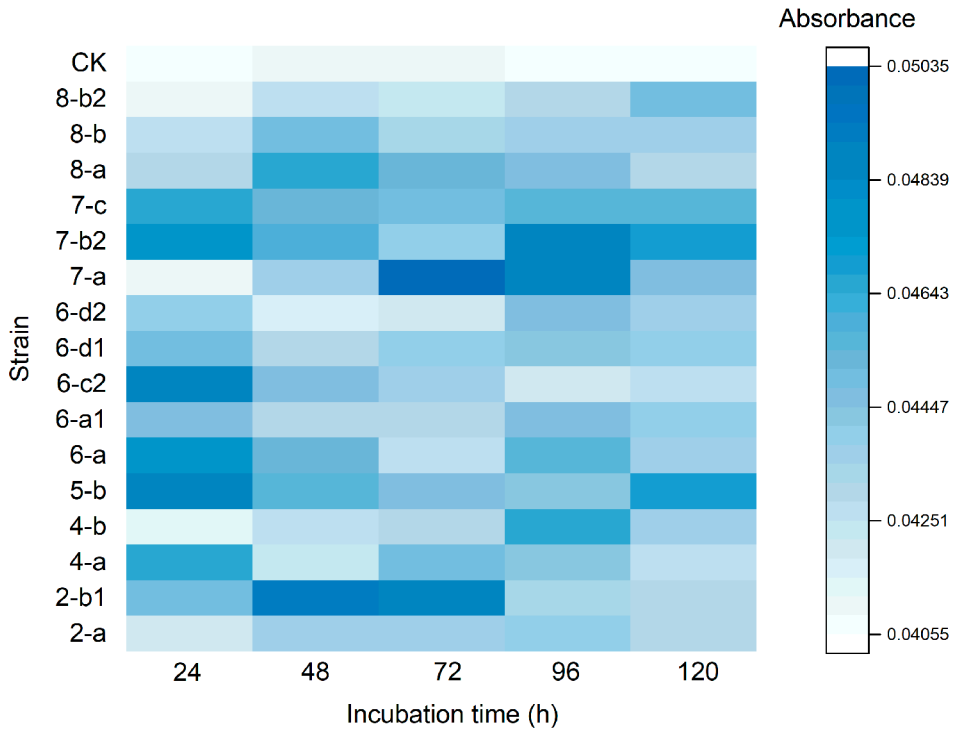

Fig. 2 Growth dynamics of isolated bacterial strains in MSM cultural solutions with PFOA (1000 mg/L)

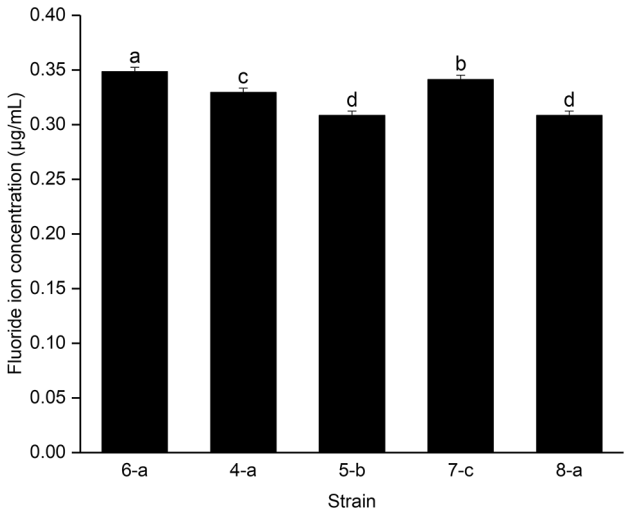

Fig. 3 Quantity of fluoride ions produced in the cultural suspensions by the degradation of bacterial strains towards PFOA during the incubation period of 31 days. Different letters (a, b, c or d) above the columns indicate statistically significant differences among bacterial culture filtrates (mean ± SD, $N = 3$)

of five bacterial isolates appeared translucent to yellow, circular in shape, convex in elevation, and regular in margin and motility. They were short, non-spore-forming and gram-negative bacilli. The results of physiological and biochemical tests on five bacterial strains are listed in Table 3, positive on growth at 41°C, oxygen requirement, fluorescent, catalase, oxidase reaction, denitrification, utilization of glucose, L-lysine and L-tryptophan; negative on growth at 4°C, Voges-Proskauer test and starch hydrolysis for all the strains, and differential reactions on utilization of trehalose, sucrose, D-ribose, mannitol, m-inositol, D-xylose, tartrate and L-valine indicated the genomic heterogeneity in genus *Pseudomonas*. The phylogenetic tree (Fig. 4) derived from 16 S rRNA gene sequences was constructed by the neighbor-joining method to clarify the relative position of the five bacterial strains with other *Pseudomonas* species with greater than 99.0% 16 S rRNA gene sequence identity. Collectively, it was difficult to conclude the accurate taxonomic statuses of the five bacterial strains according to the existing references except for the affirmation of genus *Pseudomonas*. Therefore, the whole-genome sequencing of five bacterial genomes needs to be done to determine its taxonomy.

Discussion

PFOA is an emerging persistent organic pollutant, which is difficult to degrade using conventional methods, owing to its stable physical and chemical properties. Considering the decontamination of dominant PFOA and PFOS, several emerging technologies for PFOA/PFOS degradation and removal are reported in the literature (Leung et al. 2022). Although a low defluorination efficiency of PFOA was reported (Leung et al. 2022), bioremediation can lead to a permanent dispose of chemicals and is a relatively

Table 3 Physiological and biochemical characteristics of five bacterial strains selected

Characteristic	4-a	5-b	6-a	7-c	8-a
Growth at 4°C	-	-	-	-	-
Growth at 41°C	+	+	+	+	+
Oxygen requirement	+*	+*	+*	+*	+*
Fluorescent	+	+	+	+	+
Voges-Proskauer test	-	-	-	-	-
Catalase	+	+	+	+	+
Starch hydrolysis	-	-	-	-	-
Oxidase reaction	+	+	+	+	+
Denitrification	+	+	+	+	+
Utilization of					
Glucose	+	+	+	+	+
Trehalose	+	+	-	-	+
Sucrose	+	-	+	+	-
L-Lysine	+	+	+	+	+
D-Ribose	-	+	+	+	-
Mannitol	+	+	+	-	+
m-Inositol	+	+	-	+	-
D-Xylose	-	+	-	+	+
Tartrate	+	-	+	+	+
L-Tryptophan	+	+	+	+	+
L-Valine	+	+	-	+	+

Noted: +, 90% or more strains are positive; -, 90% or more strains are negative; +*, facultative anaerobes

inexpensive choice for eliminating low concentrations of widely distributed pollutants in the environment. Therefore, it is still an important aspect to explore the microorganisms existing in nature that can degrade PFOA, especially in sludge (Chiavola et al. 2020). Monitoring fluoride ion generation using ion-selective electrodes is a quick method to assess the extent of defluorination, which is a critical step of organofluorine biodegradation by microbial strains (Amorim et al. 2014; Moreira et al. 2014). Currently, only a very few of microbial strains identified have been reported in the literature for the degradation of PFOA, which include *Ensifer adhaerens* (Chetverikov and Loginov 2019), *Acidimicrobium* sp. (Huang and Jaffé 2019; Ruiz-Urigüen et al. 2022; Huang et al. 2022), *Pseudomonas parafulva* (Yi et al. 2016), *Delftia acidovorans* (Harris et al. 2022), *Pseudomonas plecoglossicida* (Chetverikov and Sharipov 2022), etc. In terms of the results of these studies, different degrading mechanisms towards PFOA were embodied on the reductive defluorination for anaerobic *Acidimicrobium* sp. (Huang and Jaffé 2019) and the enzymatic processes in aerobic *Delftia acidovorans* (Harris et al. 2022). Our experimental results on the defluorination of the isolates towards PFOA were consistent with the PFOA degrading effects of *Ensifer adhaerens* (Chetverikov and Loginov 2019) and *Delftia acidovorans* (Harris et al. 2022) based on the changes of fluoride ion concentrations in cultural medium. Among the five isolates of facultative anaerobic *Pseudomonas* sp. focused in this study, a single growth peak appeared in the

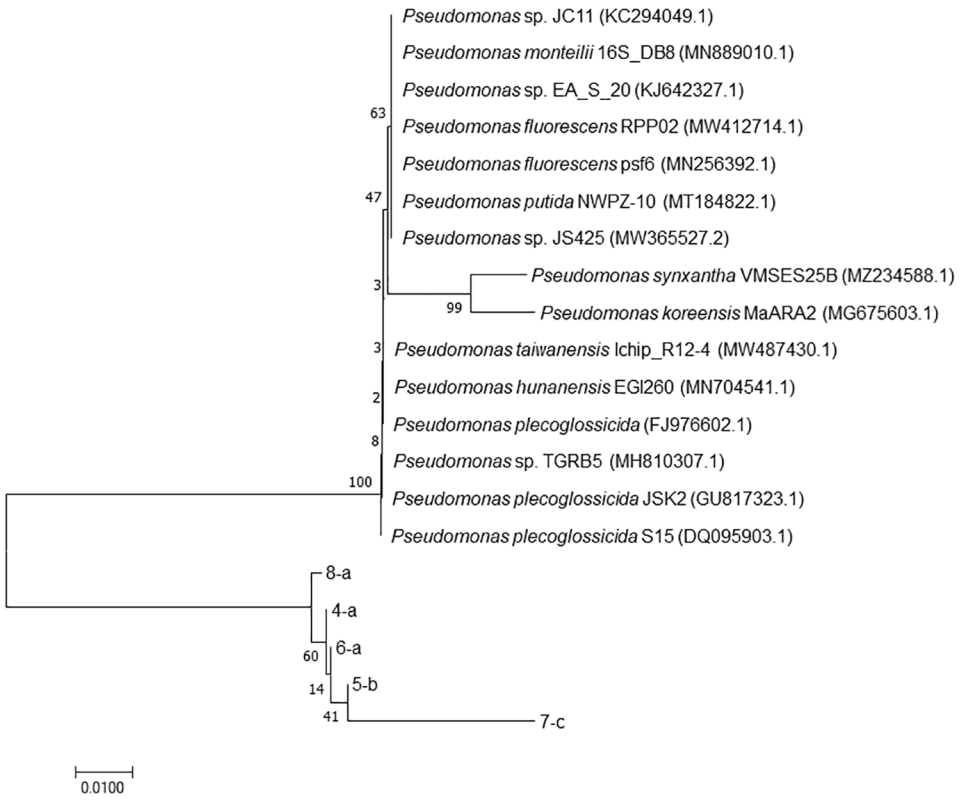

Fig. 4 The phylogenetic tree based on 16 S rRNA gene sequence with neigh-bour-joining method. Bar, 0.02 substitution per nucleotide position

early growth stage for 8-a and 5-b at 48 h and 24 h of incubation respectively, which might hint the main enzymatic processes in the degradation of PFOA similar to the function of *Delftia acidovorans* (Harris et al. 2022) under the aerobic condition. On the other hand, two growth peaks were observed for strain 4-a (24 and 72 h), 7-c (24 and 96 h) and 6-a (24 and 96 h). This might imply the serial degradation of PFOA including enzymatic oxidative metabolism (the early growth stage in the aerobic condition) and a reductive metabolism under the availability of electron donors– ammonium or hydrogen and electron acceptor-NO_3^-, MoO_4^{2-}, PFOA or by other factors (the late growth stage in the low oxygen partial pressure or the anaerobic environment) on PFOA. Meanwhile, more experiments are required to validate these assumptions. Differential fluoride ion concentration in the cultural medium among the five specific isolates verified the in-depth defluorination of strain 4-a, 7-c and 6-a surpassing 8-a and 5-b during the incubation. Especially for the isolate 7-c, a stable growth tendency occurred during incubation of 120 h, indicating its strong adaptation mechanisms, such as the capacity to modify the cellular membrane to maintain their biological functions and/or the use of efflux pumps to decrease the concentration of toxics inside the cells. A striking reduction of growth for strain 4-a, 5-b, 6-a and 8-a at the end of the incubation period suggested the potential toxicity of metabolic end products of defluorination on the bacteria, including known fluoride ions (Chetverikov and Loginov 2019; Wackett et al. 2022). More research needs to be done for the clarification of the differential metabolic pathways probably involving in the production of perfluoroheptanoic acid, perfluorobutanoic acid, shorter chain perfluorinated products, fluoride ions, acetate, et al. among the isolates towards PFOA through the genomics, transcriptomics and proteomics methods.

Conclusion

In conclusion, this present study illustrated the biodegradation of PFOA by some bacterial strains from the sludge soils. Unimodal or bimodal growth dynamics for the isolates with PFOA as the sole carbon and energy source indicated the differences in the mechanism of PFOA biodegradation. Five bacterial isolates of them, namely 4-a, 5-b, 6-a, 7-c and 8-a exhibiting the differential defluorination towards PFOA were identified as *Pseudomonas* sp. based on the cultural and biochemical characteristics, 16 S rRNA sequences and phylogenetic analysis. Nonetheless, this study can be beneficial for researchers to deepen their understanding of the defluorination of facultative anaerobic bacteria towards PFOA.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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