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Degradation Mechanism of 4-Chlorobiphenyl by Consortium of *Pseudomonas* sp. Strain CB-3 and *Comamonas* sp. Strain CD-2

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Abstract

Polychlorinated biphenyls (PCBs) are types of lasting environmental pollutants which are widely used in various industries. 4-chlorobiphenyl (4CBP) is a PCB which is harmful to the environment as well as humans. Two strains, CB-3 and CD-2, were isolated from the polluted soil of a chemical factory and could completely degrade 50 mg/L 4CBP within 12 h by co-culture. The consortium comprising strains CB-3 and CD-2 was effective in the degradation of 4CBP. 4CBP was degraded initially by strain CB-3 to accumulate 4-chlorobenzoate (4CBA) and further oxidised by strain CD-2. Based on 16S rRNA gene sequence analysis and phenotypic typing, strain CB-3 and strain CD-2 were identified as *Pseudomonas* sp. and *Comamonas* sp., respectively. The substrate spectra experiment showed that strain CB-3 could degrade PCBs with no more than three chlorine atoms. A gene cluster of biphenyl metabolism was found in the genome of strain CB-3. Besides, a dechlorination gene cluster and a gene cluster of protocatechuate (PCA) metabolic were found in the genome of strain CD-2. These gene clusters are supposed to be involved in 4CBP degradation. The ability of strains CB-3 and CD-2 to degrade 4CBP in soil was assessed by soil experiment, and 4CBP at the initial concentration of 10 mg/kg was 80.5% removed within 15 days.

Introduction

PCBs are widely used in the chemical industry due to their superior insulation, flame retardancy, thermal stability and fat solubility [10]. PCBs are listed as the priority persistent organic pollutants (POPs) in an international chemical treaty from the Stockholm Convention, which is owed to its serious hazards, such as its chemical stability, biological toxicity, bioaccumulation and mobility [3, 29]. 4CBP is a kind of low chlorinated PCB and had harmed the natural environment and human health for its high toxicity, polluted persistence and carcinogenicity [14]. The bioremediation is more safe and economical compared to the physical and chemical restoration [33], and the remediation of 4CBP-contaminated soil has attracted the attention of scholars.

To date, a lot of bacterial strains have been reported to aerobically metabolise 4CBP. For example, *Ralstonia* sp. SA-3 could degrade approximately 90% 0.66 mM 4CBP within 70 h [1], strain IR08 isolated from electrical transformer fluid-contaminated soil could degrade 0.27 mM 4CBP in less than 96 h [18], and *Bacillus* sp. MCS caused a reduction of 60% in the initial 4CBP concentration (200 mg/L) after 48 h and completely removed it after 7 days [26]. Research has shown that all of them only transformed chlorinated biphenyl to respective chlorobenzoates (CBAs) as dead-end metabolites [27]. Incomplete metabolism of CBAs by microorganisms could produce toxic metabolites indirectly [5, 31]. Several strains show complete degradation of 4CBP, such as *Cupriavidus* sp. strain SK-3, which could completely transform 0.48 mM 4CBP within 85 h [21] and *Comamonas* sp. DJ-12 (formerly *Pseudomonas* sp.), which could degrade 0.5 mM 4CBP completely after 24 h [7]. The goal of this study was to isolate bacterial strains that could degrade 4CBP completely with high efficiency, studying its degradation mechanism and characteristics, and provide more strain resources for bioremediation of 4CBP-contaminated soil.

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Materials and Methods

Chemicals and Medium

Minimal salt medium (MSM) was used: 1.0 g NH_4NO_3, 1.5 g K_2HPO_4, 0.5 g KH_2PO_4, 0.2 g $MgSO_4{\cdot}7H_2O$ and 1.0 g NaCl per litre water at pH 7.0 (1.7% agar was added to solid medium). This was in addition to Luria–Bertani broth (LB): 10.0 g tryptone, 5.0 g yeast extract and 5.0 g NaCl per litre water at pH 7.0 (1.7% agar was added to solid medium). 4CBP (98% purity) and 4CBA (99% purity) were provided by Macklin Biochemical Co., Ltd (Shanghai, China). Methanol was of chromatographic grade (Sigma-Aldrich, Shanghai, China), and organic reagents were of analytical grade (Nanjing Chemical Reagent Co., Ltd).

Strains, Isolation and Identification

Soil collected from drain outlet of a chemical factory in Jiangsu Province, P. R. China. 15 g soil samples were added to an Erlenmeyer flask containing 100 mL MSM (contained 50 mg/L 4CBP). The culture was incubated at 30 °C under aeration, on a shaker at 160 rpm for 7 days. Approximately 5 mL of enrichment culture was transferred into 100 mL of fresh MSM medium containing 50 mg/L 4CBP every 7 days. After three rounds of transfer, the rate of 4CBP removal was determined using high-performance liquid chromatography (HPLC). The enrichment culture was diluted in serial gradient (1.0×10^{-2}–1.0×10^{-6}) and spread on MSM agar plates supplemented with 50 mg/L 4CBP. These plates were incubated at 30 °C. Different forms of single colonies were picked and further purified by repeated streaking onto MSM agar plates supplemented with 50 mg/L 4CBP. Each isolate was tested for the ability to degrade 4CBP.

Strains that were effective at degrading 4CBP were characterised and identified by morphological, physiological and biochemical characteristics. For the amplification of the 16S rRNA sequence, genomic DNA of isolated was extracted, and a 16S rRNA sequence was acquired by PCR amplification using a pair of universal primers, 27F and 1492R [13]. The purified PCR product was cloned into pMD19-T vector and sequenced using an automated sequencer (model 3730; Applied Biosystems). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the EzTaxon-e server (https://www.ezbiocloud.net) [20]. Multiple alignments were performed by using the clustal_x program [34]. Gaps were edited in the BioEdit program [16], and a phylogenetic tree was constructed on the basis of the neighbour-joining method [28], maximum parsimony and maximum likelihood [12] in MEGA version 7.0 [32], with bootstrap values based on 1000 replications [11].

Biodegradation of 4CBP by Consortium of Strains CB-3 and CD-2

Strains CB-3 and CD-2 were, respectively, picked into LB broth, and cells were harvested after being cultivated to an OD_{600} of 0.7. Harvested cells were washed twice with MSM as the initial culture. Degradation of 4CBP (50 mg/L) was tested in 100 mL MSM, inoculated with an equal amount of strains CB-3 and CD-2 to an OD_{600} of 0.2 in the 250-mL Erlenmeyer flasks. In addition, the growth and degradation of strains CB-3 and CD-2 were tested individually according to the following steps: strain CB-3 was inoculated into 100 mL MSM containing 50 mg/L 4CBP and strain CD-2 into 100 mL MSM containing 100 mg/L 4CBA to an OD_{600} all of 0.08. All cultures were incubated at 30 °C, at 160 rpm on a rotary shaker for 20 h, sampling at intervals of 2 h. The OD_{600} values were tested by UV spectrophotometer, and the concentration of 4CBP and 4CBA was detected by HPLC. All treatments set three replicates, including other experiments in this study.

Degradation Characteristics of Strains CB-3 and CD-2

To determine the effect of pH, temperature and the concentration of substrate on degradation by strains CB-3 and CD-2, 1 mL corresponding initial culture was added into 100 mL MSM containing 50 mg/L 4CBP or 100 mg/L 4CBA. To determine the optimum pH, experiments were performed at pH values of 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 9.0, 9.5 and 10.0, which adjusted by HCl and NaOH solution and precisely detected by pH metre. To assess the effect of temperature, the degrading medium with the optimum pH was incubated at 15 °C, 20 °C, 25 °C, 30 °C, 37 °C and 42 °C. To determine the effect of substrate concentration, the range of substrate addition was 30, 50, 100, 150 and 200 mg/L and experimented on under optimum pH and temperature. The above experiments were on a rotary shaker at 160 rpm for 12 h. The residual substrate concentration under different conditions was measured by HPLC. The values of degradation rate are peak area ratio of decreased substrate concentration and initial substrate concentration.

To investigate the substrate spectra of strains CB-3 and CD-2, some analogues of 4CBP and 4CBA, such as biphenyl, 2-chlorobiphenyl, 3-chlorobiphenyl, 3,4-dichlorobiphenyl, 3,4,5-trichlorobiphenyl, 4-hydroxybiphenyl, diphenylmethane, 2,3,4,5-tetrachlorobiphenyl, 2,3,4,5,6-pentachlorobiphenyl, 4-iodobenzoate, 4-bromobenzoate,

4-hydroxybenzoate, benzoate, salicylate, gentisate and 4-aminobenzoate, were used to test the degradation ability of two strains by HPLC.

Draught Genome Sequencing and Prediction of the Degradation Gene Cluster

DNA manipulation was performed according to standard protocols [24]. Genomic DNA of strain CB-3 and CD-2 were extracted and delivered to Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) for draught genome sequencing using an Illumina MiSeq system. ORF annotations of the genomes of strain CB-3 and CD-2 were performed using the RAST online analysis tool with the sequence-based model [2, 22].

Bioremediation of 4CBP-Contaminated Soil by Consortium of Strains CB-3 and CD-2

A remediation test of 4CBP-contaminated soil in the laboratory environment was performed. Experiment soil was collected from 0–20 cm topsoil in Xiamafang Park (Nanjing, China), which has not been contaminated by 4CBP before. Physicochemical properties of soil are listed: the total nitrogen content is 0.66 g/kg, total phosphorus content is 0.71 g/kg, total potassium content is 4.25 g/kg, and pH is 6.87. Impurities and stones were removed from the soil by passing it through a 2-mm sieve. Soil samples were divided into non-sterile soil and sterile soil (sterilized for 30 min at 121 °C, three times). All soil samples were homogeneously treated with a solution containing 4CBP to a final concentration of 4CBP 10 mg/kg soil. Cells of strains CB-3 and CD-2 were collected by centrifugation (5000 rpm), washed twice with double distilled water (ddH_2O) and resuspended in ddH_2O to a cell density approximately 2×10^9 CFU/mL. Two strains were added into soil samples and stirred homogeneously [a final concentration of approximately 10^9 colony-forming units (CFU)/g for each strain]. Additionally, soil samples without an inoculum were used as controls. All samples were incubated at 30 °C, in a constant temperature incubator without illumination. Sterile water was added daily to ensure that the soil moisture content was not less than 25% in the process of experiment, and the detection of 4CBP content sampling was at an interval of 5 days. 4CBP was enriched via multiple extractions and detected concentration by HPLC.

To determine the effect of temperature, the initial concentration of 4CBP and the inoculation amount of strain CB-3 and CD-2 on the remediation of 4CBP-contaminated soil, the degradation efficiency of 4CBP was tested under different conditions and the concentration of 4CBP was detected after 15 days.

Treatment of Samples and Chemicals Analysis

The extraction of 4CBP in liquid medium was as follows: the samples were mixed with an equal volume of dichloromethane and shocked violently by a vortex apparatus for 3 min. The organic extracts passed through anhydrous sodium sulphate and dried naturally in a draught cupboard. Finally, all the samples were dissolved with methanol and filtered through 0.22-μm Millipore membrane filters.

The detection wavelength of UV spectrophotometer ranges from 350 to 200 nm. The concentration of 4CBP was detected by HPLC with the following parameters: chromatographic column: Syncronis C18 (4.6×250 mm$\times5$ μm) reverse phase column; mobile phase (water: methanol, 40:60) containing 0.5% acetic acid; the flow rate: 1.0 mL/min; UV detector wavelength: 250 nm and volume for sampling: 20 μL.

The structural identification of metabolites in samples was performed by LC–MS/MS, and the mass spectrum was obtained using a TripleTOF 5600 (AB SCIEX) mass spectrometer. Mass spectrometry was performed using an electrospray source in negative ion mode with MSe acquisition mode, with a selected mass range of 50 to 600 *m/z*.

The extraction of 4CBP in soil included the following: 10 g soil samples and 40 mL ddH_2O was added into a 250-mL Erlenmeyer flask and mixed for 1 h at 30 °C and 160 rpm. The mixture was left to stand still for 30 min and 20 mL of supernatant was transferred into 50-mL tube and centrifuged at 8,000 rpm to remove most of the impurity. 10 mL of supernatant from the centrifuged tube was transferred into a new 50-mL tube and mixed with an equal volume of dichloromethane, extraction, dried and dissolved with methanol.

Results and Discussion

Strains Isolation and Identification

There were 12 strains isolated from all plates, which were mixed, and their ability to degrade 4CBP was verified as true and the ability of degradation for each isolated strain was tested. 4CBP can be obviously degraded by one of twelve strains, but the final product was an unknown intermediate product. The strain that degraded 4CBP was named CB-3. After that, strain CB-3 was mixed with 11 strains of remained, respectively, to assess 4CBP degradation, and the results showed that 4CBP was catabolised completely when a pale yellow strain (named CD-2) was mixed with strain CB-3. It is preliminarily judged that the degradation of 4CBP was the synergistic action of the two strains.

Strains CB-3 and CD-2 were identified by morphological and physiological characteristics and phylogenetic

analysis of 16S rRNA gene sequences. Strain CB-3 was determined to be a gram-negative with flagellum and rod-shaped (0.59–0.61 μm × 0.90–1.1 μm), and colonies were yellow, convex and fold of edge on LB plate. Strain CB-3 tested positive for catalase, reduction of nitrates, oxidase reaction and starch hydrolysis. Meanwhile, it tested negative for hydrolysis of gelatin and indole production. Antibiotic tests indicated that strain CB-3 was resistant to penicillin, vancomycin, lincomycin and bacitracin. Strain CD-2 was determined to be a gram-negative without flagellum and short rod-shaped (0.96–1.0 × 2.0–2.3 μm). The microorganism colonies were pale yellow, flat, smooth and viscous of edge on LB. Strain CD-2 showed a negative response to the reduction of nitrates, oxidase and catalase. However, it showed a negative result for indole production and hydrolysis of gelatin and starch. Antibiotic tests showed that strain CD-2 is resistant to polymyxin, vancomycin, spectinomycin, rifampicin, lincomycin and bacitracin.

Phylogenetic analysis of 16S rRNA gene sequences suggests that strain CB-3 shows a close affiliation with the strains of genus *Pseudomonas*. Strain CB-3 had the highest similarity (99.25%) with *Pseudomonas stutzeri* ATCC 17588^T (Fig. S1). Based on the phylogenetic tree (Fig. S2), strain CD-2 grouped among *Comamonas* species and was most closely related to *Comamonas sediminis* S3^T (98.26% similarity). Therefore, strain CB-3 was tentatively identified as *Pseudomonas* sp. and strain CD-2 as *Comamonas* sp.

The GenBank accession number for the strains CB-3 and CD-2 16S rRNA gene sequences are MN099436 and MN099437, respectively.

Analysis and Identification of Metabolites of 4CBP by Strain CB-3

For the sample collected after 12 h of inoculation, only a compound was detected by HPLC, with a retention time of 3.61 min (Fig. 1a). The prominent protonated molecular ion of the product was *m/z* 157.0050 $[M+H]^+$ (Fig. 1b) and major fragments at *m/z* 113.0038 (loss of –COOH) (Fig. 1c). The isotopic ratio of ^{35}Cl and ^{37}Cl are about 3:1 in nature, while peaks at *m/z* 157.0050 and 159.0020 are 3:1 in Fig. 1b, so we speculate that the product contains a chlorine atom. With consideration of the LC–MS and LC–MS/MS spectrometry, the product was identified as 4CBA.

Biodegradation Characteristics of Consortium of Strains CB-3 and CD-2

During the early period of the process of 4CBP degradation, the concentration of 4CBP was decreasing slowly, and 4CBA accumulated constantly. In the later period of the process, 4CBP and 4CBA were degraded rapidly, which indicated that degradation of 4CBP and 4CBA may be induced by substrates (Fig. 2a). When drawing degradation curves by sampling at intervals, for strain CB-3 after 12 h of incubation, 50 mg/L 4CBP was completely degraded and the culture OD_{600} had increased from 0.08 to 0.12 (Fig. 2b). For strain CD-2 after 14 h of incubation, 100 mg/L 4CBA was completely degraded and the culture OD_{600} had increased from 0.08 to 0.14 (Fig. 2c).

In addition, a series of substrate analogues were used to detect the degradation ability of two strains. Only PCBs within three chlorine atoms could be transformed by strain CB-3; for this phenomenon, metabolic intermediates may have higher cytotoxicity, like (polychloro) 2,3-dihydro-2,3-dihydroxybiphenyl and (polychloro) 2,3-dihydroxybiphenyl [6]. Strain CB-3 could also degrade 4-hydroxybiphenyl and diphenylmethane; it proved that the enzymes of the biphenyl catabolic pathway are very versatile (Table 1) [23]. Strain CD-2 not only degraded 4CBA but also 4-iodobenzoate and 4-bromobenzoate, and other structural analogues, such as benzoate, 4-hydroxybenzoate, could not degrade salicylate, gentisate and 4-aminobenzoate (Table 2).

Effect of pH, Temperature and Concentration of Substrate on 4CBP Degradation by Strain CB-3 and 4CBA Degradation by Strain CD-2

The ability of strains to degrade pesticides is influenced by different environmental conditions. The result showed that the optimum temperature for 4CBP degradation was determined to be 30 °C. It's worth noting that the ability on the degradation of strain CB-3 was lost quickly when the temperature exceeded 37 °C. The maximum degradation rate was observed at pH 7.0, and strain CB-3 had a high-efficiency degradation on partial alkalinity condition (pH values of 8.0–10.0). As the 4CBP concentration increased consistently, the degradation rate of strain CB-3 declined continuously. The degradation rate of strain CB-3 was only 18.3% when the concentration of 4CBP reached 200 mg/L (Fig. S3).

For strain CD-2, the optimum temperature for 4CBA degradation was determined to be 30 °C, and the weaker degradation rate at high temperature. Different from strain CB-3, strain CD-2 was more resistant to acidic environments, but the optimum pH values were 7.0 yet. The result showed that the strain CD-2 could degrade 100 mg/L 4CBA, but the degradation was inhibited when the concentration of the substrate exceeded 200 mg/L (Fig. S4), indicating that a high-concentration 4CBA probably has intensive cytotoxicity.

Prediction of 4CBP Catabolic Pathways and Related Gene

The draught genome sequence of strain CB-3 contains 49 scaffolds (> 500 bp), constituting a total size of 4.31 MB

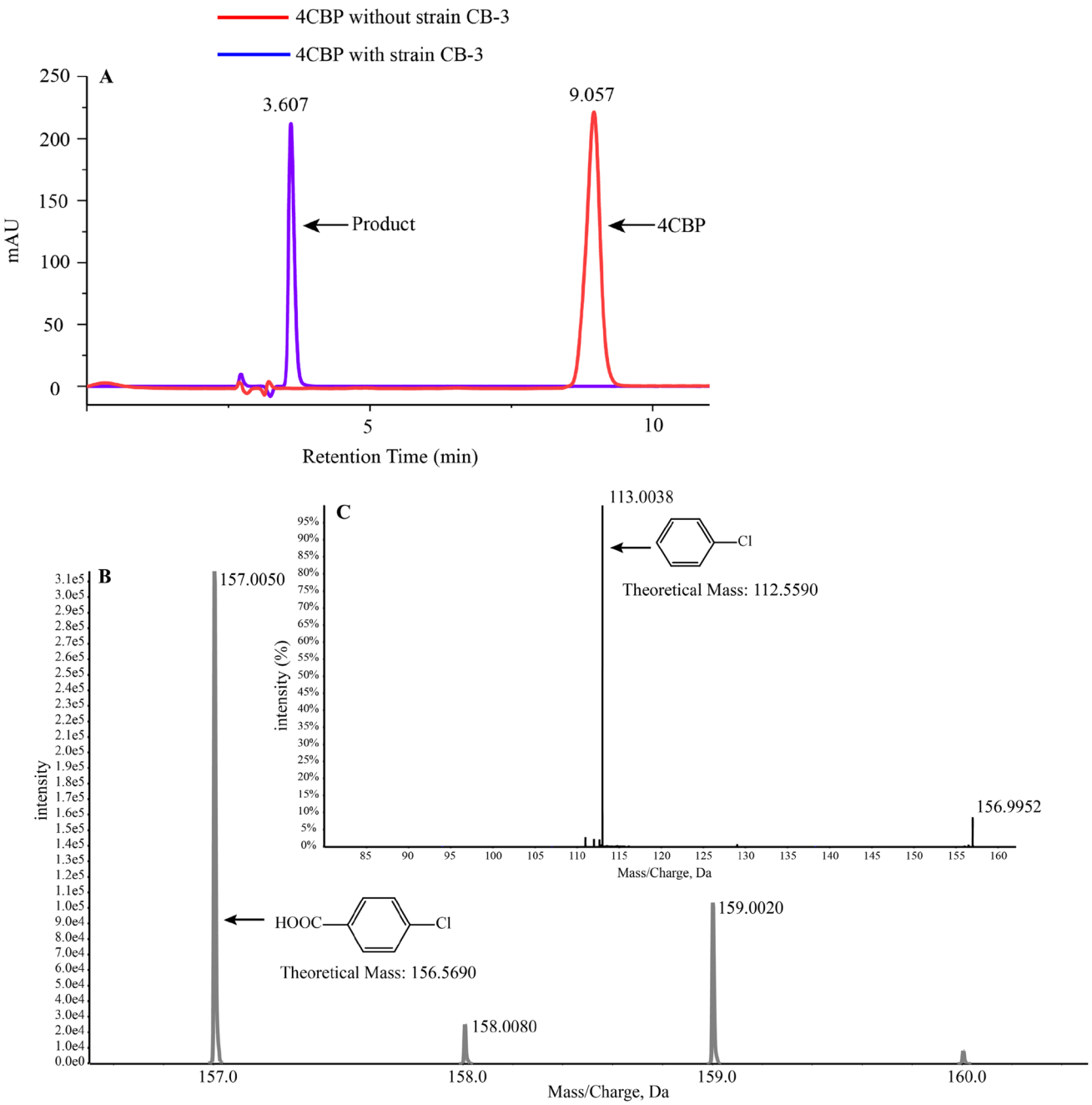


Fig. 1 HPLC-MS/MS analysis of the metabolite of 4CBP degradation by strain CB-3. **a** HPLC analysis of the degradation product, **b** MS analysis of degradation product and **c** MS/MS analysis of degradation product

(4073 ORFs), of which the G + C content is 64.24 mol%. The draught genome sequence of strain CD-2 contains 51 scaffolds (> 1000 bp), constituting a total size of 4.83 MB (4489 ORFs), of which the G + C content is 64.95 mol%.

Based on the annotations of all genes in strain CB-3 using RAST, a gene cluster of biphenyl upstream metabolic consisting of *ORF1179* to *ORF1167* was considered to be involved in 4CBP catabolism. Based on using BLASTP, the *bph* gene cluster in strain CB-3 shows the highest identities (at the amino acid sequence level) to strain LB400 (Table. S1). In this gene cluster, except the upstream metabolism gene of biphenyl (*bphA1A2A3A4BCD*), while contained, *bphX0* encodes a glutathione S-transferase and *bphX1X2X3* encodes a metabolisation of enzymes system of 2-hydroxypenta-2,4-dienoate; *bphX1X2X3* is an enzyme required for conversion of the aliphatic end product of *bphABCD*-encoded catabolism of biphenyls to Krebs cycle intermediates [17]. This could explain why strain CB-3

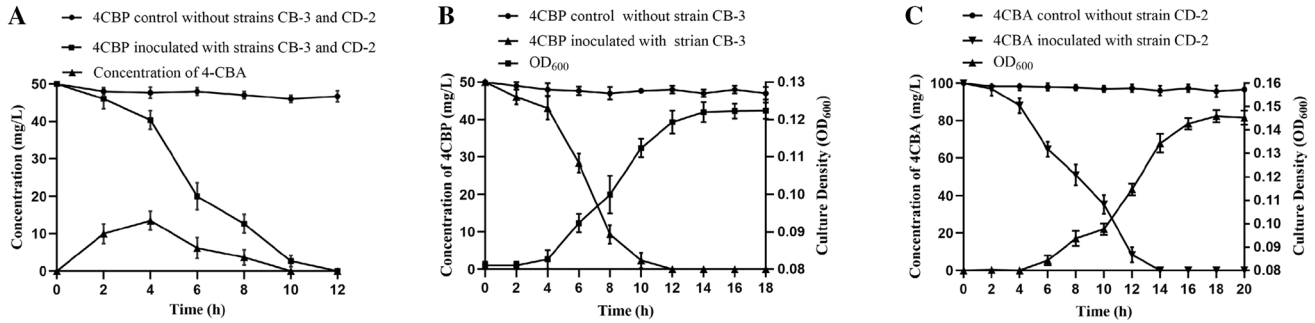


Fig. 2 4CBP and 4CBA degradation and cell growth of strains CB-3 and CD-2. **a** Degradation of 4CBP in MSM by co-culture of *Pseudomonas* sp. strain CB-3 and *Comamonas* sp. strain CD-2. **b** Degradation of 4CBP in MSM by *Pseudomonas* sp. strain CB-3. **c** Degradation of 4CBA in MSM by *Comamonas* sp. strain CD-2

cannot completely degrade 4CBP but can utilise 4CBP as the sole carbon source for growth.

In the degradation of halogenated aromatics, the key step is the removal of the halogen substituent. In general, there are two pathways for halogenated aromatics degradation: one involving the initial direct removal of the halogen substituent from the aromatic ring via reductive, hydrolytic or oxygenolytic mechanisms and the other involving removal of the halogen substituent following ring cleavage [15]. The six ORFs which were found in the genome of strain CD-2 were considered to be responsible for the dechlorination of 4CBA. The gene cluster of dechlorination has 100% identities with *fcbBAT1T2T3C* in *Comamonas* sp. DJ-12 [8], in which *fcbT1T2T3* are three transport proteins. No relevant dechlorination genes were retrieved in strain CB-3, and this may explain why strain CB-3 cannot completely degrade 4CBP. Furthermore, multiple 4-hydroxybenzoate 3-monooxygenase genes and a PCA metabolic gene cluster *pmcTEFDABC* also exist in the genome of strain CD-2. The PCA metabolic gene cluster destroys PCA via PCA 4,5-cleavage pathway, and this was reported in *Comamonas* sp. DJ-12 [19]. Based on the above-described information, the consortium of two strains' degrading pathway was speculated as follows: 4CBP degrading was initiated by a gene cluster of *bphABCD* in strain CB-3 to accumulate 4CBA, which was destroyed by strain CD-2 via the action of dechlorination and further degraded by PCA metabolic gene cluster.

Bioremediation of 4CBP-Polluted Soil

The rate of degradation was most rapid in the first 15 days, which resulted in 80.5% removal of 4CBP and a 4CBP half-time of 7.5 days in the bioremediation system (Fig. 3). What we need to pay attention to is that 4CBP reduced slightly in non-sterile soil compared to sterile soil, and this means that 4CBP may be degraded by indigenous microorganisms slowly. Some articles reported that bioremediation in soil is influenced by indigenous microflora [4], but there has not been a significant difference in non-sterile and sterile soil in this study. 4CBP in soil cannot be completely removed since pollutants are sorbed by solids [35]. 4CBA cannot be detected in the experiment of soil degradation, proving that 4CBA is degraded rapidly, as soon as it is produced.

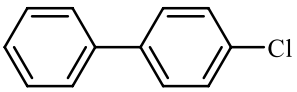
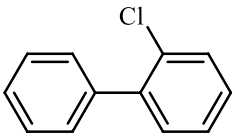
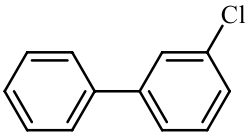
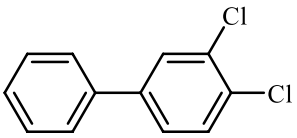
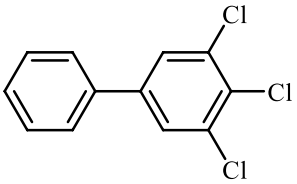
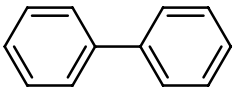
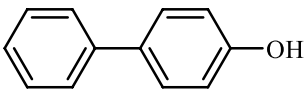
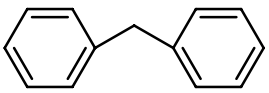
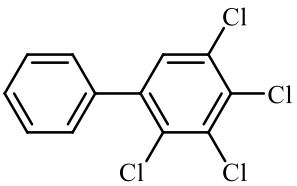
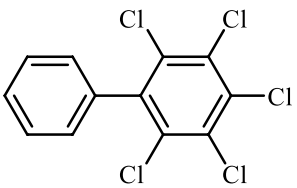
The optimum temperature for 4CBP degradation in soil was determined to be 30 °C. The degradation efficiency showed a downward trend as the amount of 4CBP increased. As the inoculation amount of strain CB-3 increased, the degradation efficiency also improved (Fig. S5).

Hitherto, many reported PCB-degrading strains were applied to the bioremediation of soil. This includes the consortium of genetically modified strains *Burkholderia xenovorans* LB400 (*ohb*) and *Rhodococcus jostii* RHA1 (*fcb*) which degraded a 57% mixture of Aroclor in soil at 30 days [25], the bioaugmentation system of *Rhodococcus ruber* P25 and *Microbacterium* sp. B51 which degraded 72.2% PCBs at 30 days and 96.4% at 90 days [9] and the consortium of the strains *Arthribacter* sp. B1B and *Ralstonia eutrophus* H850 which degraded 55–59% PCBs over 126 days [30]. Compared with above bioremediation system, the consortium of strains CB-3 and CD-2 is provided with a shorter remediation period to remove almost 4CBP-contaminated soil, thereby achieving soil purification.

Conclusion

In this study, strains CB-3 and CD-2 were isolated from the soil of a chemical factory and identified as members of the genera *Pseudomonas* and *Comamonas*, respectively. Strains CB-3 and CD-2 degraded 4CBP via synergistic action with high efficiency. Functional genes were annotated in the genome of strains CB-3 and CD-2 via RAST online service, and complete biphenyl metabolic gene clusters *bphABCD* and *benABCD* were found in genome of strain CB-3. We also found a dechlorination gene cluster *fcbBAT1T2T3C* and a PCA metabolic gene cluster *pmcABCDEFT* in the genome

Table 1 Degradation substrate spectra of strain CB-3

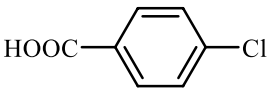
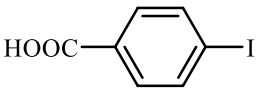
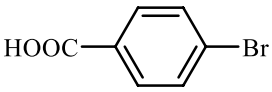
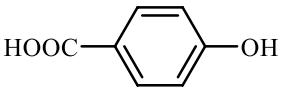
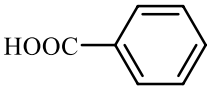
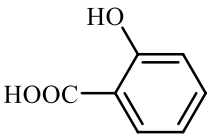
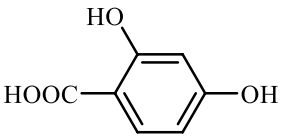
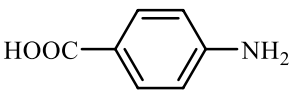
Substrate	Structural formula	Ability of degradation[a]
4-Chlorobiphenyl		+
2-Chlorobiphenyl		+
3-Chlorobiphenyl		+
3,4-Dichlorobiphenyl		+
3,4,5-Trichlorobiphenyl		+
Biphenyl		+
4-Hydroxybiphenyl		+
Diphenylmethane		+
2,3,4,5-Tetrachlorobiphenyl		ND[b]
2,3,4,5,6-Pentachlorobiphenyl		ND[b]

[a]"+" represents this substrate could be degraded

[b]Not detectable

of strain CD-2. It can be considered that the genes of degradation were derived from the horizontal gene transfer between bacteria due to the relatively high identities with reported genes. The bioremediation of 4CBP by consortium of strains CB-3 and CD-2 was tested in the laboratory, and residual contents of 4CBP in experiment soil, 30 days after the experiment, amounted to below 10%. To summarise, strains CB-3 and CD-2 degraded most of the 4CBP in a

Table 2 Degradation substrate spectra of strain CD-2

Substrate	Structural formula	Ability of degradation[a]
4-Hydroxybenzoate		+
4-Iodobenzoate		+
4-Bromobenzoate		+
4-Hydroxybenzoate		+
Benzoate		+
Salicylate		ND[b]
Gentisate		ND[b]
4-Aminobenzoate		ND[b]

[a]"+" represents this substrate could be degraded

[b]Not detectable

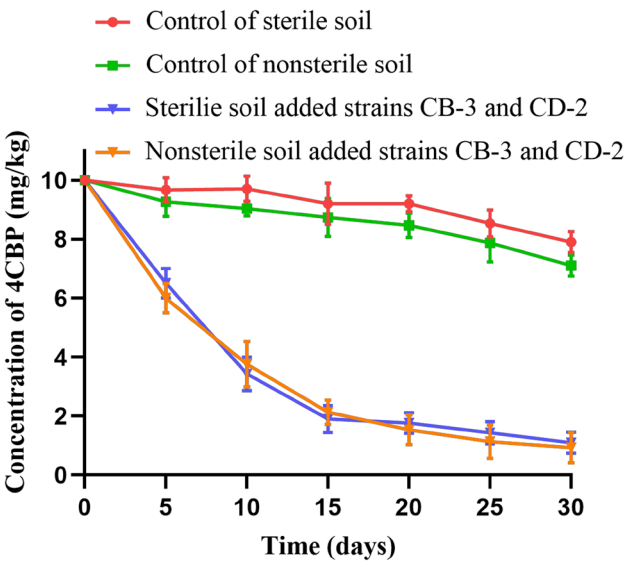


Fig. 3 Biodegradation of 4CBP by *Pseudomonas* sp. strain CB-3 and *Comamonas* sp. strain CD-2 in sterile soil and non-sterile soil

short period and no remain of chlorobenzoate, which proved the potential for using the two strains for bioremediation processes in 4CBP-contaminated soil.

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Compliance with Ethical Standards

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

Research Involving Human Participants and/or Animals This article does not contain any studies with human participants or animals performed by any of the authors.

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