# Different roles of dioxin-catabolic plasmids in growth, biofilm formation, and metabolism of *Rhodococcus* sp. strain p52

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*Supplemental material Content*

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Figure S8: Cluster heat map for the gene expression levels of the strain p52, p52 (pDF01⁻, pDF02), and p52 (pDF01⁻, pDF02⁻);

Figure S9: Scatter plots of the differentially expressed genes in strain p52 after plasmid loss.

Table S1: The primers used in this study.

*Measurement of* strain p52 *Growth on different concentrations of dibenzofuran*

The growth of strain p52 on dibenzofuran at different concentrations were evaluated using 250 mL Erlenmeyer flasks, each containing 50 g (dry weight) of sterilized soil that had been sieved through a 2 mm mesh. Dibenzofuran stock solution was mixed with water and sprayed to the soil samples at concentrations of 0 mg/kg, 100 mg/kg, and 1000 mg/kg (soil). Then, strain p52 was inoculated into the soil to achieve a final concentration of $10^6$ to $10^7$ CFU/g (soil). During the experiment, soil samples were collected, and soil suspensions were prepared. The suspensions were serially diluted and spread onto dibenzofuran-supplemented agar plate to assess bacterial growth. Three replicates were set up for each sample.
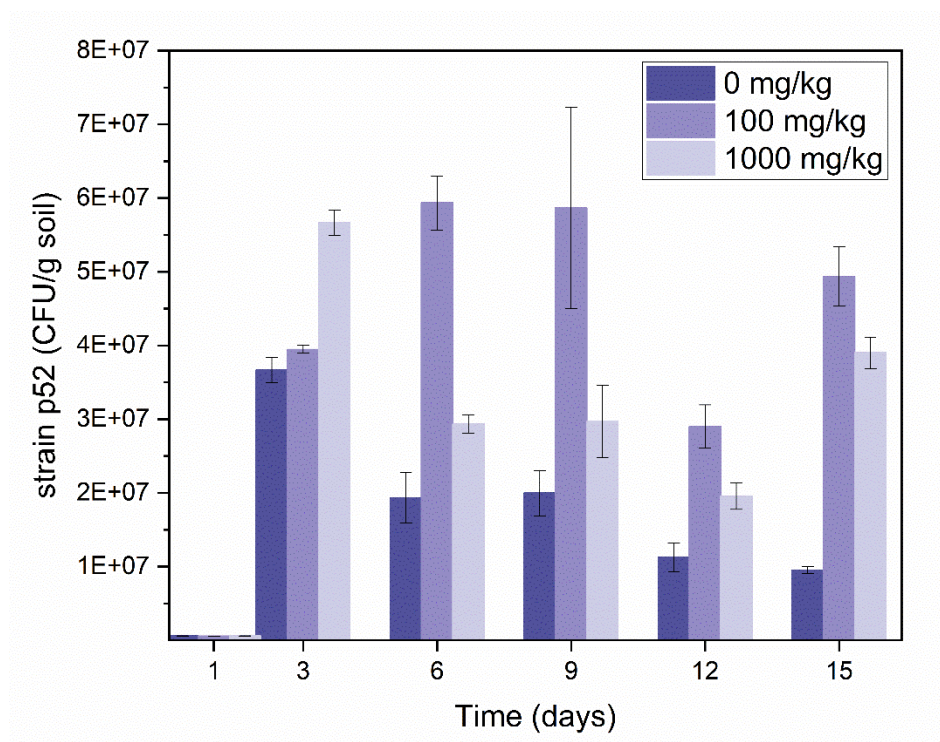
**Figure S1.** Growth of strain p52 on different concentrations of dibenzofuran in the soil.
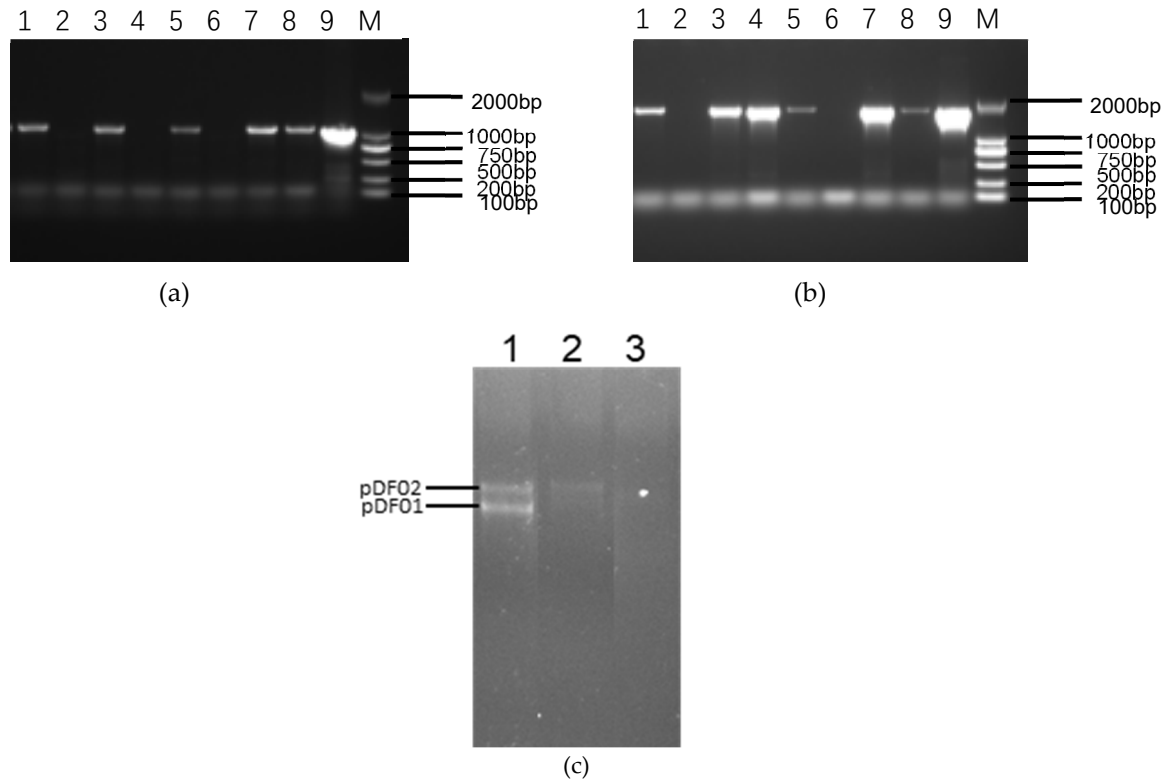
**Figure S2**. Confirmation of plasmid loss. (a) Electrophoresis analysis of the amplification product targeting the angular dioxygenase gene *dfdA* (1.06 kb) located on pDF01. (b) Electrophoresis analysis of the amplification product targeting the angular dioxygenase gene *dbfA* (1.84 kb) located on pDF02. DNA Maker is shown in Lane M, and Lane 9 is the amplification result of plasmid-bearing clone of p52 as a positive control. The detected clones are shown in Lanes 1−8. (c) Electrophoresis analysis of plasmid DNA extracted from strain p52, p52 (pDF01⁻, pDF02), and p52 (pDF01⁻, pDF02⁻). Lanes 1, 2, 3 correspond to strain p52, p52 (pDF01⁻, pDF02), and p52 (pDF01⁻, pDF02⁻), respectively.
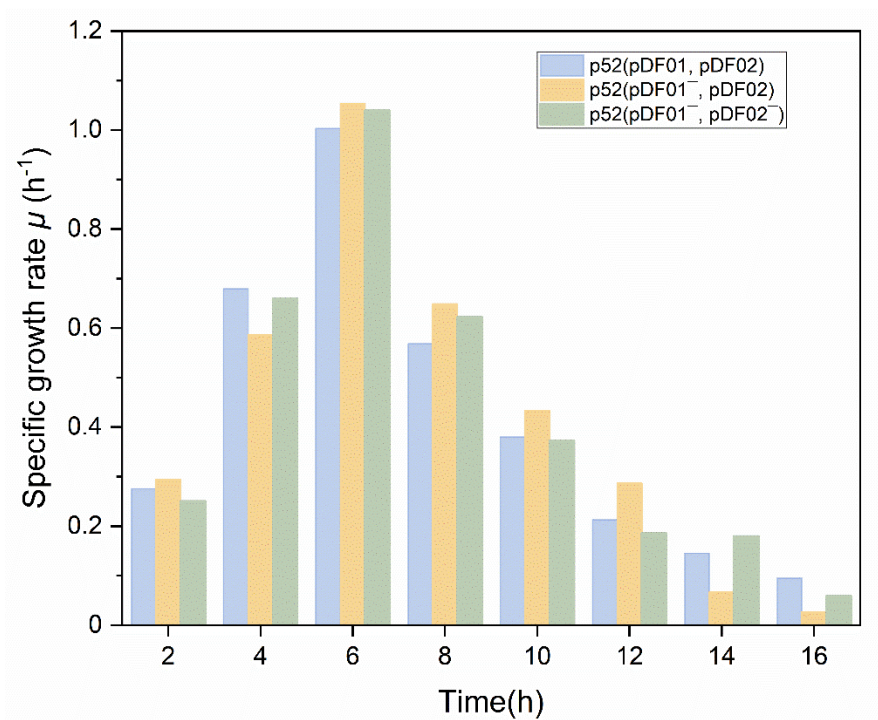
**Figure S3.** Specific growth rate of the clones of strain p52, p52 (pDF01⁻, pDF02), and p52 (pDF01⁻, pDF02⁻) without selective pressure.
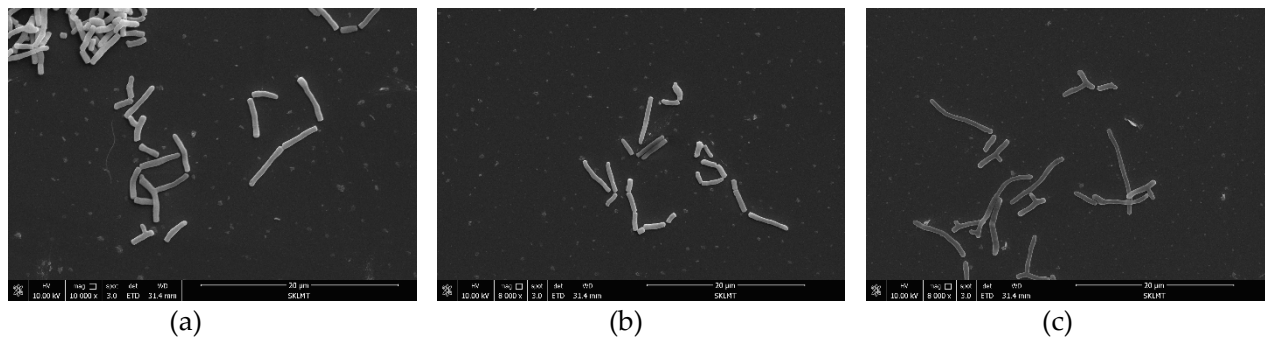
(a)                          (b)                          (c)

**Figure S4**. SEM images of the clones of (a) strain p52, (b) p52 (pDF01⁻, pDF02), and (c) p52 (pDF01⁻, pDF02⁻) at the mid-logarithmic phase.

**Figure S5**. Morphological analysis of the clones of strain p52 at early and late logarithmic phase on a population level using flow cytometry. The clones of strain p52, p52 (pDF01⁻, pDF02) and p52 (pDF01⁻, pDF02⁻) are in blue, yellow, and green lines, respectively. The upper three figures represent the strains in the early logarithmic phase ($OD_{600}$ = 0.5) (a-c), while the lower three figures represent the strains in the late logarithmic phase ($OD_{600}$ = 1.7) (d-f). The normalized frequency of forward scatter area to estimate cell size is shown in (a)&(d). The normalized frequency of aspect ratio to estimate cell shape is shown in (b)&(e). The normalized frequency of side scatter intensity to estimate cellular granularity is shown in (c)&(f).

**Figure S6**. Relative expression level of the genes involved in cell division by RNA-seq in the two comparison groups, p52 (pDF01⁻, pDF02) vs p52, and p52 (pDF01⁻, pDF02⁻) vs p52. The gene ID and name are listed on the left and right, respectively. The relative expression level is expressed as $\log_2$(fold change) and reflected by the color as labeled on the top right.
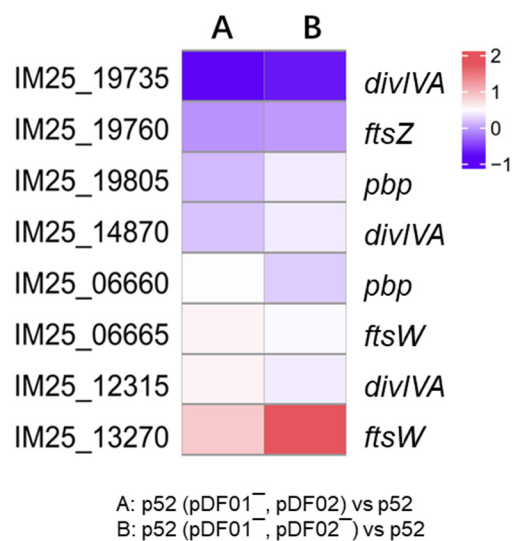
**Gene name and description**

*virB4*: Type IV secretion system protein VirB4
*virD4*: Type IV secretion system coupling protein VirD4
*mobF*: Conjugal transfer protein TraA
*fic*: Cell filamentation protein Fic
*arsR*: ArsR family transcriptional regulator
*cueR*: MerR family transcriptional regulator
*arsB*: Arsenic resistance protein
*arsA*: Arsenical pump-driving ATPase
*cmtR*: ArsR family transcriptional regulator
*cadD*: Cadmium transporter
*copC*: Copper resistance protein CopC
*dfdC*: Alpha/beta hydrolase
*dfdB*: Biphenyl 2,3-dioxygenase
*paaX*: PaaX family transcriptional regulator
*dfdA1*: Aromatic ring-hydroxylating dioxygenase subunit alpha
*dfdA2*: Aromatic-ring-hydroxylating dioxygenase subunit beta
*dfdA3*: Dibenzofuran dioxygenase ferredoxin component
*dfdA4*: Ferredoxin reductase
*parA*: Partitioning protein ParA
*rep*: Replication protein
*repA*: Replication protein RepA
*repB*: Replication protein RepB
*parA*: Chromosome partitioning protein ParA
*parB*: Chromosome partitioning protein ParB
*uvrD*: ATP-dependent helicase
*relE*: Plasmid stabilization protein
*dbfA1*: Rieske (2Fe-2S) protein
*dbfA2*: Aromatic-ring-hydroxylating dioxygenase subunit beta

(a)

(b)

A:RNA processing and modification
B:Chromatin structure and dynamics
C:Energy production and conversion
D:Cell cycle control, cell division, chromosome partitioning
E:Amino acid transport and metabolism
F:Nucleotide transport and metabolism
G:Carbohydrate transport and metabolism
H:Coenzyme transport and metabolism
I:Lipid transport and metabolism
J:Translation, ribosomal structure and biogenesis
K:Transcription
L:Replication, recombination and repair
M:Cell wall/membrane/envelope biogenesis

N:Cell motility
O:Posttranslational modification, protein turnover, chaperones
P:Inorganic ion transport and metabolism
Q:Secondary metabolites biosynthesis, transport and catabolism
R:General function prediction only
S:Function unknown
T:Signal transduction mechanisms
U:Intracellular trafficking, secretion, and vesicular transport
V:Defense mechanisms
W:Extracellular structures
X:Mobilome: prophages, transposons
Y:Nuclear structure
Z:Cytoskeleton
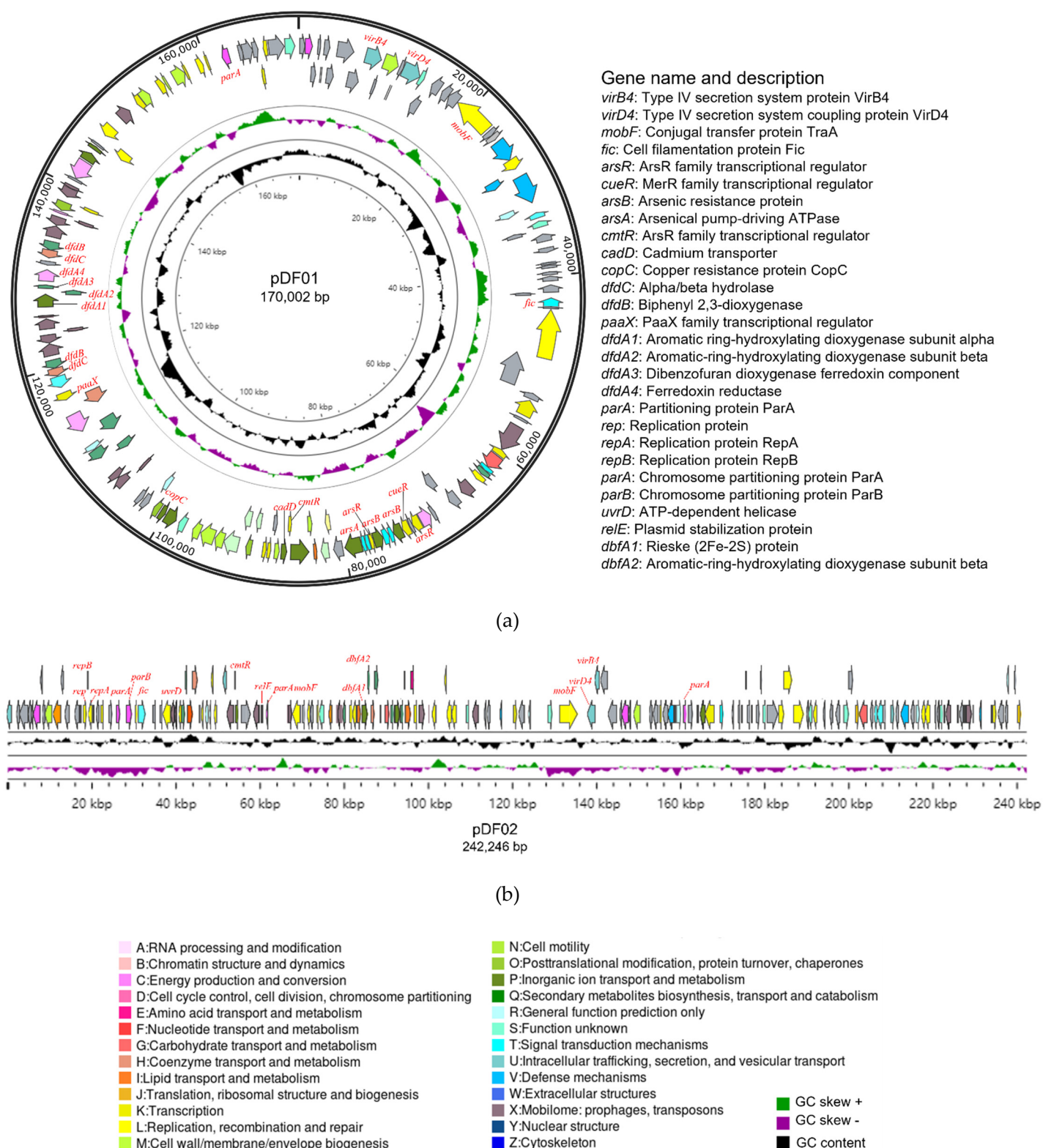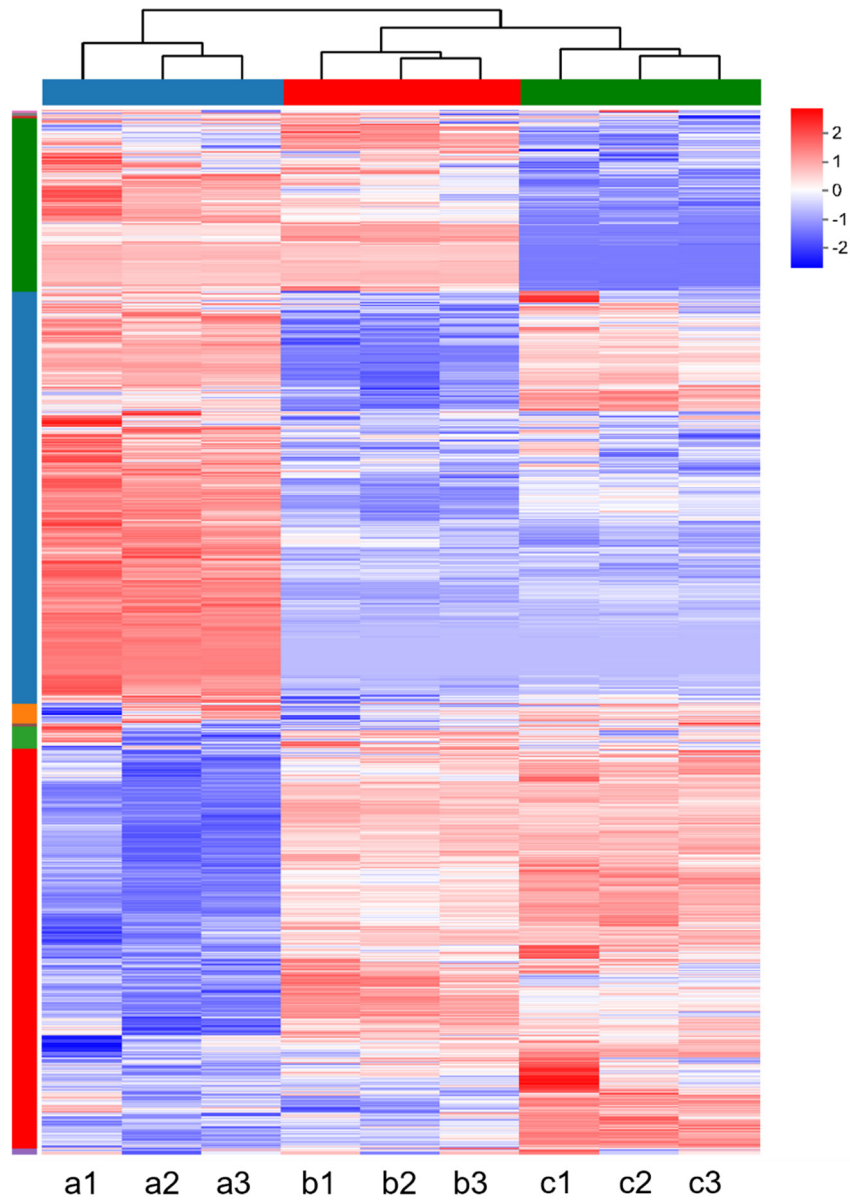
GC skew +
GC skew -
GC content

**Figure S7**. Genetic maps of (a) pDF01 and (b) pDF02, with genes assigned to COG categories and depicted in different colors. The names and descriptions of partial genes are shown on the right. Rings inside represent GC content (black) and GC skew (green and purple). The COG category assignments are present at the bottom of the figure.

p52 (pDF01, pDF02): a1, a2, a3

p52 (pDF01⁻, pDF02): b1, b2, b3

p52 (pDF01⁻, pDF02⁻): c1, c2, c3

**Figure S8**. Cluster heat map for the gene expression levels of the strain p52, p52 (pDF01⁻, pDF02), and p52 (pDF01⁻, pDF02⁻). Each sample was replicated three times biologically. The color represents the gene expression levels through normalizing the data. Logarithmic transformation was applied to (TPM+1), followed by Z-score standardization.
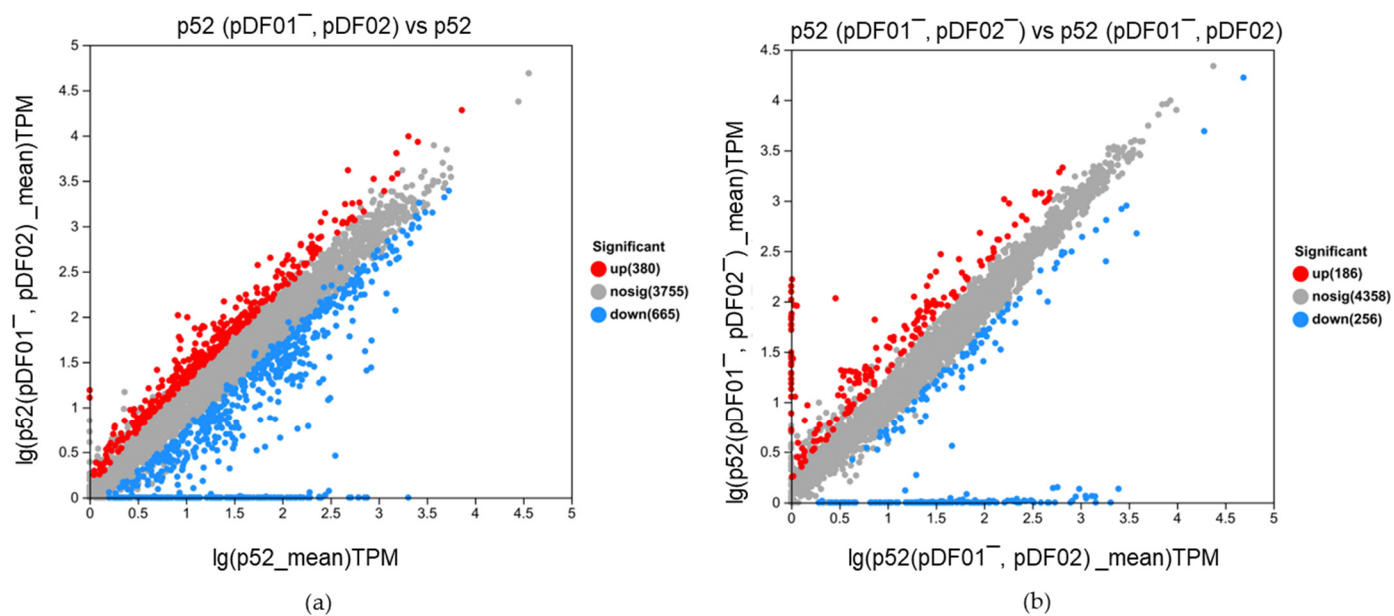
**Figure S9.** Scatter plots of the differentially expressed genes in strain p52 after plasmid loss. The DEGs in group p52 (pDF01⁻, pDF02) vs p52 is shown in (a) and that in group p52 (pDF01⁻, pDF02⁻) vs p52 (pDF01⁻, pDF02) is shown in (b).

**Table S1**. The primers used in this study.

| Targets | Oligonucleotide sequence (5′ to 3′) | Amplicon size (bp) | Location |
|---|---|---|---|
| **Primers used for PCR** | | | |
| *dfdA* | F: GCAGTCTGTACCGACGCT<br>R: GAGTGCGACGGGATGGAC | 1061 | pDF01 |
| *dbfA* | F: GCTCATGACCAGCATTAGCG<br>R: GGGCCTCAGAAGAAGATGGAG | 1841 | pDF02 |
| **Primers used for qPCR** | | | |
| Reference gene | | | |
| *rpoB* | F: TGGGTGACTTCCCGATGA<br>R: TGACCTTGACGCTGTGC | 153 | chromosome |
| Dibenzofuran degradation genes on pDF01 and pDF02 | | | |
| *dfdA1* | F: CTTCTTCTCGACCTACAT<br>R: GGCAGACCTTCATCCCAC | 110 | pDF01 |
| *dfdA2* | F: CGACGACGACAAACCGAA<br>R: CGATAGACGAGGAAGTGGG | 171 | pDF01 |
| *dfdA3* | F: GGACGAAGGTCTGTGCC<br>R: ACGAGTGCGACGGGAT | 234 | pDF01 |
| *dfdA4* | F: CTTCGTCAGTCCGTTCAC<br>R: TGGAGCCGATGTCCGTAT | 156 | pDF01 |
| *dfdB* | F: GGCGAGAAGACGACCAGC<br>R: CGAAGCCAGCACTACCAA | 163 | pDF01 |
| *dfdC* | F: CGAAGGGCTGAAAGTGA<br>R: ATGCCACGGACGGTAT | 228 | pDF01 |
| *IM25_24385* | F: TGGGTGACTTCCCGATGA<br>R: TGACCTTGACGCTGTGC | 153 | pDF01 |
| *IM25_24375* | F: TCCGGCACTCTCTCTGCTTT<br>R: TTTCATCACGGGTCGCTGG | 106 | pDF01 |
| *IM25_24380* | F: CTGACCGGCTACACCGAC<br>R: ACACGATCTTTCCCGGTGTG | 136 | pDF01 |
| *dbfA1* | F: AGGCGATCGAGTACGTCAAC<br>R: ATTGGAAGAGCCCGAAGACG | 153 | pDF02 |
| *dbfA2* | F: CCAAGCGGATGGAGCG<br>R: GATGGCACGGGGCAAC | 272 | pDF02 |
| *IM25_23265* | F: GTGGTGGAGATTGGCCTCTT<br>R: GGTCCACTGCTCCTTCATCC | 180 | pDF02 |
| *IM25_23270* | F: TCATTACATCATGCCCGTCCG<br>R: ACGATGATTTCGTCGGGGTT | 219 | pDF02 |
| Genes associated with biofilm formation | | | |
| *galU* | F: AAGAAGAAGCAGGAGTGCGTAA<br>R: GTTCGATGCCCGGCGTGT | 135 | chromosome |
| *pgm* | F: GTTCGATGCCCGGCGTGT<br>R: AGCAGGGCGAGGACGA | 226 | chromosome |
| *galE* | F: CGACGTTGAAGTACCGCAGAC<br>R: TACGGCGAACCCGACAGC | 154 | chromosome |
| *dgc* | F: TGCTGGGTCGTCTCGAAGTG<br>R: GCATCTGCTGCTGGTGGTG | 210 | chromosome |
| *galT* | F: CGAAGGTGACCCACTCCC | 156 | chromosome |

| | R: GCCGGGAACTGATCTATT | | |
|---|---|---|---|
| *pde* | F: CGTGGTGTACCGCGGAGT<br>R: CGCAGACGCACAAGTAGTTTCA | 229 | chromosome |
| *galK* | F: GCCCTGCCCGCAACAACT<br>R: GTGAGGTCGAAGACGCCA | 159 | chromosome |