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Chemotactic screening of imidazolinone-degrading bacteria by microfluidic SlipChip

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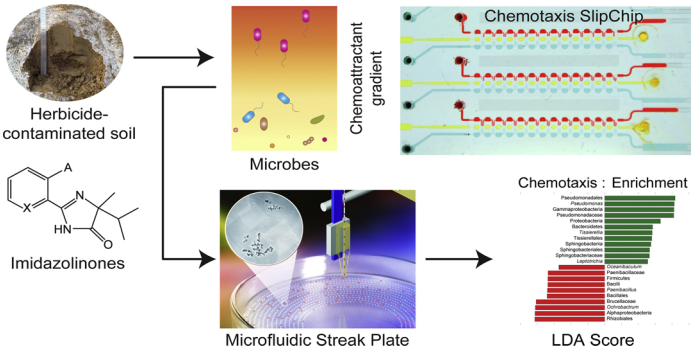
GRAPHICAL ABSTRACT



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ABSTRACT

The group of imidazolinone herbicides, widely used for weed control, is hazardous to some sensitive rotational crops. Thus, rapid elimination of imidazolinones from contaminated soil is significant for the environment. Biodegradation studies have demonstrated the ability of chemotaxis to enhance the biodegradation of pollutants. In this study, we used our newly developed SlipChip device for chemotactic sorting and a microfluidic streak plate device for bacterial cultivation as a new pipeline for screening imidazolinone degraders. The degradation efficiencies of an enrichment consortium and a chemotaxis consortium were determined by HPLC-MS/MS. Both consortia degraded all tested imidazolinones, with the highest efficiency (71.8%) for imazethapyr, and the chemotaxis consortium degraded these compounds approximately 10% more efficiently than the enrichment consortium. Moreover, the community diversities of the enrichment consortium and the chemotaxis consortium were analyzed by 16S rRNA gene amplicon sequencing. The results indicated that members of genus *Ochrobactrum* primarily contribute to the degradation of imidazolinones. This work proved that chemotaxis toward biodegradable pollutants increases their bioavailability and enhances the biodegradation rate. It also provided a new way to screen effective pollutant degraders and can be applied for the selective isolation of other chemotactic species from environmental samples.

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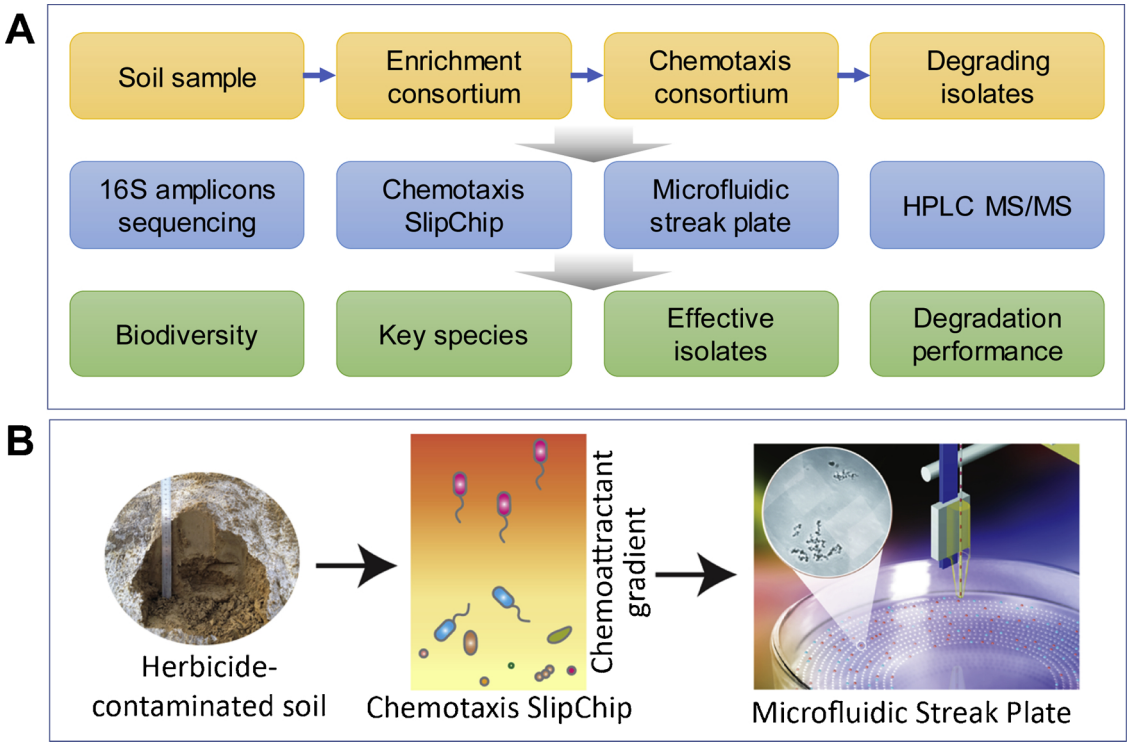

Fig. 1. Chemotactic screening of imidazolinone-degrading bacteria. (A) Pipeline of this study in brief including methods used and prospective outputs. (B) Increasing screening efficiency using consortia-level separation by chemotaxis SlipChip and single-cell isolation by microfluidic streak plate.

1. Introduction

The excessive use of synthetic herbicides for weed control in agriculture has caused serious environmental effects due to their toxicity and persistence in the soil [1–3]. Among them, imidazolinones are a class of herbicides that control a broad spectrum of weeds and are used throughout the world on legumes, cereals, forests, and plantation crops [4]. These herbicides are widely used for their low-dose usage and high efficacy, but they also show high soil persistence with half-lives ranging from 20 to 180 days depending on soil conditions (moisture, pH, organic matter) and soil types [5,6], and concentrations varied from 5.3 to 293.5 μg/kg [7,8], which would have carryover effect on subsequent rotational crops [9]. Therefore, rapid elimination of imidazolinones from the contaminated soil is of great significance for the environment and ecological systems.

Microorganisms play a major role in the biodegradation of various herbicides. The separation and identification of degrading bacteria as well as understanding the underlying biological process is of great importance and the basis for the development of effective inoculants for bioremediation. To isolate herbicide-degrading bacteria, enrichment cultures of samples from contaminated sites are generally utilized, and cultivation on agar plates is used to obtain pure isolates. Without exception, imidazolinones are also primarily degraded by microbial activities and to a lesser extent through hydrolysis and photodegradation [10,11]. Only a few bacterial species that can degrade imazaquin [12], imazethapyr [13] or imazamox [14] have been reported. Given the structural diversity of imidazolinones, bacterial species that can degrade a broad spectrum of imidazolinones are highly important. However, strains or microbial consortia that can simultaneously degrade a variety of imidazolinones have rarely been reported.

Many factors influence the biodegradation rates of pollutants, and sufficient contact of microbes with pollutants is an important one. Bacterial chemotaxis can bring motile and chemotactic microorganisms toward biodegradable pollutants and thus increase their bioavailability and enhance biodegradation rates [15–17]. This behavior has been demonstrated by the biodegradation of herbicide 2,4-dichlorophenoxyacetate by *Ralstonia eutropha* JMP134(pJP4) [18], cyclic nitramine explosives by *Clostridium* sp. EDB2 [19], naphthalene degradation by *Pseudomonas putida* G7 [17,20], and mass transfer of polycyclic aromatic hydrocarbons by *Tetrahymena pyriformis* [21]. Thus, chemotaxis-enhanced biodegradation studies afford a more targeted strategy for exploiting pollutant-degrading microbial resources.

Conventionally, bacterial chemotaxis is analyzed by swarm plate assays and capillary assays, but these tests are mostly qualitative and semiquantitative assays and time consuming. Over the past few years, microfluidic technologies have been rapidly developed and widely utilized in biological studies [22], including in bacterial chemotaxis studies [23–29]. Microfluidic-based chemotaxis systems can generate accurately controlled gradients and enable the dynamic observation and quantitative study of bacterial chemotaxis [25]. In 2014, we developed an instrument-free microfluidic SlipChip device [30] that can perform diffusion-based chemotaxis assays and selectively separate chemotactic species from a mixture [31]. Recently, this SlipChip technique has been applied to quantitatively measure the chemotaxis of rhizobacteria *Bacillus amyloliquefaciens* SQR9 toward 99 root-exudate compounds to systematically identify chemoattractants and their sensing chemoreceptors [32]. Importantly, the SlipChip device allows the simple harvesting of chemotactic bacteria by a manual pipettor, which opens up new opportunities for the enrichment of functional species from microbial communities in the environment.

Furthermore, conventional isolation and cultivation of degrading species on agar plates is laborious, costly and not efficient. The recent development of droplet-based microfluidics provides a high-throughput platform for bacterial cultivation and sorting [33–39]. Compartmentalization of cell(s) in droplets may reduce cross contamination and is suitable for the recovery of rare species and slow-growing species in long-term incubation [37,40].

In this study, we developed a new pipeline to screen imidazolinone-degrading consortia with high throughput using microfluidic techniques (Fig. 1A). Two newly developed techniques were used to increase

the efficiency of the screening process: one is a chemotactic sorting (ChemoSort) SlipChip device (Fig. 1B & C) [31], and the other is the high-throughput microfluidic streak plate (MSP) technique (Fig. 1B), which compartmentalizes single cells for growth and analysis in nanoliter sessile droplets [41]. The enrichment consortium was derived from soil samples, and the chemotaxis consortium was sorted from the enrichment consortium by SlipChip. The efficiencies of imidazolinone degradation by both consortia and isolated strains were determined. They could degrade all six imidazolinones with efficiencies varying from 23.8% to 71.8% in 7 days. Moreover, the microbial community composition and diversity of both consortia were analyzed by 16S rRNA gene amplicon sequencing. The combination of this data with an analysis of microbial abundance and differential genera between enrichment consortium and chemotaxis consortium identified consortium members that contribute to the increased efficiency of imidazolinone degradation.

2. Materials and methods

2.1. Chemicals and media

All chemicals and reagents were purchased from commercial sources (Text S1). Stock solutions of six imidazolinones, including imazapyr, imazamox, imazapic, imazamethabenz-methyl, imazethapyr, and imazaquin were prepared separately with methanol at 5 mg/mL. Mineral salts medium (MSM, pH 7.0) [42] with the addition of all six imidazolinones (each at 50 mg/L) as the sole carbon and energy source was used for screening and growth. Luria-Bertani (LB) agar plates were applied for the isolation of single clones, and LB broth was used for the cultivation of *Escherichia coli* and isolated pure clones.

2.2. Preliminary tests of chemotaxis-based screening system

Preliminary tests were conducted to test the effectiveness of the screening system. Several strains described below were selected to prepare a mixed microbial suspension that included species with different chemotaxis and degradation abilities to confirm the effectiveness of the chemotaxis-based screening system. *Comamonas testosteroni* CNB-1, which has the ability to degrade and chemotaxis toward benzoate; CNB-1Δ20, which lacks the chemotaxis ability but still can degrade benzoate [43]; and *E. coli* RP437 (pDsRed.T4), which can neither degrade nor show chemotaxis toward benzoate and thus acts as a noise strain, were used. Chemotaxis assays were conducted separately as described previously [31]. In these assays, benzoate was chemoeffector, cell suspension was loaded into a SlipChip, and the migration of cells in the presence of a benzoate gradient was monitored under an inverted fluorescence microscope (Ti-Eclipse, Nikon, Japan). The number of cells in benzoate microwells and phosphate-buffered saline (PBS) microwells was counted individually, and the chemotaxis index was calculated.

2.3. Imidazolinone determination in soil and liquid culture

The determination of imidazolinones in soil samples was performed as described by Kemmerich et al. [7]. Briefly, 5 g of soil in a 50-mL polypropylene tube was extracted with 10 mL of 0.5 M ammonium acetate solution. The tube was shaken for 1 min and centrifuged for 5 min at 3400 rpm. Then, 2 mL of the extract was transferred to a 15-mL polypropylene tube containing 125 mg of primary secondary amine sorbent (40–60 μm, Agilent, USA), shaken for 1 min and centrifuged for 5 min at 3500 rpm. The extract was filtered with a 0.22-μm nylon filter, and the pH was adjusted to 3.0 with 6 M HCl. Prior to injection into the high-performance liquid chromatography (HPLC)-mass spectrometry (MS)/MS system, the extract was diluted 5× with ddH_2O.

For liquid culture, samples (10 mL) were adjusted to a pH of 2–3 with 88% formic acid and centrifuged for 5 min at 12,000 rpm; the supernatants were transferred to a new polypropylene tube and then extracted three times each with 10, 10, and 5 mL of dichloromethane. The extracts were pooled, dried over anhydrous sodium sulfate and concentrated to dryness with a rotary evaporator. The final residue was redissolved in 1 mL of methanol and analyzed by HPLC-MS/MS as described in Text S2.

2.4. Soil sampling, enrichment, and ChemoSorting

Three imidazolinone-contaminated soil samples (5–10 cm from the surface) were collected from an abandoned site of a chemical factory in Jing County, Hebei Province, China (see sampling sites and geographical information in Fig. S1). The soil samples were mixed thoroughly to obtain a composite soil sample for the following experiments. Ten-gram soil samples were added to 50 mL of saline solution to prepare soil suspensions. Then, 10 mL of soil suspension was transferred to 100 mL of MSM supplemented with imidazolinones (50 mg/L, individually) and incubated at 30 °C on a rotary shaker (150 rpm). Ten percent of the culture were transferred to fresh MSM-imidazolinone broth every week. The enrichment was maintained for one month prior to further ChemoSorting. The consortium obtained by enrichment was designated the enrichment consortium.

ChemoSorting was performed on a SlipChip with modified design (Fig. 1C) and was the same as described previously [31]. We applied 200 mg/L imidazolinone mixture as a chemoeffector; after chemotaxis for 30 min, chemotactic cells, which had reached the chemoeffector microwells, were collected from the device by pipette in clean bench. The collected cells were transferred to 100 mL of MSM supplemented with imidazolinones (100 mg/L, individually) and incubated as described above. The obtained consortium was designated the chemotaxis consortium.

2.5. MSP cultivation of imidazolinone-degrading bacteria

The enrichment consortium and chemotaxis consortium were cultured with imidazolinones for approximately 2 days at 30 °C to the postexponential growth phase and diluted to a concentration of approximately 3–5 × 10^4 cells/mL, then loaded into a gastight syringe as samples. The MSP cultivation was conducted as described by Jiang et al. [41]. Approximately 4000 droplets generated from 30 μL of cell suspension were written onto the surfaces of polystyrene Petri dishes (9-cm in diameter) pretreated with 3-aminopropyl triethoxysilane (APTES) silanization and filled with mineral oil. Then, the plates were incubated at 30 °C. Droplets containing growing cells were picked individually into 96-well plates preloaded with 200 μL of MSM-imidazolinone broth. After another 2 days of incubation at 30 °C monitored with an EnSpire Multimode Plate Reader (PerkinElmer, USA), the culture solutions were diluted, and 200-μL diluents of each well were spread onto MSM-imidazolinone agar plates to acquire pure clones.

2.6. Identification of isolated strains

The isolated pure single clones were identified by 16S rRNA gene sequencing. Universal primers 27 F (5′-AGAGTTTGATCCTGGCT CAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) were used to amplify the bacterial 16S rRNA gene by PCR. The products were purified and sequenced. The 16S rRNA gene sequences were deposited into GenBank databases, compared to known GenBank sequences using BLAST [44] and compared to known sequences on the EzBioCloud's database (http://www.ezbiocloud.net/) using the Identify service [45].

2.7. Degradation of imidazolinones by microbial consortia and isolated strains

The enrichment consortium, the chemotaxis consortium and isolated strains grown in LB broth were centrifuged at 6000 ×*g* for 10 min. The cell pellets were washed twice and suspended in MSM. The optical

density at 600 nm (OD_{600}) of the cell suspension was measured. In addition, samples of MSM medium with 6 imidazolinones at a final concentration of 50 mg/L were then inoculated with individual cell suspensions. The initial OD_{600} of cultures were adjusted to approximately 0.02. Cultures were incubated at 30 °C on a rotary shaker at 100 rpm. Each experiment was performed in triplicate, and the control experiment, without inoculation, was carried out under the same conditions.

Aliquots (10 mL) were collected at days 0, 1, 2, 4, and 7 for residue analysis. Sample preparation of liquid culture was performed by the method mentioned above in section 2.3, and the residue concentration was likewise determined. The efficiencies of imidazolinone degradation by consortia and strains was calculated, see details in Text S2.

2.8. 16S rRNA gene amplicon sequencing

Total genome DNA was extracted from samples with the E.Z.N.A. Mag-Bind Soil DNA Kit (Omega Bio-Tek, GA, USA). The V3 and V4 regions of the 16S rRNA genes were PCR-amplified with the 341 F (5′ – CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) primers [46] with barcodes. The PCR products were used to construct DNA libraries and sequenced on an Illumina HiSeq2500 platform by the PE250 protocol. Details about amplicon processing are provided in Text S3. To detect significant differences in relative abundances between the chemotaxis consortium and the enrichment consortium, we used linear discriminant analysis (LDA) effect size (LEfSe) to identify metagenomic biomarker(s) [47]. The abundances in LEfSe were normalized using the '-o 1000000' flag, and the threshold on the logarithmic LDA score for discriminative features was set to 3.0 (using '-l 3.0').

3. Results and discussion

3.1. Validation of ChemoSorting on SlipChip

To confirm the effectiveness of the chemotaxis-based screening system, we performed a control experiment using *C. testosteroni* CNB-1 and benzoate (as chemoattractant). After chemotaxis for 30 min, the chemotaxis index (I_{30}, in the range from 0% to 100%) [31] of each strain was calculated by counting the bacteria entering the top and bottom microwells. A nonchemotactic or nonmotile strain will show an I_{30} close to 50%, while a greater I_{30} value represents positive chemotaxis and a lower I_{30} value represents a repellent response. As shown in Fig. 2A, when the concentration gradient of benzoate was generated, 195 ± 20 CNB-1 cells migrated toward benzoate, and 7 ± 1 cells migrated toward buffer, showing strong chemotaxis to benzoate, with a high chemotaxis index of 96.3 ± 0.5% (Fig. 2B). The mutant CNB-1Δ20 cells in both microwells were equivalent (21 ± 5 cells in benzoate microwells *vs.* 23 ± 4 cells in buffer microwells), showing a nonchemotactic response to benzoate and a chemotaxis index of 46.8 ± 2.2% (Fig. 2B). These results mentioned above were consistent with those previously reported by Ni, *et al.* [43] and Huang, *et al.* [48] *E. coli* RP437 showed a behavior similar to that of CNB-1Δ20, and the cell number in buffer was much greater than that of CNB-1Δ20 cells, indicating a repellent response as the chemotaxis index was only 35.9%, lower than 50%. As the results described above, the SlipChip can separate and screen out the chemotactic bacteria based on the I_{30} values of different species. The majority of cells collected by SlipChip were chemotactic bacteria. In addition, the noise bacteria were filtered out by the repellent response or by the enrichment of degradative species. Therefore, the chemotaxis-based screening system can be applied for the screening of chemotactic imidazolinone-degrading species.

3.2. The community diversity of imidazolinone-degrading bacteria

To ensure that bacterial consortia and strains that effectively degrade imidazolinones were obtained, we first analyzed the concentration of imidazolinones in original soil samples. Three soil samples were mixed, and the concentrations of imidazolinones were determined by HPLC-MS/MS as described in Section 2.3: imazapyr, 25.6 μg/kg soil; imazamethabenz-methyl, 0.945 μg/kg soil; imazethapyr, 4.56 μg/kg soil; and imazaquin, 0.728 μg/kg soil. Next, the degradative species from the soil sample were enriched in MSM broth with 100 mg/L imidazolinones individually. Then, the enrichment consortium was sorted on a SlipChip by the chemotactic response to a mixture of imidazolinones as described above. Due to the limited number of cells we can obtain from a SlipChip, we designed a multichannel SlipChip that has a higher processing throughput and performed a brief scale-up cultivation after ChemoSorting. The community structure of soil samples, the enrichment consortium and the chemotaxis consortium were investigated via 16S rRNA gene amplicon sequencing.

Microbial compositions were compared and visualized using principal coordinates analysis (PCoA) of weighted UniFrac distances, which revealed a distinct grouped clustering of the enrichment consortium

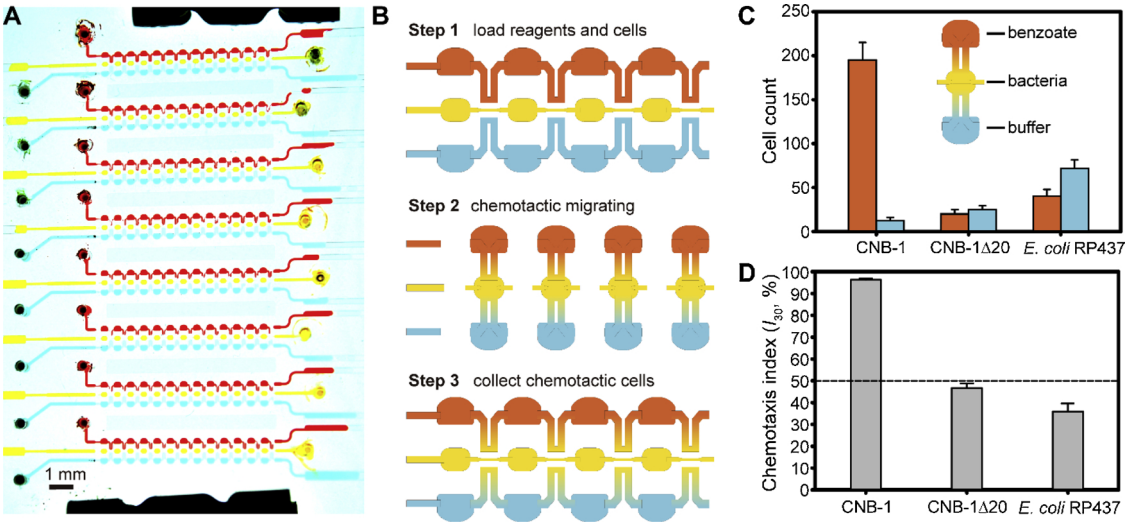

Fig. 2. Validation of chemotaxis-based screening system. (A–B) Picture and schematics of operation of an 8-channel Chemotaxis SlipChip. Scale bar: 1 mm. (C) Bar graphs representing cell numbers of strains in benzoate microwells and corresponding cell numbers in buffer microwells. (D) The chemotaxis index of bacterial strains responding to benzoate.

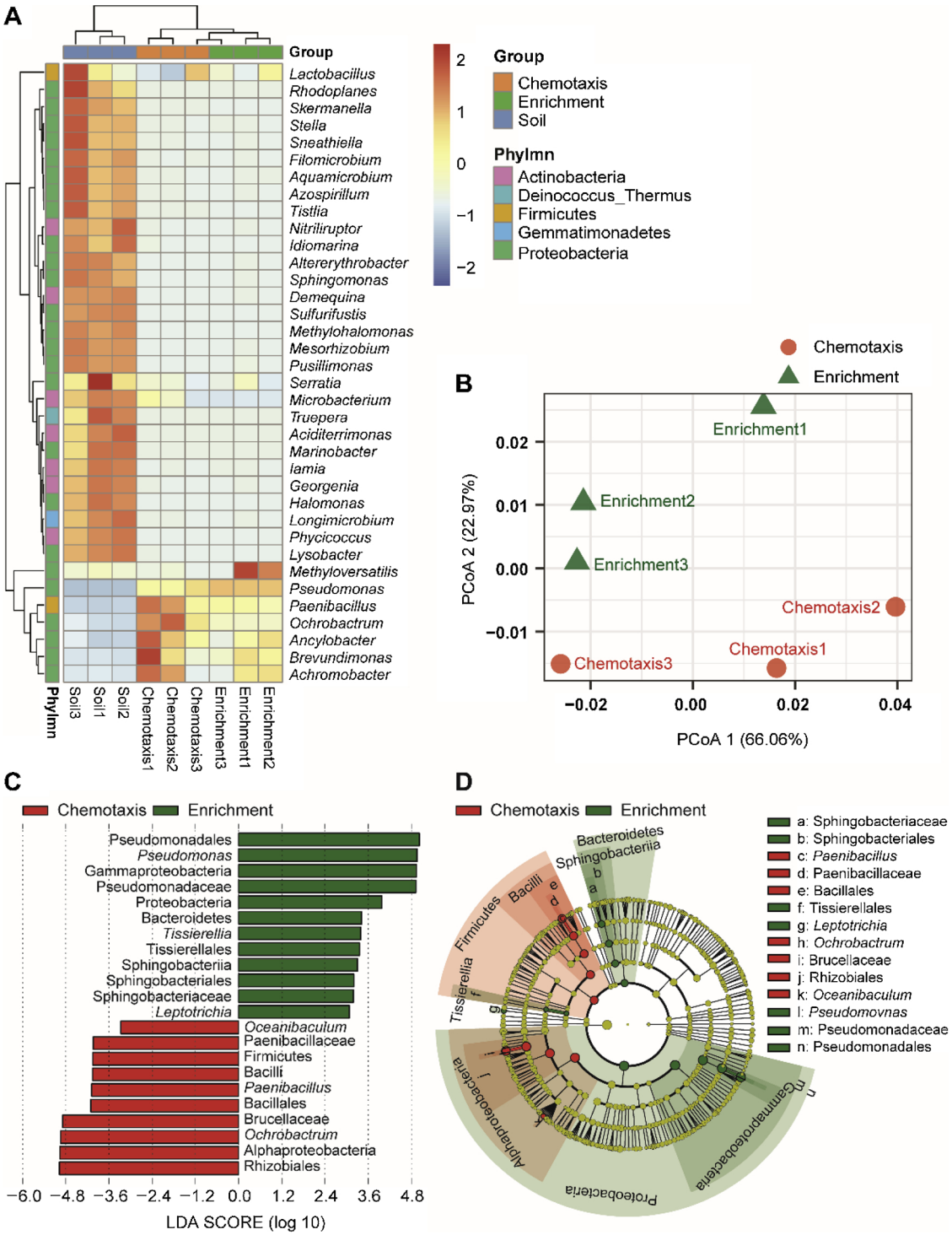

Fig. 3. Comparison of the microbial diversity and abundance of the enrichment consortium and the chemotaxis consortium. (A) Hierarchical cluster analyses reflecting the major differences in relative abundances across the soil, enrichment consortium, and chemotaxis consortium (data filtered for those taxa with relative abundance > 0.1%). (B) Principal coordinates analysis (PCoA) of weighted UniFrac distances depicting the clusters of bacterial communities within enrichment and chemotaxis samples. (C–D) Bacterial taxa differentially abundant between the chemotaxis consortium and the enrichment consortium were analyzed by LEfSe (C), and their polygenetic distribution of bacterial lineages is shown in a cladogram (D).

and the chemotaxis consortium composition relative to the soil samples (Fig. 3A). Enrichment by imidazolinones greatly altered the composition of the soil microbial community. Although the chemotaxis consortium was derived from the enrichment consortium, the microbial composition of the chemotaxis consortium was different from that of the enrichment consortium (Fig. 3B). The relative abundance of bacterial genera revealed that 7 genera were significantly enriched in the enrichment consortium: *Methyloversatilis*, *Pseudomonas*, *Paenibacillus*, *Ochrobactrum*, *Ancylobacter*, *Brevundimonas*, and *Achromobacter* (Fig. 3A). Strikingly, all of these except *Pseudomonas* were not reported to degrade imidazolinones, and these bacterial genera highly enriched by imidazolinones are potentially new imidazolinone degraders [12–14]. LEfSe analysis revealed that the genera of *Ochrobactrum*, *Paenibacillus* and *Oceanibaculum* were relatively more abundant in the

chemotaxis consortium than the enrichment consortium (Fig. 3C and D), which suggested that members of these genera might contribute to the enhanced degradation of imidazolinones. The genera *Ochrobactrum* and *Pseudomonas*, with LDA scores higher than 5, were considered the most differentially abundant bacterial taxa that dominated the chemotaxis consortium and the enrichment consortium, respectively.

3.3. Cultivation and identification of isolated strains

To obtain the degrading species from the consortia with high efficiency, we used MSP to compartment the enrichment consortium and the chemotaxis consortium as single cells into sessile droplets grown on the surfaces of Petri dishes. Compartmentalization of microbial species in a community in nanoliter droplets by MSP greatly increases the effective concentration of rare species and decreases the time required to reach detection thresholds [41]. Droplets containing growing cells were picked individually into 96-well plates for further cultivation. After another 2 days of incubation at 30 °C, the culture solutions were diluted and spread onto MSM-imidazolinone agar plates for acquiring pure clones. The 16S rRNA genes of isolated clones were sequenced and analyzed. We obtained 156 species belonging to 15 genera according to phylogenetic analysis, of which *Pseudomonas*, *Stenotrophomonas*, and *Ochrobactrum* were the dominant genera. *Pseudomonas* degrades imidazolinones [13], but *Stenotrophomonas* and *Ochrobactrum* have rarely been recorded to have such activity.

3.4. Degradation of imidazolinones by consortia and strains

To determine and compare the efficiency of degrading a broad spectrum of imidazolinones, the mixture of six imidazolinones, each with a concentration of 50 mg/L, were added to the enrichment consortium, the chemotaxis consortium and selected strains as the sole carbon source, and these samples were grown for 7 days. Ten strains, belonging to *Achromobacter*, *Brevundimonas*, *Lysinibacillus*, *Microbacterium*, *Ochrobactrum*, *Pseudomonas*, *Rhizobium* and *Stenotrophomonas*, were selected based on their growth in MSM supplemented with imidazolinones (data not shown) for further determination of their efficiencies of imidazolinone degradation. At designed time intervals, sample aliquots were collected, and the concentrations of the herbicides were then determined by HPLC-MS/MS.

The analysis of the culture aliquot by HPLC-MS/MS revealed that the efficiency of imidazolinone degradation by the enrichment consortium ranged from 54.2% (imazaquin) to 61.9% (imazamethabenz-methyl) and that that by the chemotaxis consortium ranged from 64.9% (imazaquin) to 71.8% (imazethapyr) (Fig. 4 and Table 1). The chemotaxis consortium degraded imidazolinones approximately 10% more efficiently than the enrichment consortium. The difference in degradation efficiencies of chemotaxis consortium and enrichment consortium is consistent with previous studies that bacterial chemotaxis could increase biodegradation rates [15–17,20]. The reason behind this may be due to changes in the microbial structure and abundance of the chemotaxis consortium, although the chemotaxis consortium was derived from the enrichment consortium. The differentially enriched bacterial genera in the chemotaxis consortium including *Paenibacillus*, *Ochrobactrum*, *Ancylobacter*, *Brevundimonas*, and *Achromobacter* are all motile bacteria, their relative abundance was higher than that of the enrichment consortium. Motile and chemotactic microorganisms possess the advantage of chemotaxis to access degradable substrates quickly and function as active carrier for these substrates [21] to overcome mass transfer limitation and therefore enhance their bioavailability, and consequently increase the overall biodegradation rates. Meanwhile, the efficiencies of selected strains degrading imidazolinones differed greatly; *Lysinibacillus mangiferihumi* has the lowest degradation efficiency, degrading only 23.8% of the starting imazapyr. The degradation efficiencies of *Ochrobactrum anthropi*, *Microbacterium oxydans*, *Pseudomonas umsongensis* and *Stenotrophomonas maltophilia* were above 50%, and *Microbacterium oxydans* had the highest degradation efficiency (62.0%, imazamethabenz-methyl), approaching that of the enrichment consortium (Table 1).

The degrading efficiencies of strains we obtained were compared with those of reported imidazolinone-degrading bacteria. Wang et al. [12] isolated an *Arthrobacter crystallopoietes* strain that completely degrades imazaquin in 10 days at the concentration of 50 mg/L. Huang, et al. [13] reported a *Pseudomonas* sp. strain IM-4 that degrades approximately 73.4% of 50 mg/L imazethapyr within 7 days. A clinical bacterium, *Acinetobacter baumannii* IB5, was recently described to degrade 98.6% of 400 mg/L imazamox within 48 h [14]. However, strains or microbial consortia that can degrade all six imidazolinones have rarely been reported. In this study, the efficiencies of isolated strains degrading imidazolinones varied from 23.8% to 62.0%, which was slightly lower than previously reported degradation efficiencies, but the isolated strains can degrade a wider range of imidazolinones than those reported strains.

Species of the genus *Ochrobactrum* were suggested to play an important role in the degradation of imidazolinones according to the microbial community analysis (Fig. 3C and D); the degradation efficiencies of genus *Ochrobactrum* were not the highest among those selected strains, being slightly lower than those of *M. oxydans*, *P. umsongensis* and *S. maltophilia* (Fig. 4C and Table 1). *M. oxydans* is a member of the genus *Microbacterium* with the ability to degrade alginate and laminarin [49]. This report is the first indicating that *M. oxydans* degrades imidazolinones. Members of the genus *Pseudomonas* are well known for their capacity to degrade many environmental contaminants, including a range of herbicides [50]. *S. maltophilia* is a multiple-drug-resistant bacterium, but the antimicrobial resistance properties confer the ability to degrade pesticides to this species [51]. The genus *Ochrobactrum* comprises many opportunistic human pathogens but has potential applications in bioremediation. *O. anthropi* is the type species of the genus, which can degrade various aromatic hydrocarbons and pesticides [52]. *O. tritici* also showed bioremediation potential for pyrethroid pesticides [53]. Members of the genera studied in this work showed great efficiencies of imidazolinone degradation. We anticipate that they could serve as new microbial inoculants for herbicide degradation. Compared with isolated strains, microbial consortia have greater ability to degrade imidazolinones, which is consistent with known advantages of microbial consortia for the bioremediation of pollutants.

4. Conclusions

In this study, for the first time, a function-targeted chemotactic screening pipeline that combines a ChemoSort SlipChip and single-cell cultivation MSP techniques is developed and successfully applied in the enrichment and sorting of imidazolinone-degrading microbes. We obtained an enrichment consortium, a chemotaxis consortium and 156 strains, representing 15 genera, cultivated by MSP. Both consortia and selected strains degrade all six tested imidazolinones, with varied efficiency, within 7 days. The chemotaxis consortium degraded imidazolinones by ~70% in 7 days of cultivation. Although the chemotactic consortium only degrades ~10% more efficiently than the enrichment consortium, the chemotactic motility of the chemotaxis consortium may help it actively migrate toward the herbicides in the environment and greatly increase the overall degradation effect on large scales. Biodiversity analysis revealed that selective enrichment greatly changed the composition and structure of the soil microbial community. In addition, the genus *Ochrobactrum* was significantly enriched by a chemotaxis-based SlipChip and was considered to play an important role in imidazolinone degradation.

This study offers an effective solution to screen imidazolinone-degrading bacteria by taking advantage of their chemotactic characteristics. Correspondingly, it can also be applied for the selective enrichment and isolation of other chemotactic communities and species from

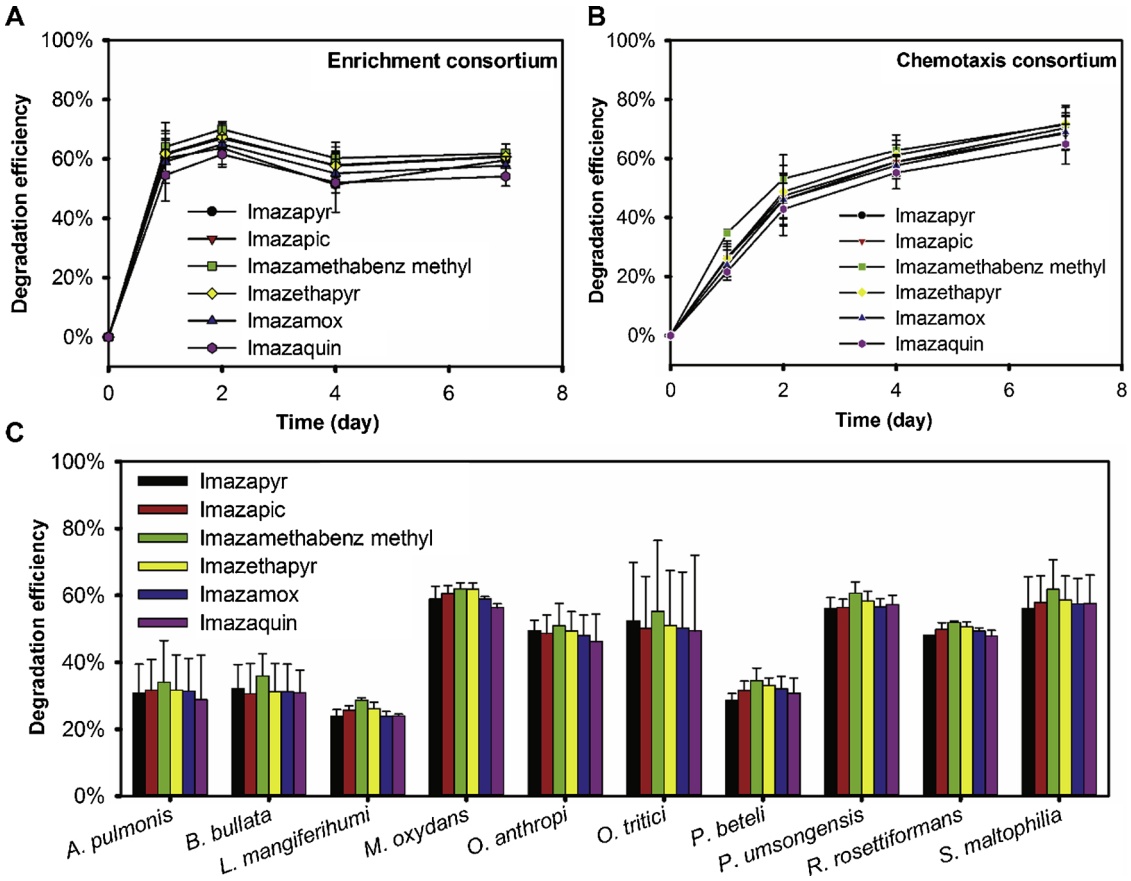

Fig. 4. Efficiency of imidazolinone degradation by the enrichment consortium (A), the chemotaxis consortium (B) and isolated strains (C).

Table 1
The efficiencies of microbial imidazolinone degradation[a].

Sample/Species	Imazapyr	Imazapic	Imazamethabenz-methyl	Imazethapyr	Imazamox	Imazaquin
Consortia						
Chemotaxis consortium	68.5 ± 5.6	70.4 ± 5.1	71.5 ± 6.0	71.8 ± 6.3	68.9 ± 5.7	64.9 ± 6.8
Enrichment consortium	59.6 ± 1.9	61.0 ± 1.9	61.9 ± 3.1	60.7 ± 1.1	57.8 ± 3.0	54.2 ± 3.3
Strains						
Achromobacter pulmonis[b]	30.7 ± 8.7	31.7 ± 9.2	34.0 ± 12.5	31.7 ± 10.5	31.4 ± 9.8	28.8 ± 13.3
Brevundimonas bullata[b]	32.2 ± 7.2	30.6 ± 9.0	35.9 ± 6.7	31.2 ± 8.4	31.2 ± 8.2	30.9 ± 6.8
Lysinibacillus mangiferihumi[c]	23.8 ± 2.0	25.6 ± 1.4	28.7 ± 0.7	26.1 ± 2.0	23.9 ± 1.4	24.0 ± 0.5
Microbacterium oxydans[c]	58.9 ± 3.9	60.6 ± 2.4	62.0 ± 1.8	61.8 ± 1.8	58. 9 ± 0.8	56.4 ± 1.1
Ochrobactrum anthropi[b]	49.4 ± 3.3	48.7 ± 5.4	50.9 ± 6.6	49.3 ± 5.8	48.0 ± 6.1	46.2 ± 8.2
Ochrobactrum tritici[b]	52.4 ± 17.5	50.2 ± 15.5	55.2 ± 21.2	51.1 ± 16.4	50.3 ± 16.8	49.4 ± 22.6
Pseudomonas beteli[b]	28.5 ± 2.2	31.6 ± 2.8	34.6 ± 3.7	33.1 ± 2.3	32.0 ± 3.7	30.8 ± 4.6
Pseudomonas umsongensis[c]	56.1 ± 3.2	56.4 ± 2.5	60.7 ± 3.3	58.3 ± 3.0	56.6 ± 2.5	57.3 ± 2.7
Rhizobium rosettiformans[c]	48.0 ± 0.1	49.8 ± 2.0	51.9 ± 0.4	50.6 ± 1.5	49.3 ± 1.0	47.9 ± 1.7
Stenotrophomonas maltophilia[b]	56.1 ± 9.5	57.9 ± 8.0	61.8 ± 8.8	58.6 ± 7.2	57.5 ± 7.6	57.6 ± 8.6

[a] Data in the table are presented as a percentage (%) and shown as the means ± SD.
[b] Isolated by ChemoSorting and microfluidic streak plate.
[c] Isolated by agar plate.

environmental and clinical communities or from host-associated microbiomes. When coupled with metagenomic analyses, this method will facilitate dissecting the ecological functions of chemotactic species in their original habitats.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2018.12.029.

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