

1

2

Supporting Information for

3

4 **New Metabolites in Dibenzofuran Cometabolic Degradation by a**

5 **Biphenyl-Cultivated *Pseudomonas putida* Strain B6-2**

6

7 Qinggang Li, Xiaoyu Wang, Guangbo Yin, Zhonghui Gai,

8 Hongzhi Tang, Cuiqing Ma, Zixin Deng, and Ping Xu *

9

10 11 pages

11 2 tables

12 6 figures

Table S1. Carbon utilization of strain B6-2

substrate	growth	substrate	growth
Water	–	Itaconic Acid	–
α -Cyclodextrin	–	α -Ketobutyric Acid	+
Dextrin	+	α -Ketoglutaric Acid	+
Glycogen	\pm	α -Ketovaleric Acid	+
Tween 40	–	D,L-Lactic Acid	+
Tween 80	\pm	Malonic Acid	\pm
N-Acetyl-D-Galactosamine	+	Propionic Acid	+
N-Acetyl-D-Glucosamine	+	Quinic Acid	–
Adonitol	+	D-Saccharic Acid	\pm
L-Arabinose	+	Sebacic Acid	–
D-Arabitol	–	Succinic Acid	+
D-Cellobiose	+	Bromosuccinic Acid	+
i-Erythritol	+	Succinamic Acid	–
D-Fructose	+	Glucuronamide	\pm
L-Fucose	\pm	L-Alaninamide	+
D-Galactose	+	D-Alanine	+
Gentiobiose	+	L-Alanine	+
α -D-Glucose	+	L-Alanyl-Glycine	+
m-Inositol	+	L-Asparagine	+
α -D-Lactose	+	L-Aspartic Acid	+

Lactulose	+	L-Glutamic Acid	+
Maltose	+	Glycyl-L-Aspartic Acid	+
D-Mannitol	+	Glycyl-L-Glutamic Acid	–
D-Mannose	+	L-Histidine	+
D-Melibiose	+	Hydroxy-L-Proline	–
b-Methyl-D-Glucoside	+	L-Leucine	±
D-Psicose	+	L-Ornithine	–
D-Raffinose	+	L-Phenylalanine	–
L-Rhamnose	–	L-Proline	+
D-Sorbitol	+	L-Pyroglutamic Acid	±
Sucrose	+	D-Serine	+
D-Trehalose	+	L-Serine	+
Turanose	+	L-Threonine	+
Xylitol	+	D,L-Carnitine	–
Pyruvic Acid Methyl Ester	+	g-Aminobutyric Acid	–
Acetic Acid	+	Urocanic Acid	±
cis-Aconitic Acid	+	Inosine	+
Citric Acid	+	Uridine	+
Formic Acid	–	Thymidine	+
D-Galacturonic Acid Lactone	±	Phenylethylamine	–
D-Galacturonic Acid	+	Putrescine	–
D-Gluconic Acid	+	2-Aminoethanol	–

D-Glucosaminic Acid	–	2,3-Butanediol	–
D-Glucuronic Acid	+	Glycerol	+
a-Hydroxybutyric Acid	±	D,L-a-Glycerol phosphate	+
b-Hydroxybutyric Acid	+	a-D-Glucose-1-Phosphate	+
g-Hydroxybutyric Acid	–	D-Glucose-6-Phosphate	–

- 1 +, Positive. –, Negative. ±, Weak growth. Plate type: GN2, incubation time:
- 2 16-24h. Experiments were carried out using Biolog Microbial Identification System
- 3 (Biolog, California, USA).
- 4 According to these results, strain B6-2 was assigned to *Pseudomonas* sp.

1

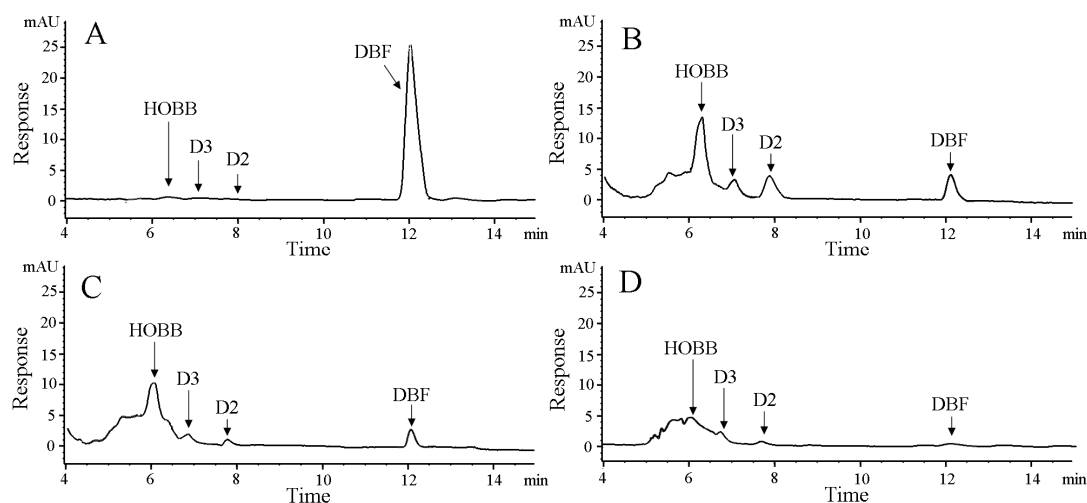
Table S2. Physical and biochemical characteristics of strain B6-2

characteristics	results
Bacteriological tests:	
Shape of cells	2.0-3.5 μm length; 0.6-0.8 μm width
Motility	+
Flagella	> 1 at polar
Gram reaction	–
Lysis in 3% KOH	+
Growth at 41°C	–
Biochemical tests:	
Aminopeptidase	+
Oxidase	+
Catalase	+
Arginine dihydrolase	+
Urease	–
Denitrification	–
Lecithinase	–
Hydrolysis of gelatine	–
Hydrolysis of esculin	–
Hydrolysis of tween 80	–

2 +, Positive. –, Negative. Experiments were carried out at Deutsche Sammlung
3 von Mikroorganismen und Zellkulturen GmbH (DSMZ).

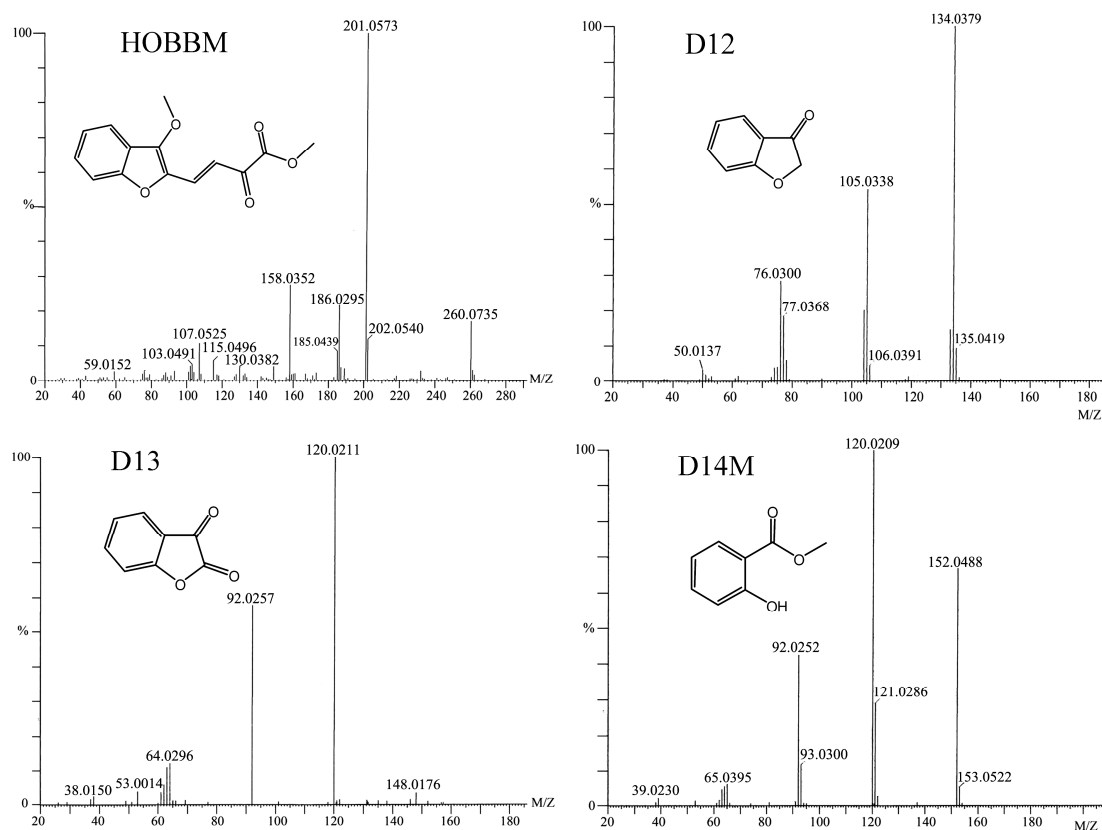
4 The data shown in table S2 are also characteristics of *Pseudomonas* sp.

1



2

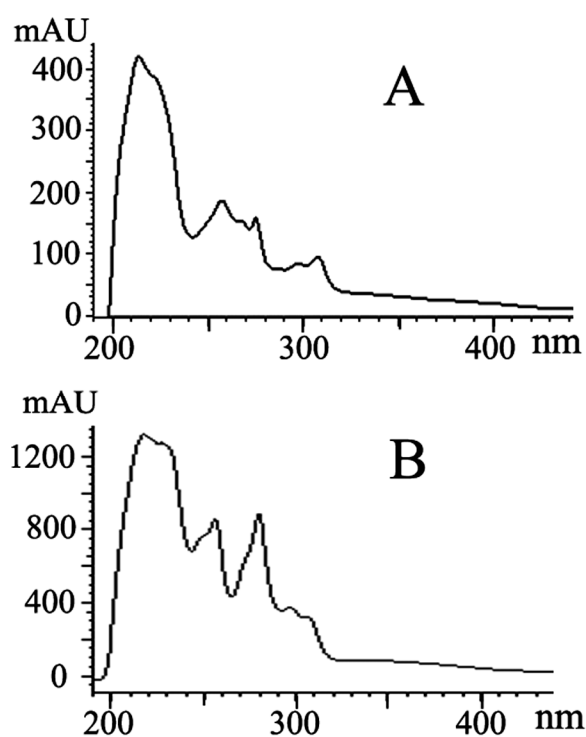
3 Figure S1. Portions of HPLC profiles of DBF degradation samples by the
 4 BP-grown B6-2 cell suspension. A, after 0-h incubation; B, after 5-h incubation; C,
 5 after 11-h incubation; D, after 24-h incubation. DBF, dibenzofuran; HOBB (D1),
 6 2-hydroxy-4-(3'-oxo-3'*H*-benzofuran-2'-yliden)but-2-enoic acid; D2,
 7 1-hydroxydibenzofuran; D3, 3-hydroxydibenzofuran. The mobile phase for HPLC
 8 analyses was methanol–water (70:30) at a flow rate 0.5 ml/min, and HOBB could not
 9 be separated under this conditions. To calculate the amount of HOBB, another
 10 mobile phase (acetonitrile–1 mmol/L H₂SO₄ [14:86] at a flow rate 0.7 ml/min) was
 11 applied, and the results are shown in Figure 1 in the article.



1

2 Figure S2. HR-MS data of dimethylated
3 2-oxo-4-(3'-hydroxy-3'*H*-benzofuran-2'-yl)but-3-enoic acid (HOBMM),
4 benzofuran-3(2*H*)-one (D12), benzofuran-2,3-dione (D13) and methylated salicylic
5 acid (D14M).

1

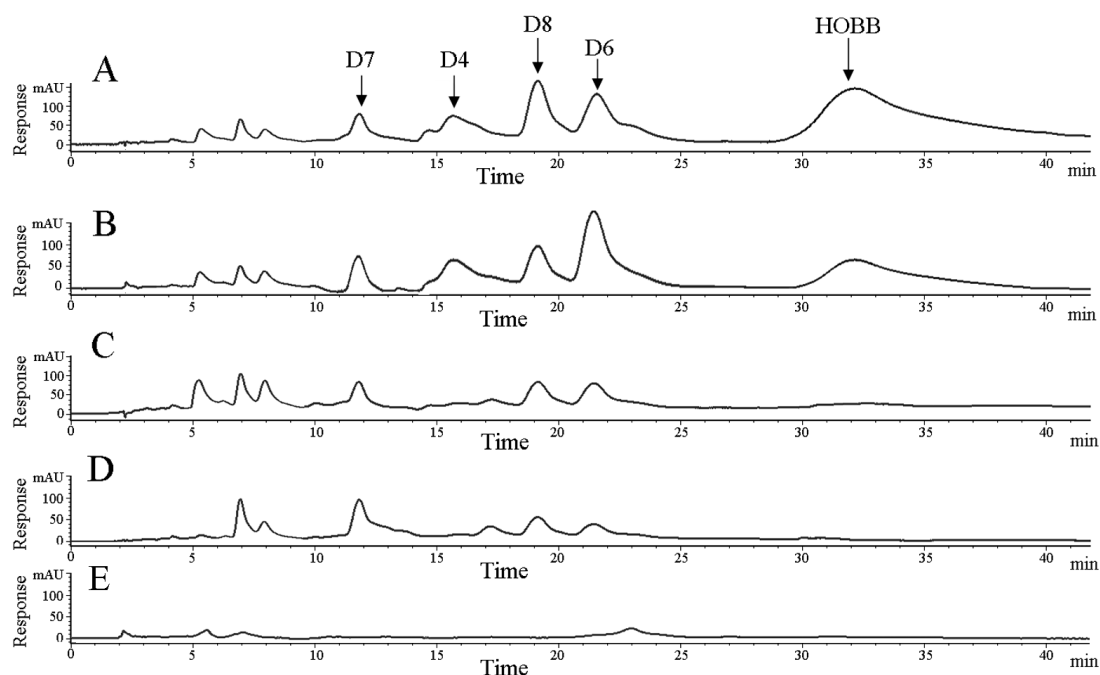


2

3

4 Figure S3. UV-Vis spectra of 1-hydroxydibenzofuran (A) and
5 4-hydroxydibenzofuran (B)

1

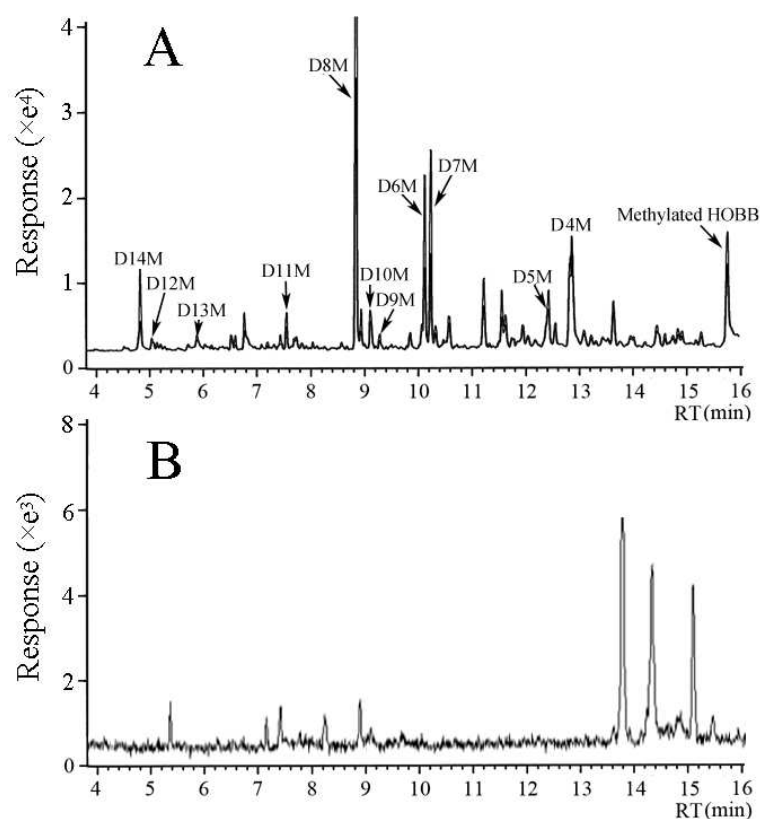


2

3

4 Figure S4. HPLC profiles of HOBB degradation samples by BP-grown B6-2 cell
 5 suspension. A, after 4-h incubation; B, after 8-h incubation; C, after 16-h incubation;
 6 D, after 24-h incubation; E, control sample without HOBB (a mixture of cultures after
 7 4-h and 16-h incubation). The identificaiton of major metabolites D4, D6, D7 and
 8 D8 are described in the article. D4, 2-hydroxy-4-(3'-oxobenzofuran-2'-yl)butanoic
 9 acid; D6, 3-(3'-oxobenzofuran-2'-yl)propanoic acid; D7,
 10 3-hydroxy-3-(3'-oxobenzofuran-2'-yl)propanoic acid; D8,
 11 2-(3'-oxobenzofuran-2'-yl)acetic acid.

12 As shown in Figure S4, the major metabolites (D4, D6, D7 and D8) of HOBB were
 13 not detected in control sample.



1

2 Figure S5. Portions of the total ion current chromatograms from the HR-MS
3 analyses of the diazomethane derivatized HOBB-degradation sample (A) and control
4 sample without HOBB (B).

5 Figure S5A has been shown in the article (Figure 3B). For comparison with
6 Figure S5B, it is also shown here. It may be due to the effects of HOBB and its
7 metabolites on B6-2 cell activities that the peaks in Figure S5A and Figure 5B are
8 rather different. However, according to the different retention times and MS profiles
9 (data for compounds in the control sample not shown) of HOBB metabolites in Figure
10 S5A and compounds in Figure S5B, it is obvious that the metabolites of HOBB were
11 not produced in the control sample.

12

