Supporting Information

Genetic bioaugmentation of activated sludge with dioxin-catabolic plasmids harbored by *Rhodococcus* sp. strain p52

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Summary

Supporting information contains the composition of trace element solution in the synthetic wastewater, the operation (Table S1) and performance of the second experiment in SBR with different bioaugmentation manner (Figure S1), features of isolated transconjugants (Table S2) and phylogenetic analysis (Figure S2), and rarefaction curve of high-throughput 16S rRNA gene sequencing (Figure S3). Figure S1, Figure S2, and Figure S3 are available free of charge on the ACS Publication Website.

Table of Contents

1. Composition of trace element solution in synthetic wastewater

The trace element solution contained (g L^{-1}): $MnCl_2 \cdot 4H_2O$ 0.24, $ZnCl_2$ 0.24, H_3BO_3 0.3, KI 0.06, $NaMoO_4 \cdot 2H_2O$ 0.3, $CoCl_2 \cdot 6H_2O$ 0.116, and $CuSO_4 \cdot 5H_2O$ 0.06.

2. Experimental information for SBR bioaugmentation in a manner of repeated inoculation of strain p52 (Experiment II)

The reactors were set up and operated under similar conditions as described in the Material and Methods. The hydraulic retention time was 12 h or 24 h alternated according to effluent properties. Feeding in each cycle was supplemented with dibenzofuran (75–125 mg L^{-1} adjusted on the basis of effluent quality) after the reactors were pre-operated for 10 days. Bioaugmentation was conducted in a repeated low-dosage manner. Specifically, strain p52 was inoculated into the bioaugmented reactor three times with a cell density around 10^6 cells mL^{-1} each time at a frequency of 20–30 days according to the effluent properties. Strain p52 was inoculated initially after 10 days of pre-operation, in accordance with the first supplemental feeding of dibenzofuran. The difference of running condition between two batch experiments is listed in Table S1.

Table S1. SBRs running difference between two experiments

	Experiment I	Experiment II
Strain p52-inoculation times	1	3
Inoculation dosage/time	$(3.8\pm0.9)\times10^6$ CFU mL^{-1} (day 1)	$(1.0\pm0.3)\times10^6$ CFU m L^{-1} (days 1, 20, 51)
Cycle time	12 h (days 1–50); 24 h (days 51–100)	12 h (days 1–12, 20–50, 58–77); 24 h (days 13–19, 51–57)
Dibenzofuran in influent (mg L^{-1})	120	75 (days 1–30); 120 (days 31–50); 100 (days 51–77)

3. Performance of bioaugmented SBR in the manner of repeated inoculation of strain p52 (Experiment II)

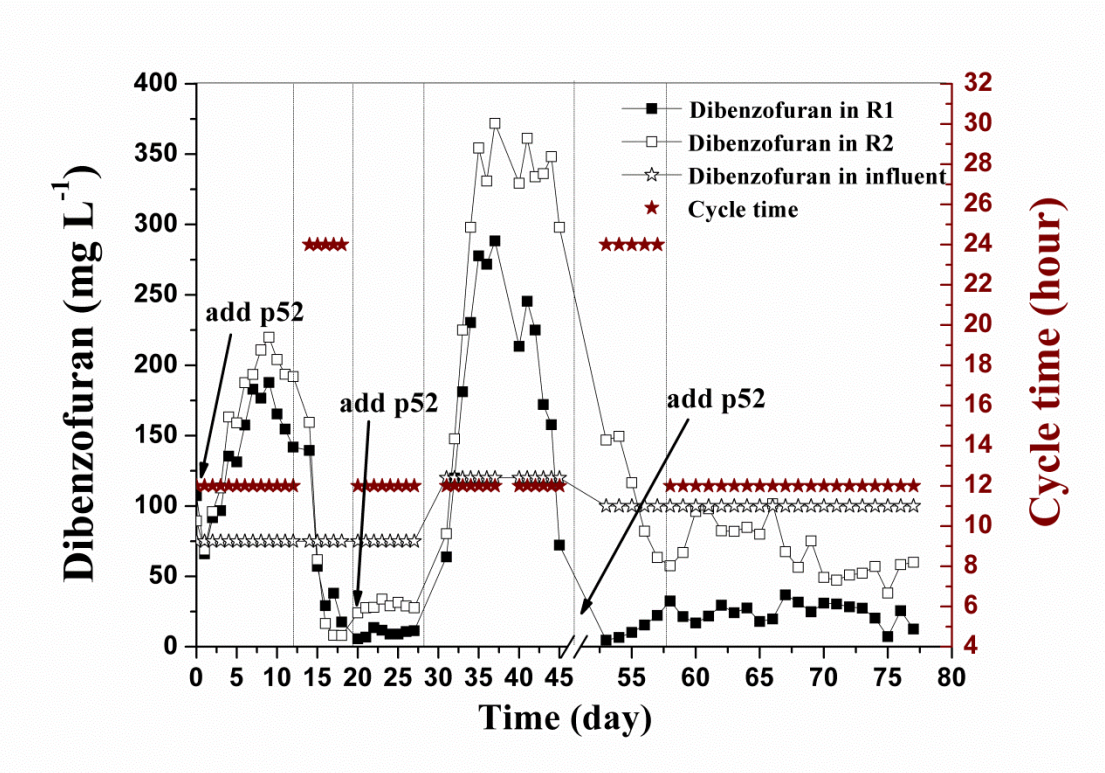

Figure S1. Dibenzofuran removal in the bioaugmented SBR (R1) and non-bioaugmented control (R2). The cycle time was 24 h or 12 h as indicated by solid asterisks. The dibenzofuran concentration in influent is marked by empty asterisks. Inoculation with *Rhodococcus* sp. strain p52 is indicated by arrows.

4. Features of transconjugants isolated from the bioaugmented SBR

Table S2. The isolated transconjugants during SBR operation

Transconjugants affiliated genus	Colony numbers of the isolated transconjugants							Colony morphology
	Day2 ×2,000 /mL	Day 3 ×2,000 /mL	Day 6 ×5,000 /mL	Day 10 ×20,000 /mL	Day 20 ×20,000 /mL	Day 30 ×20,000 /mL	Total	
Klebsiella	3	5	8	9	5	6	36	Round, white with regular edge; colony diameter 1.5–2.0mm
Arthrobacter	2	1	3	3	2	5	16	Round, yellow, low convex with regular edge; colony diameter 1.5–2.0mm
Corynebacterium	2	1	2	1	2	0	8	Round, white, flat with regular edge; colony diameter < 0.5 mm
Enterobacter	0	0	1	2	0	1	4	White with irregular edge; colony diameter 0.5–1.0mm
Total isolates	7	7	14	15	9	12	64	
*Colonies/plate	54±10	50±7	22±8	22±8	34±3	37±5		

*The colonies counted on the selective plates did not include the donor strain *Rhodococcus* sp. strain p52.

5. Phylogenetic analysis of the isolated transconjugants

Sixteen bacterial 16S rRNA gene sequences were retrieved from NCBI nucleotide sequence database based on BLAST search, which are closely related to the transconjugants. A total of 23 bacterial 16S rRNA gene sequences, complied in

FASTA format, were aligned using Clustal W version 2.1. Each aligned sequence was trimmed to a partial sequence of 1300 bp. The trimmed sequences were realigned, and the final alignment was converted to MEGA format for phylogenetic analyses. A neighbor-joining phylogenetic tree was constructed using MEGA version 6.06 according to the online manual. An evolutionary distance matrix for the neighbor-joining method was then generated using the maximum composite likelihood substitution model. Bootstrap analysis was based on 1000 replicates.

A phylogenetic tree for the isolated transconjugants and the donor strain p52 is shown in Figure S2.

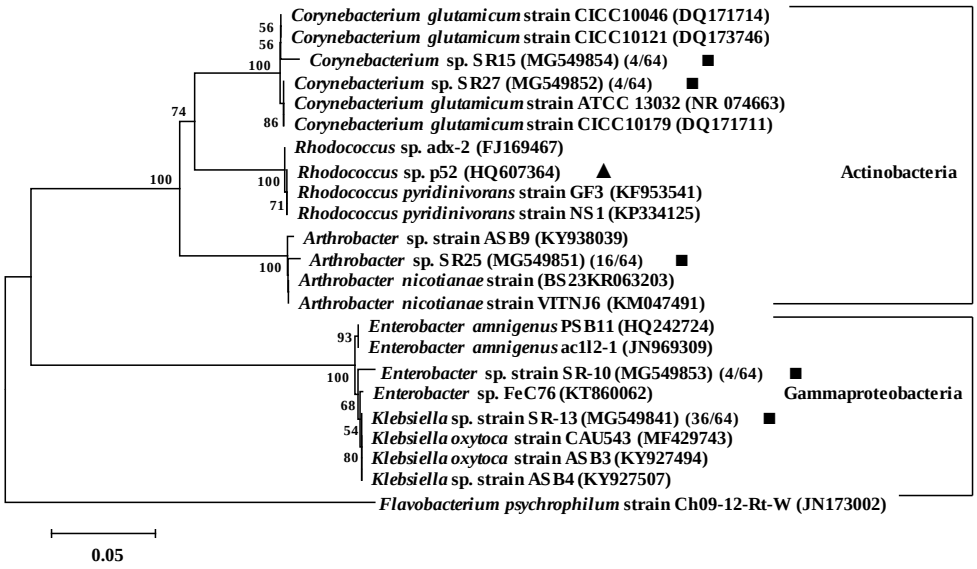

Figure S2. Phylogenetic trees of the isolated transconjugants and *Rhodococcus* sp. strain p52. The isolated transconjugants in the present study are indicated by the symbol (■). The ratio indicates the numbers of isolates belong to the same species to that of total isolated transconjugants. *Rhodococcus* sp. strain p52 is indicated by the symbol (▲). Bootstrap values above 50% are shown at the branch nodes (1000 replicates). *Flavobacterium psychrophilum* strain Ch09-12-Rt-W is used as an outgroup. The scale bar represents 0.05 nucleotide substitutions per sequence position.

6. Rarefaction curve of high-throughput 16S rRNA gene sequencing

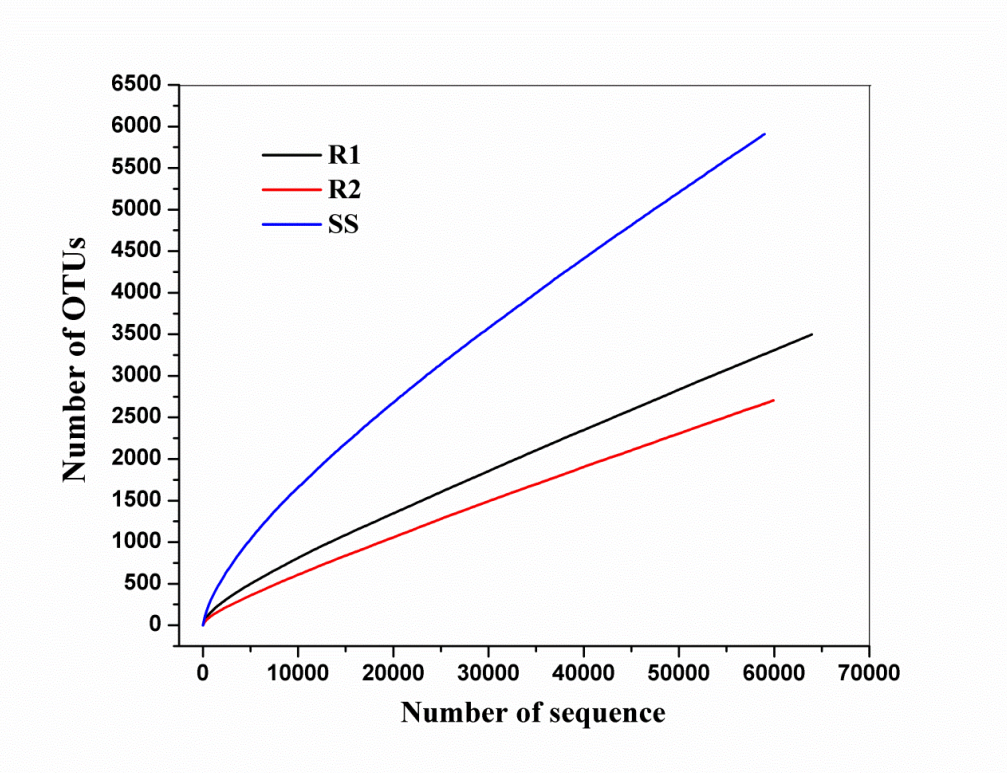

Figure S3. Rarefaction curve of 16S rRNA gene sequences of bioaugmented SBR sludge (R1), non-bioaugmented SBR sludge (R2), and seed sludge (SS) with OTUs clustered at 97% sequence identity.