J. Microbiol. Biotechnol. (2016), 26(5), 891–899
http://dx.doi.org/10.4014/jmb.1511.11070

jmb

Bioaugmentation with GFP-Tagged *Pseudomonas migulae* AN-1 in Aniline-Contaminated Aquifer Microcosms: Cellular Responses, Survival and Effect on Indigenous Bacterial Community S

Yongsheng Zhao[1], Dan Qu[1], Rui Zhou[1,2], Yunge Ma[1], Hao Wang[1], and Hejun Ren[1]*

[1]*Key Laboratory of Groundwater Resources and Environment of the Ministry of Education, College of Environment and Resources, Jilin University, Changchun 130021, P.R. China*
[2]*Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA94720, USA*

Received: November 26, 2015
Revised: January 18, 2016
Accepted: February 18, 2016

First published online
February 24, 2016

**Corresponding author*
Phone: +86-431-88502606;
Fax: +86-431-88502606;
E-mail: renhejun@126.com

Supplementary data for this paper are available on-line only at http://jmb.or.kr.

pISSN 1017-7825, eISSN 1738-8872

The recently isolated aniline-degrading bacterium *Pseudomonas migulae* AN-1 was tagged with green fluorescent protein (GFP) to investigate its bioaugmentation potential against aniline-contaminated groundwater through microcosm experiments. The survival and cellular response of GFP-tagged AN-1 introduced in a lab-scale aquifer corresponded directly with aniline consumption. During the process, the GFP-tagged AN-1 biomass increased from 7.52×10^5 cells/ml to 128×10^5 cells/ml and the degradation rate of aniline was 6.04 mg/l/h. GFP-tagged AN-1 was moderately hydrophobic (41.74%–47.69%) when treated with 20–100 mg/l aniline and exhibited relatively strong hydrophobicity (55.25%–65.78%) when the concentration of aniline was ≥100 mg/l. The membrane permeability of AN-1 increased followed by a rise in aniline below 100 mg/l and was invariable with aniline above 100 mg/l. Pyrosequencing analysis showed that the relative abundance of *Proteobacteria* (accounted for 99.22% in the non-bioaugmentation samples) changed to 89.23% after bioaugmentation with GFP-tagged AN-1. *Actinobacteria* increased from 0.29% to 2.01%, whereas the abundance of *Firmicutes* barely changed. These combined findings demonstrate the feasibility of removing aniline in aquifers by introducing the strain AN-1 and provide valuable information on the changes in the diversity of dominant populations during bioaugmentation.

Keywords: Aniline, bioaugmentation, cellular responses, GFP-tagged *Pseudomonas migulae* AN-1, survival

Introduction

Aniline has been widely used in the manufacturing of dyes, medicines, and pesticides and is commonly detected in groundwater [33]. As a result of its severely toxic, mutagenic, and persistent properties, aniline is considered an environmental hazard and listed as a prior pollutant by the USA and China. Therefore, the removal of aniline from contaminated aquifers is critically necessary [36, 38]

The remediation of aniline-contaminated soil and wastewater has been attempted using biological and abiological strategies [13, 32]. Bioaugmentation by using biodegradative activities of indigenous or introduced microorganisms has received increasing interest for in situ bioremediation of contaminated sites [34], because of its environmental benefits compared with physical and chemical methods such as adsorption [39] and oxidation [16], which have high capital and operating costs and production of secondary pollutants [2, 10].

However, studies have frequently observed that bioaugmentation is prone to failure because the activity and number of introduced microorganisms decrease shortly after adding them to the field site [12]. The survival of introduced microorganisms in complex ecosystems is regarded as key for a successful bioremediation [5, 35]. Numerous factors may make the survival of introduced

microorganisms poor and deteriorate their biodegradation ability. The main factor is the interaction between microorganisms and the toxic pollutants in the environment, which is generally associated with cellular responses, such as cell surface hydrophobicity (CSH) and cellular membrane permeability [11]. In addition, the fate of introduced microorganisms as exogenous agents depends on competition with indigenous microorganisms. Therefore, their effect and the risks they pose on indigenous microbial communities during the bioaugmentation process need to be evaluated.

We have recently characterized a novel strain, *Pseudomonas migulae* AN-1, which is a psychrotrophic bacterium that can degrade high concentrations of aniline at 10°C (up to 2,202 mg/l) [19]. The cold-adapted and high aniline-degrading capacities make this strain an excellent candidate for the bioremediation of aniline-contaminated groundwater. However, its usefulness during bioaugmentation processes remains unclear.

In this study, the aniline-degrading *P. migulae* AN-1 was tagged with green fluorescent protein (GFP) to analyze its survival and degradation activity in aquifer microcosms. Simultaneously, a pyrosequencing technique was employed to assess the change in bacterial community dynamics caused by the introduced AN-1. The cellular response to aniline toxic stress was also investigated. These will help enrich the basic information and theoretical basis for the remediation of aniline-contaminated sites via bioaugmentation technology.

Materials and Methods

Chemicals and Media

Standards of aniline (99.5% purity) and chromatographic-grade methanol were purchased from Burdic & Jackson, USA. All the other chemicals and reagents used were of pure analytical grade and available commercially.

The mineral salt medium (MSM) contained 0.28 mg/l KH_2PO_4, 0.25 mg/l $MgSO_4 \cdot 7H_2O$, 0.07 mg/l $CaCl_2 \cdot 2H_2O$, 0.02 mg/l $FeCl_3 \cdot 6H_2O$, 1.8 mg/l $MnCl_2 \cdot 4H_2O$, 4.5 mg/l $Na_2B_4O_7 \cdot 10H_2O$, 0.22 mg/l $ZnSO_4 \cdot 7H_2O$, 0.05 mg/l $CuCl_2 \cdot 2H_2O$, 0.03 mg/l $NaMoO_4 \cdot 2H_2O$, and 0.01 mg/l $CoSO_4 \cdot 7H_2O$. The Luria–Bertani (LB) medium contained 10.0 g/l tryptone, 5.0 g/l yeast extract, and 10.0 g/l NaCl [19].

The lactose induction medium contained 3 mg/l KH_2PO_4, 12.8 mg/l $Na_2HPO_4 \cdot 7H_2O$, 0.5 mg/l NaCl, 1 mg/l NH_4Cl, 0.5 mg/l $MgSO_4$, 0.01 mg/l $CaCl_2$, and 5 mg/l lactose. The β-galactosidase buffer was composed of 0.24 mg/l KH_2PO_4, 2.9 mg/l $Na_2HPO_4 \cdot 7H_2O$, 8 mg/l NaCl, 0.2 mg/l KCl, 0.25 mg/l $MgSO_4 \cdot 7H_2O$, and 3.9 ml/l β-mercaptoethanol [41]. The phosphate buffer consisted of 1.093 g/l Na_2HPO_4, 0.3175 g/l $NaH_2PO_4 \cdot H_2O$, 5.0 g/l NaCl, and 1% Triton X-100, which was slightly modified according to Liu *et al*. [20].

Construction of GFP-Tagged *P. migulae* AN-1

The plasmids pBBRGRP-45 and pRK2013 (Table 1) were provided by Professor Yanzheng Gao (Institute of Organic Contaminant Control and Soil Remediation, Nanjing Agricultural University, China). The isolation and preservation of *P. migulae* AN-1 (Amp^r, 50 mg/l), which can degrade aniline, was recently described by our laboratory [19]. The plasmid pBBRGRP-45 was transformed into AN-1 via triparental conjugation, as described by Sun *et al*. [31].

Stability of Plasmid

The assay for segregational plasmid stability was performed as described by Xiong *et al*. [40]. Briefly, a pre-culture of GFP-tagged AN-1 was grown at 30°C and 120 rpm in LB medium containing 50 mg/l Amp and 50 mg/l Km to ensure that the initial seed culture contained only the transformant. Logarithmic-phase cultures were then diluted 1:100 and used to inoculate LB broth without antibiotics. GFP-tagged strains were grown in LB broth without antibiotics and transferred at 24 h intervals at a 1:100 dilution into fresh medium for 10 consecutive days. Under these growth conditions, the cell number at the beginning of inoculation was 10^5–10^6 CFU/ml, whereas at the end of incubation was 10^8–10^9 CFU/ml. Cultures went through approximately 10 generations per transfer according to the formula of Ma *et al*. [21]. At the end of the culture transfer process, culture samples were diluted and spread onto LB agar plates with or without antibiotics. The plates were incubated until colonies appeared. Plasmid loss was calculated as the ratio between the plate counts of green fluorescent colonies and total counts on nonselective plates.

Plasmid Burden Testing

To determine the metabolic burden of the GFP-expressing plasmid in the GFP-tagged strain, the growth properties and aniline degradation abilities of GFP-tagged AN-1 and its parental strain were compared. The test stains were cultured overnight in 100 ml of LB liquid medium without antibiotics at 30°C and

Table 1. Strains and plasmids for used triparental conjugation.

Strain[a] or plasmid	Genotype or phenotype	Source or reference
pBBRGFP-45	pBBR-MCS2 vector with 1.4 kb foreign fragment (*gfp*)	[31]
pRK2013	*mob*$^+$, *tra*$^+$, Km^r, helper plasmid	[31]
Pseudomonas migulae AN-1	Aniline degradation, Amp^r, 50 mg/l	This laboratory

[a]*Escherichia coli* DH5α containing the plasmid pBBRGFP-45 (Km^r, 50 mg/l), *Escherichia coli* DH5α containing the plasmid pRK2013 (Km^r, 50 mg/l), and strain AN-1 (Amp^r, 50 mg/l).

120 rpm, and then harvested by centrifugation (8,000 ×*g*, 10 min at 4°C). After washing twice with sterile water, 1% of the suspension (OD_{600} = 1.0) was inoculated into 100 ml of sterilized liquid MSM with 1,280 mg/l aniline as the sole carbon source in a 250 ml Erlenmeyer flask. Batch experiments were carried out using a gyratory incubator shaker at 10°C. Samples were withdrawn at a regular time interval during the experiments to determine biomass production and aniline consumption. All the experiments in this study were performed in triplicates, and the uninoculated medium was used as a control.

Cellular Membrane Permeability

The membrane permeability of GFP-tagged AN-1 was determined by measuring the concentration of β-galactosidase released into the culture medium using o-nitrophenyl-β-D-galactoside (ONPG) as a substrate [29]. The bacteria precultured overnight in LB medium were collected, washed, and suspended in 0.9% NaCl solution. The suspension was added into a lactose induction medium and incubated at 20°C. Subsequently, the cells were collected, washed, and suspended in β-galactosidase buffer. Afterward, the cells were treated with 20, 50, 100, 250, 450, 650, or 850 mg/l aniline in the presence of 50 mg/l ONPG at 10°C. The production of *o*-nitrophenol was detected at 504 nm after 40 min cultivation.

Measurement of the Relative CSH

The relative CSH of the GFP-tagged AN-1 during growth on aniline was determined using the microbial adherence to hydrocarbon method [43]. Briefly, AN-1 grown in MSM with 20, 50, 100, 250, 450, 650, or 850 mg/l aniline was harvested, washed three times, and resuspended in sterile water to an OD_{600} of 0.6 (A_0). Then 3 ml samples of bacterial suspensions were placed in glass tubes with 400 μl of xylene and vortexed for 2 min. After a 15 min phase separation, the lower aqueous phase was removed and its OD_{600} was determined (A_1). The values were then expressed as the percentage of bacteria adhering to hydrocarbon (A) compared with the control suspension, that is, $A = [(A_0 - A_1) / A_0] \times 100$.

Microcosm Experiments

Aquifer sediments and groundwater with no exposure to aniline were collected in Jilin City, China. Each microcosm was artificially contaminated with aniline to a concentration of 738.15 mg/l and was established by placing 20 g of sediments and 10 ml of groundwater in a 100 ml Erlenmeyer flask. The microcosm was inoculated with GFP-tagged AN-1 (7.52×10^5 cells/ml) and incubated at 10°C in the dark. The uninoculated microcosms were used as controls under the same conditions. Biomass production and aniline consumption were periodically determined.

To calculate the biomass of GFP-tagged AN-1, 50 ml of phosphate buffer was added into the flasks containing the microcosms. The flasks were shaken for 10 min to dissociate the bacteria bound to the surface of the granular material. Then, 20 ml of the supernatant was filtered onto black Millipore membrane filters (25 mm diameter, 0.2 μm pore size), which were mounted on glass slides. Three filters were prepared per sample, and a minimum of 10 microscopic fields were scanned on each filter. The GFP-tagged bacteria were enumerated at a precision of ±10% using an EVOS fluorescence microscope (Advanced Microscopy Group, USA) and Image-Pro Plus 6.0 (Media Cybernetics, USA).

Aniline in the microcosms was extracted using 20 ml of methanol. The mixture was shaken in a shaker for 10 min with the temperature controlled at 20°C, and the supernatant was filtrated with a 0.22 μm pore filter. Then, the aniline concentration was quantitatively determined with high-performance liquid chromatography (Agilent 1100, USA) equipped with a C18 column (Waters, Pacific Grove, USA) according to the method described by Liu *et al*. [19].

Pyrosequencing and Data Analysis

To analyze the bacterial communities of microcosms, DNA was extracted from 5 g of non-bioaugmentation and bioaugmentation microcosm samples using the Soil DNA kit (OMEGA, Bio-Tek, USA) according to the manufacturer's instruction. Primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were employed to amplify the bacterial 16S rRNA gene fragments from the extracted DNA. The PCR products were pooled, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Scientific, USA), and quantified with Quanti Fluor-ST (Promega, USA).

The pyrosequencing was performed commercially by Majorbio Pharmaceutical Technology Co., Ltd. (Shanghai, China) on a Roche GS 454 FLX platform (http://www.majorbio.com). The sequences with a similarity of over 97% were classified into one operational taxonomic unit (OTU) according to the distance matrix using the Usearch ver. 7.1 software and following the Schloss standard operating procedure [27]. Rarefaction curves, Alpha-diversity, and species richness estimators were generated with the Mothur ver. 1.30.1 software for each sample. Using the BLASTN software with an e-score cutoff of 0.01, taxonomic classification was performed against the RDP database ver. 10.18 and the Greengenes web service (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi). After the phylogenetic allocation of the sequences at the phylum, class, and genus levels, the relative abundance of a given phylogenetic group was set as the number of sequences affiliated with that group divided by the total number of sequences per sample. Venn diagrams with shared and unique OTUs were used to depict the similarities among the microbial communities.

Results and Discussion

Stability and Degradation Performance of GFP-Tagged *P. migulae* AN-1

GFP is a useful and sensitive marker for distinguishing introduced strains from complex microcosms [1]. For this

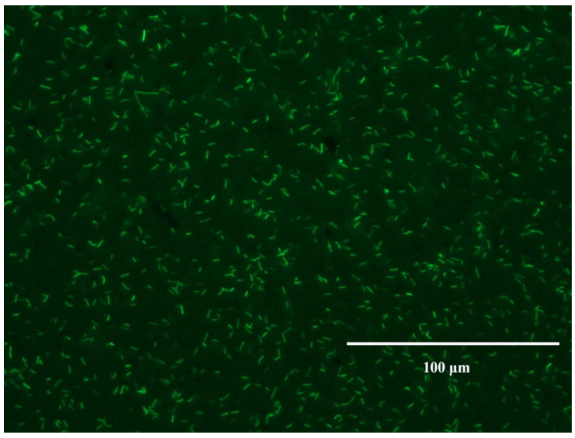

Fig. 1. Fluorescence of the GFP-tagged AN-1 strain under fluorescence microscopy.

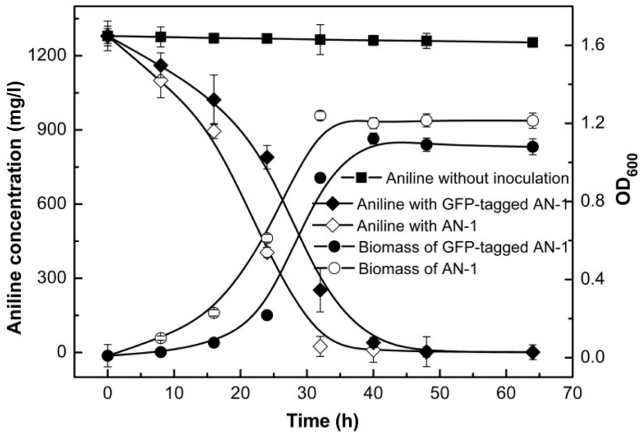

Fig. 2. Aniline degradation and growth curves of GFP-tagged AN-1 and the parental strain.
Error bars represent standard deviations of three replicates.

reason, we transformed the plasmid pBBRGRP-45 into *P. migulae* AN-1. The cells carrying the plasmid were detected by fluorescence microscopy, as shown in Fig. 1. The strain exhibited bright green fluorescence. The numbers of GFP-tagged AN-1 colonies growing on LB plates with and without antibiotics were very similar, and nearly all of the colonies appeared green (less than 3.0% plasmid loss) after 10 transfers (approximately 100 generations) (data not shown). This result suggested that the plasmid pBBRGRP-45 was stable in GFP-tagged AN-1. It indicated that AN-1 was successfully tagged with the GFP gene and showed stable *gfp* activity. This result provided a solid foundation for the monitoring of AN-1 survival and dynamics in aquifer microcosms, although great caution should be further taken when using marker genes in bacterial behavior and physiology studies [40]. Fig. 2 shows that GFP-tagged AN-1 degraded aniline a little slower than its parental strain. The aniline degradation rate of GFP-tagged AN-1 and its parental strain were 46.84 and 54.40 mg/l/h, respectively. In addition, the final biomass was different between the tagged and untagged AN-1, although the same aniline was consumed. After 48 h, the OD_{600} of GFP-tagged AN-1 was 1.09, which was 89.74% of the untagged AN-1.

During the degradation of aniline, GFP-tagged AN-1 exhibited a slight reduction in biomass yield and degradation rate compared with its plasmid-free counterpart (AN-1). This behavior indicated that the GFP-tagging process imparted a metabolic burden to the cells. Some previous studies showed that GFP plasmids do not interfere with the growth of host cells [28, 40]. By contrast, the results of our study agree with those of Seoane *et al*. [28]. The mechanisms by which the fitness cost of plasmid carriage operates have been reviewed by Slater *et al*. [30] but are still unclear. Three groups of hypotheses regarding such cost have been formulated: as an effect of plasmid-encoded protein expression [26], as an effect of the replication and transfer of the plasmid DNA [4], and as an effect of disorders in the cellular regulatory mechanisms [6]. Although GFP-tagged AN-1 degraded aniline slower than its parental strain and exhibited fewer generations, it still demonstrated high aniline-degrading capacity in high concentrations at low temperatures. The slight difference in growth did not change the nature of AN-1 fundamentally. Thus, using GFP-tagged AN-1 to investigate its survival in the microcosms was feasible.

Cellular Responses of GFP-Tagged *P. migulae* AN-1 during Aniline Biodegradation

In order to explore the cellular responses of GFP-tagged AN-1 to aniline toxic stress, CSH and membrane permeability were investigated. CSH is believed to be an important response to various environmental factors, and one of the factors governing bacterial adhesion, uptake, and degradation of organic compounds [3, 25, 44]. According to the recommendation of Mattos–Guaraldi *et al.* [22], strains were considered strongly hydrophobic when the values were >50%, moderately hydrophobic when the values were in the range of 20%–50%, and hydrophilic when the values were <20%.

After the degradation of 20–850 mg/l aniline, the hydrophobic alteration of GFP-tagged AN-1 was recorded and plotted (Fig. 3). GFP-tagged AN-1 was moderately

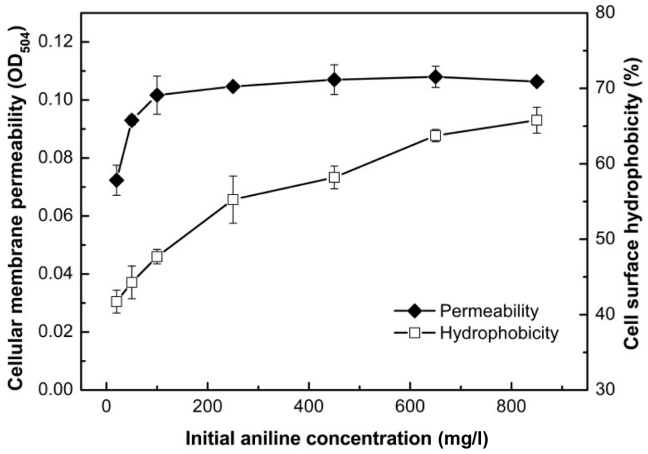

Fig. 3. Effect of aniline concentration on cell surface hydrophobicity and cellular membrane permeability.
Error bars represent standard deviations of three replicates.

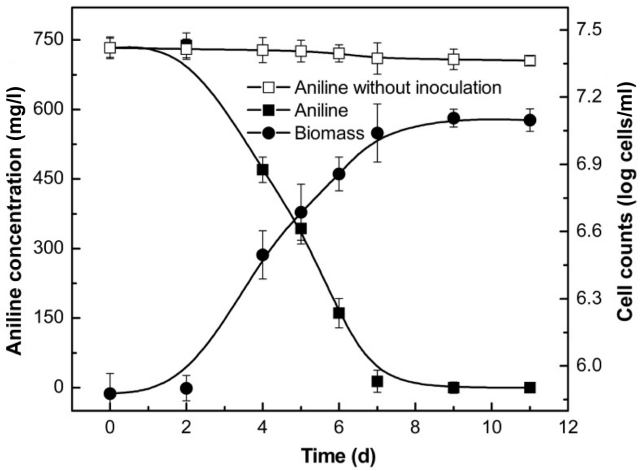

Fig. 4. Aniline degradation and growth curves of GFP-tagged AN-1 in aquifer microcosms.
Error bars represent standard deviations of three replicates.

hydrophobic (41.74%–47.69%) when treated with 20–100 mg/l aniline and exhibited relatively strong hydrophobicity (55.25%–65.78%) when the concentration of aniline was >100 mg/l. With the concomitant increase in aniline concentration to 850 mg/l, hydrophobicity reached the maximum value. Farrell and Quilty [7] reported that *P. putida* CP1 cells were more hydrophobic when they were incubated in high concentrations of mono-chlorophenol because of their toxic effects, which made the cell biomass decrease. Zhang *et al*. [43] showed that the high hydrophobicity of *Rhodococcus* sp. CN6 could enhance its adherence to pollutant surfaces. For GFP-tagged AN-1 in the present work, hydrophobicity increased with an increase in aniline concentration. This result is a clear indication that GFP-tagged AN-1 can adapt to aniline toxicity stress.

Cellular membrane permeability is important in illustrating the interaction between AN-1 and aniline. When the membrane permeability increased, not only was the pollutant easily transported into the cell across the membrane but also more intracellular degradation enzymes leaked to the outside of the cell to contact with the pollutant and thus promote pollutant removal [29, 41, 42]. Moreover, metabolites of the pollutant via intracellular degradation could be released into the extracellular environment and bioaccumulation was reduced [9]. Therefore, cellular membrane permeability has strong correlations of metabolite transport and bioaccumulation in the degradation process. Hence, the membrane permeability of GFP-tagged AN-1 was determined by measuring the concentration of β-galactosidase released into the culture medium using ONPG as a substrate. As shown in Fig. 3, when the cells were treated with aniline below 100 mg/l, the cytoplasmic β-galactosidase was progressively released. Hence, the increase in permeability was followed by a rise in aniline concentration. However, after treatment with aniline above 100 mg/l, the membrane permeability of AN-1 was invariable. These results indicated that the membrane permeability of GFP-tagged AN-1 was not perfectly positively correlated with aniline concentration and AN-1 could autoregulate membrane permeability.

Survival and Degradation Activity of GFP-Tagged *P. migulae* AN-1 in Aquifer Microcosms

Successful bioaugmentation primarily depends on the sufficient survival and activity of the introduced strain in the environment [23]. To evaluate whether GFP-tagged AN-1 could survive and degrade aniline when coexisting with indigenous microorganisms in aquifer environments, a microcosm-based study was carried out. As shown in Fig. 4, the concentration of aniline in the microcosms without inoculation barely changed. It demonstrates removal of aniline was not the cause of volatilization or adsorption. During 2 days of inoculation, GFP-tagged AN-1 was in an adapted phase. Afterward, as the GFP-tagged AN-1 biomass increased, the concentrations of aniline in the microcosms were reduced. On the ninth day, aniline was undetectable, and GFP-tagged AN-1 increased from 7.52×10^5 cells/ml on day 1 to 128×10^5 cells/ml. The degradation rate of aniline was 6.04 mg/l/h. Under the microscope, the green fluorescence of GFP-tagged AN-1 was strong, and cellular morphology was normal (data not shown).

Table 2. OTU richness and diversity indices of the sediment bacterial community.

Samples	OTUs	Ace	Chao	Shannon	Simpson	Coverage
Non-bioaugmentation	73	73.85	74.00	0.52	0.85	99.99%
Bioaugmentation	76	76.98	76.43	0.68	0.80	99.99%

Competition with indigenous microorganisms is crucial to the survival of microorganisms introduced into aquifers. Some inoculants such as *Rhodococcus* sp. D310-1::*gfp* [40] and *P. fluorescens* 2P24 [8] disappeared after being introduced into the soil. This phenomenon is generally explained as the predation of the newly introduced and unprotected bacteria by protozoa [24]. In this study, the number of GFP-tagged AN-1 cells increased after inoculation, showing their adaptive capacity to the microcosm environment. Furthermore, the survival and adaptation of GFP-tagged AN-1 corresponded directly with aniline consumption. This relationship suggests that *P. migulae* AN-1 is a potentially useful tool for the in situ bioremediation of aniline-contaminated aquifers by bioaugmentation.

Impact of GFP-Tagged AN-1 on Microbial Community Diversity

The pyrosequencing of the non-bioaugmentation (microcosms without inoculation on the eleventh day) and bioaugmentation (microcosms inoculated with GFP-tagged AN-1 on the eleventh day) samples was conducted to evaluate the interactions of GFP-tagged AN-1 with the indigenous bacterial community. After pyrosequencing and sequence quality filtering, 64,823 valid reads were retained with averages of 449 bp read length. The rarefaction curves reached a saturation plateau (Fig. S1), and the coverage estimations were 99.99% for both samples. Furthermore, 73 and 76 OTUs (97% similarity cutoff) were identified in the non-bioaugmentation and bioaugmentation

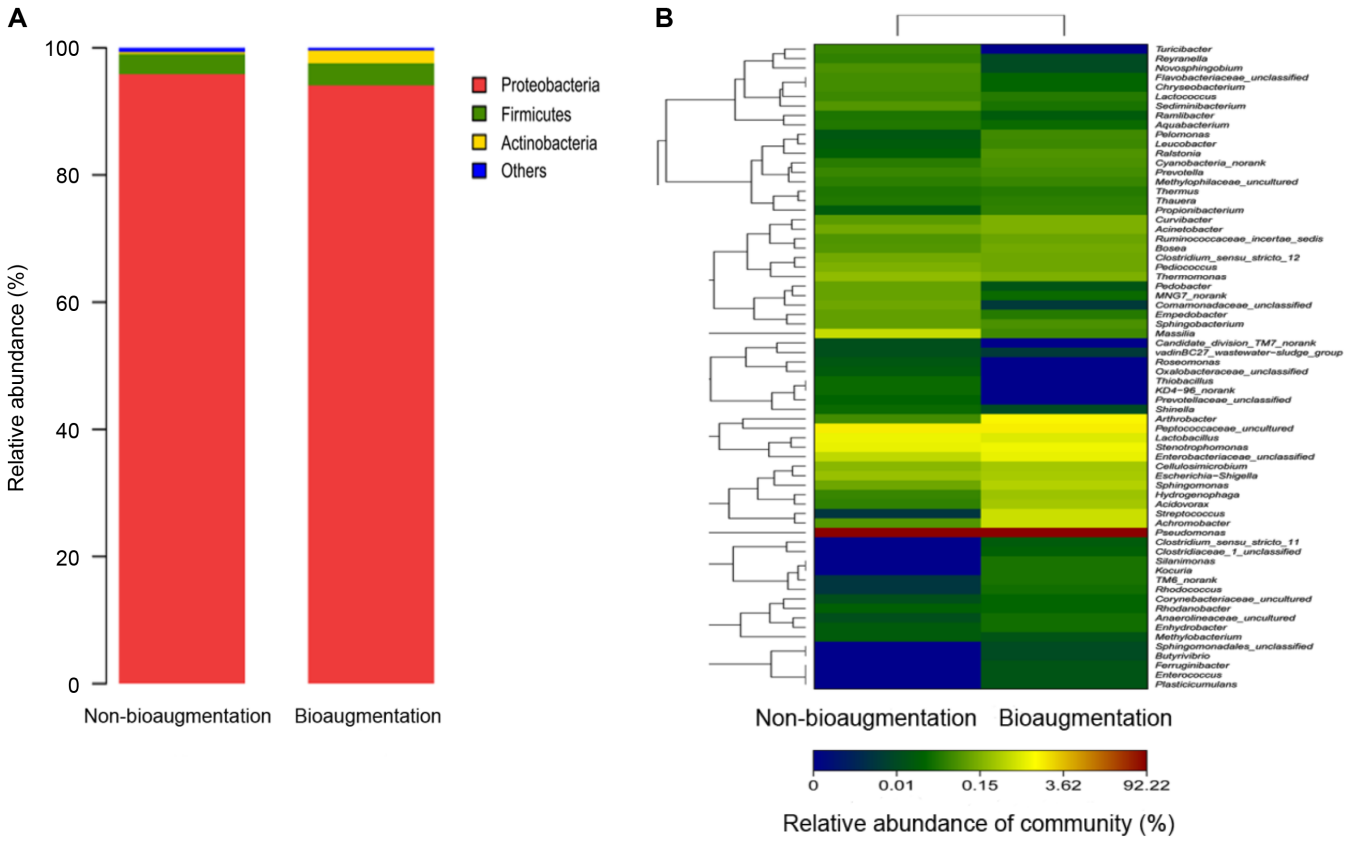

Fig. 5. Comparison of the bacterial communities at the phylum (**A**) and genus (**B**) levels.
The heatmap plot (**B**) depicts the relative abundance of each bacterial family intensity, with the legend indicated at the bottom. The genera determined in the samples and their cluster plots are labeled on the left and right, respectively.

samples, respectively. This result agrees with the calculations of Chao and Ace indices (Table 2). These findings suggest that the sequencing capability in the study was clearly large enough to capture the complete diversity of the communities.

The comparison of the diversity indices (Shannon or Simpson index) indicated that the bioaugmentation samples exhibited significantly higher richness and diversity in the bacterial community than the non-bioaugmentation samples. To examine the dynamic changes in the bacterial community structure, taxonomic classification was performed on all the valid reads, leading to the identification of 9 bacterial phyla and 68 genera in total. Among them, *Proteobacteria*, *Firmicutes*, and *Actinobacteria* (relative abundance >2%) were detected as the predominant phyla (Fig. 5A), which commonly occur in organic contaminant treatment systems [14, 17, 18]. Notably, the relative abundance of *Proteobacteria* (accounted for 99.22% in the non-bioaugmentation samples) changed to 89.23% after bioaugmentation with GFP-tagged AN-1. *Actinobacteria* increased from 0.29% to 2.01%, whereas the abundance of *Firmicutes* barely changed.

To further analyze the bacterial structure in the two samples, a heatmap was generated at the genus level (Fig. 5B). The cluster plot shows that the bioaugmentation treatment resulted in a bacterial community pattern that distinctively differed from that of the non-bioaugmentation treatment. *Pseudomonas* was the dominant genus before and after bioaugmentation, and its abundance was promoted because of the inoculation of GFP-tagged AN-1. The genus *Arthrobacter* displayed great changes and its relative abundance increased from 0.06% to 1.54% after bioaugmentation. In fact, via aerobic biodegradation, aniline can be metabolized to catechol, and subsequently undergoes metabolic transformations through *or-*, *tho-* or *meta*-ring cleavage pathways in order to enter central metabolism [14]. Some metabolites such as phenolic compounds and monocyclic aromatics are then released to the extracellular environment. The newly generated by-product utilized as carbon sources results in the biomass growth of some indigenous degrading bacteria such as *Arthrobacter* [15, 37]. Not only did the relative abundance of some genera become rich, but also species increased in microcosms through bioaugmentation. A Venn diagram was used to compare the species levels before and after bioaugmentation with GFP-tagged AN-1 (Fig. S2). According to the diagram, 8 and 11 species were unique, respectively, and 65 species were shared by the two samples. Therefore, bioaugmentation with GFP-tagged AN-1 could shift bacterial community structures to adapt to the shock of aniline and prevent the inhibition of indigenous bacteria by aniline. It could be a feasible bioremediation strategy against aniline-contaminated groundwater.

Acknowledgments

The authors are grateful to Professor Yanzheng Gao (Institute of Organic Contaminant Control and Soil Remediation, Nanjing Agricultural University, China) for providing the plasmids pBBRGRP-45 and pRK2013. This work was financially supported by the Key Project of National Natural Science Foundation of China (Grant No. 41530636), National Natural Science Foundation of China (Grant No. 41471252), and Scientific Frontier and Interdisciplinary Research Project of Jilin University, Outstanding Youth Cultivation Plan of Jilin University.

References

1. Ahn YB, Beaudette L, Lee H, Trevors J. 2001. Survival of a GFP-labeled polychlorinated biphenyl degrading psychrotolerant *Pseudomonas* spp. in 4 and 22°C soil microcosms. *Microb. Ecol.* **42:** 614-623.
2. Aksu Z, Balibek E. 2010. Effect of salinity on metal-complex dye biosorption by *Rhizopus arrhizus*. *J. Environ. Manage.* **91:** 1546-1555.
3. Bastiaens L, Springael D, Wattiau P, Harms H, Verachtert H, Diels L. 2000. Isolation of adherent polycyclic aromatic hydrocarbon (PAH)-degrading bacteria using PAH-sorbing carriers. *Appl. Environ. Microbiol.* **66:** 1834-1843.
4. Björkman J, Andersson DI. 2000. The cost of antibiotic resistance from a bacterial perspective. *Drug Resist. Updates* **3:** 237-245.
5. Cunliffe M, Kertesz MA. 2006. Effect of *Sphingobium yanoikuyae* B1 inoculation on bacterial community dynamics and polycyclic aromatic hydrocarbon degradation in aged and freshly PAH-contaminated soils. *Environ. Pollut.* **144:** 228-237.
6. Diaz Ricci JC, Hernández ME. 2000. Plasmid effects on *Escherichia coli* metabolism. *Crit. Rev. Biotechnol.* **20:** 79-108.
7. Farrell A, Quilty B. 2002. Substrate-dependent autoaggregation of *Pseudomonas putida* CP1 during the degradation of mono-chlorophenols and phenol. *J. Ind. Microbiol. Biotechnol.* **28:** 316-324.
8. Gao G, Yin D, Chen S, Xia F, Yang J, Li Q, Wang W. 2012. Effect of biocontrol agent *Pseudomonas fluorescens* 2P24 on soil fungal community in cucumber rhizosphere using T-RFLP and DGGE. *PLoS One* **7:** e31806.
9. Gao J, Ye J, Ma J, Tang L, Huang J. 2014. Biosorption and biodegradation of triphenyltin by *Stenotrophomonas maltophilia* and their influence on cellular metabolism. *J. Hazard. Mater.*

276: 112-119.
10. Ghosh A, Sinha K, Saha PD. 2013. Central composite design optimization and artificial neural network modeling of copper removal by chemically modified orange peel. *Desalin. Water Treat.* **51:** 7791-7799.
11. Huang J, Ye J, Ma J, Gao J, Chen S, Wu X. 2014. Triphenyltin biosorption, dephenylation pathway and cellular responses during triphenyltin biodegradation by *Bacillus thuringiensis* and tea saponin. *Chem. Eng. J.* **249:** 167-173.
12. Jeong S, Moon HS, Shin D, Nam K. 2013. Survival of introduced phosphate-solubilizing bacteria (PSB) and their impact on microbial community structure during the phytoextraction of Cd-contaminated soil. *J. Hazard. Mater.* **263:** 441-449.
13. Jin Q, Hu Z, Jin Z, Qiu L, Zhong W, Pan Z. 2012. Biodegradation of aniline in an alkaline environment by a novel strain of the halophilic bacterium, *Dietzia natronolimnaea* JQ-AN. *Bioresour. Technol.* **117:** 148-154.
14. Kahng HY, Kukor JJ, Oh KH. 2000. Characterization of strain HY99, a novel microorganism capable of aerobic and anaerobic degradation of aniline. *FEMS Microbiol. Lett.* **190:** 215-221.
15. Karigar C, Mahesh A, Nagenahalli M, Yun DJ. 2006. Phenol degradation by immobilized cells of *Arthrobacter citreus*. *Biodegradation* **17:** 47-55.
16. Karthikeyan S, Gupta V, Boopathy R, Titus A, Sekaran G. 2012. A new approach for the degradation of high concentration of aromatic amine by heterocatalytic Fenton oxidation: kinetic and spectroscopic studies. *J. Mol. Liq.* **173:** 153-163.
17. Liang B, Cheng HY, Kong DY, Gao SH, Sun F, Cui D, *et al.* 2013. Accelerated reduction of chlorinated nitroaromatic antibiotic chloramphenicol by biocathode. *Environ. Sci. Technol.* **47:** 5353-5361.
18. Liu S, Ma Q, Wang B, Wang J, Zhang Y. 2014. Advanced treatment of refractory organic pollutants in petrochemical industrial wastewater by bioactive enhanced ponds and wetland system. *Ecotoxicology* **23:** 689-698.
19. Liu YB, Qu D, Wen YJ, Ren HJ. 2015. Low-temperature biodegradation of aniline by freely suspended and magnetic modified *Pseudomonas migulae* AN-1. *Appl. Microbiol. Biotechnol.* **99:** 5317-5326.
20. Liu Y, Yang CH, Li J. 2007. Influence of extracellular polymeric substances on *Pseudomonas aeruginosa* transport and deposition profiles in porous media. *Environ. Sci. Technol.* **41:** 198-205.
21. Ma L, Zhang G, Doyle MP. 2011. Green fluorescent protein labeling of *Listeria*, *Salmonella*, and *Escherichia coli* O157:H7 for safety-related studies. *PLoS One* **6:** 1-7.
22. Mattos-Guaraldi AL, Formiga LCD, Andrade AFB. 1999. Cell surface hydrophobicity of sucrose fermenting and nonfermenting *Corynebacterium diphtheriae* strains evaluated by different methods. *Curr. Microbiol.* **38:** 37-42.
23. Mohan SV, Falkentoft C, Nancharaiah YV, Sturm BSM, Wattiau P, Wilderer PA, *et al.* 2009. Bioaugmentation of microbial communities in laboratory and pilot scale sequencing batch biofilm reactors using the TOL plasmid. *Bioresour. Technol.* **100:** 1746-1753.
24. Rosenberg K, Bertaux J, Krome K, Hartmann A, Scheu S, Bonkowski M. 2009. Soil amoebae rapidly change bacterial community composition in the rhizosphere of *Arabidopsis thaliana*. *ISME J.* **3:** 675-684.
25. Rosenberg M, Gutnick D, Rosenberg E. 1980. Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiol. Lett.* **9:** 29-33.
26. Rozkov A, Avignone-Rossa C, Ertl P, Jones P, O'Kennedy R, Smith J, *et al.* 2004. Characterization of the metabolic burden on *Escherichia coli* DH1 cells imposed by the presence of a plasmid containing a gene therapy sequence. *Biotechnol. Bioeng.* **88:** 909-915.
27. Schloss PD, Gevers D, Westcott SL. 2011. Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. *PLoS One* **6:** e27310.
28. Seoane J, Sin G, Lardon L, Gernaey KV, Smets BF. 2010. A new extant respirometric assay to estimate intrinsic growth parameters applied to study plasmid metabolic burden. *Biotechnol. Bioeng.* **105:** 141-149.
29. Shi G, Yin H, Ye J, Peng H, Li J, Luo C. 2013. Effect of cadmium ion on biodegradation of decabromodiphenyl ether (BDE-209) by *Pseudomonas aeruginosa*. *J. Hazard. Mater.* **263:** 711-717.
30. Slater FR, Bailey MJ, Tett AJ, Turner SL. 2008. Progress towards understanding the fate of plasmids in bacterial communities. *FEMS Microbiol. Ecol.* **66:** 3-13.
31. Sun K, Liu J, Gao Y, Jin L, Gu Y, Wang W. 2014. Isolation, plant colonization potential, and phenanthrene degradation performance of the endophytic bacterium *Pseudomonas* sp. Ph6-gfp. *Sci. Rep.* **4:** 5462.
32. Sun W, Li Y, McGuinness LR, Luo S, Huang W, Kerkhof LJ, *et al.* 2015. Identification of anaerobic aniline-degrading bacteria at a contaminated industrial site. *Environ. Sci. Technol.* **49:** 11079-11088.
33. Travis AS. 1997. Poisoned groundwater and contaminated soil: the tribulations and trial of the first major manufacturer of aniline dyes in Basel. *Environ. Hist.* **2:** 343-365.
34. Tyagi M, da Fonseca MMR, de Carvalho CC. 2011. Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. *Biodegradation* **22:** 231-241.
35. Van Veen JA, Van Overbeek LS, Van Elsas JD. 1997. Fate and activity of microorganisms introduced into soil. *Microbiol. Mol. Biol. Rev.* **61:** 121-135.
36. Wang L, Barrington S, Kim JW. 2007. Biodegradation of pentyl amine and aniline from petrochemical wastewater. *J. Environ. Manage.* **83:** 191-197.
37. Wang P, Qu Y, Zhou J. 2009. Biodegradation of mixed phenolic compounds under high salt conditions and salinity

fluctuations by *Arthrobacter* sp. W1. *Appl. Biochem. Biotechnol.* **159:** 623-633.

38. Wen Y, Yang Y, Ren H, Du X, Yang X, Zhang L, Wang X. 2015. Chemical–biological hybrid reactive zones and their impact on biodiversity of remediation of the nitrobenzene and aniline contaminated groundwater. *Chem. Eng. J.* **280:** 233-240.
39. Wu GQ, Zhang X, Hui H, Yan J, Zhang QS, Wan JL, Dai Y. 2012. Adsorptive removal of aniline from aqueous solution by oxygen plasma irradiated bamboo based activated carbon. *Chem. Eng. J.* **185:** 201-210.
40. Xiong M, Hu Z, Zhang Y, Cheng X, Li C. 2013. Survival of GFP-tagged *Rhodococcus* sp. D310-1 in chlorimuron-ethyl-contaminated soil and its effects on the indigenous microbial community. *J. Hazard. Mater.* **252-253:** 347-354.
41. Ye J, Zhao H, Yin H, Peng H, Tang L, Gao J, Ma Y. 2014. Triphenyltin biodegradation and intracellular material release by *Brevibacillus brevis*. *Chemosphere* **105:** 62-67.
42. Yong YC, Zhong JJ. 2010. Recent advances in biodegradation in China: new microorganisms and pathways, biodegradation engineering, and bioenergy from pollutant biodegradation. *Process Biochem.* **45:** 1937-1943.
43. Zhang J, Sun Z, Li Y, Peng X, Li W, Yan Y. 2009. Biodegradation of *p*-nitrophenol by *Rhodococcus* sp. CN6 with high cell surface hydrophobicity. *J. Hazard. Mater.* **163:** 723-728.
44. Zikmanis P, Shakirova L, Auzina L, Andersone I. 2007. Hydrophobicity of bacteria *Zymomonas mobilis* under varied environmental conditions. *Process Biochem.* **42:** 745-750.