Supplementary Materials: Removal and Ecotoxicity of 2,4-D and MCPA in Microbial Cultures Enriched with Structurally-Similar Plant Secondary Metabolites

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Supplementary A

Text S1. Soil characteristics: The soil was collected from a crop field in Wólka Wojslawska, Zduńska Wola district, Lodz voivodship, Central Poland (51°38'45.169" N, 18°56'14.582" E). The soil was obtained using a manual composite technique in which soil samples were taken from random sites within the area, and six to eight subsamples were mixed thoroughly in the laboratory. The general physico-chemical parameters of the soil are presented in Table 1 (Supplementary)

Text S2. Soil extract preparation: The soil extract was prepared according to Strzelczyk (1968) with some modifications. Briefly, 500 g of soil was sieved through a 2 mm sieve and flooded with 1.5 L of water. The suspension was mixed and left at room temperature on a gentle shaker for 24 hours. After that, the soil extract was decanted and filtered through Whatman paper. The extract used for sterile samples (controls) was further filtered through a Corning Disposable Vacuum Filter (0.22 μL).

Table S1. Mean and Standard Deviation for Physical and Chemical Properties of Soil; *Significant Differences at $\alpha \leq 0.05$ According to the Mann-Whitney U-Test [36].

Properties	Unit	Control Soil
pH (H_2O)		5.58
pH (KCl)		4.31
N	%	0.10 ± 0.00
C		0.98 ± 0.02
S		0.02 ± 0.00
P		620 ± 20
K		1010 ± 170
P_2O_5		1515 ± 5.00
K_2O		345 ± 1.60
Na		110 ± 0.00
Mg		690 ± 20
Ca	mg/kg	770 ± 60
Fe		4130 ± 100
Cr		10.18 ± 2.95
Mn		208.51 ± 9.92
Ni		4.07 ± 0.13
Cu		12.78 ± 0.37
Zn		41.00 ± 3.20
Cd		1.23 ± 0.11
Pb		13.53 ± 1.99

Supplementary B

Text S3. GC-MS analysis: The injection volume of 2 μL was selected for all analyzes. Helium (purity 99.999995%) was used as a carrier gas and was supplied by Air Products (Warsaw, Poland). The flow of carrier gas was 1 mL min^{-1}, with constant flow conditions being observed throughout. Chromatographic separation in standard conditions was performed; the temperature programme ran from 70 °C to 240 °C (at 12 °C min^{-1}) with a total duration of 14.17 min. The mass spectrometer was operated in selected-ion-monitoring (SIM) mode with solvent delay of 5 min. MS conditions were following: ion source temp. 200 °C, interface temp. 250 °C, ionization voltage 70 eV, emission current 150 μA. Three specific ions (141, 77 and 200) were selected for MCPA pesticide and were used to identify the compound. The first ion, the more intensive one, was used for measurement and the other two for confirmation. For presence of MCPA residues in tested samples, before validation study, mass spectrometer operated in the SCAN mode was firstly applied. Thus, false positives and additional errors were eliminated.

Supplementary C

Table S2. Target Genes, PCR Rimers and Their Optimal Annealing Temperature.

Target Gene	Primer (5'–3′)	Fragment Size (bp)	Optimal Annealing Temperature (°C)	Literature
16S rRNA	F:CCTACGGGAGGCAGCAG R: ATTCCGCGGCTGGCA	154	50	[1]
16S rRNA	F:AGAGTTTGATCCTGGCTCAG R: GGTTACCTTGTTACGACTT	1300–1400	55	[2]
tfdAα	F:CSGAGTTCKSCGACATGCG R:GCGGTTGTCCCACATCAC	350	66	[3]
tfdA Class I	F:GTGAGCGTCGTCGCAAAT R:GCATCGTCCAGGGTGGTC	856	56	[4]
tfdA Class II	F:TGAGCATCAATTCCGAATACC R:AAGACTGACCCCGTGGACT	882	53	[4]
tfdA Class III	F:TGAGCATCACTTCCGAATACC R:ACAGCGTCGTCCAACGTC	856	56	[4]
tfdA	F:GAGCACTACGCRCTGAAYTCCCG R:CTTCGGCCACCGGAAGGCCT	210	64	[3]

Supplementary D

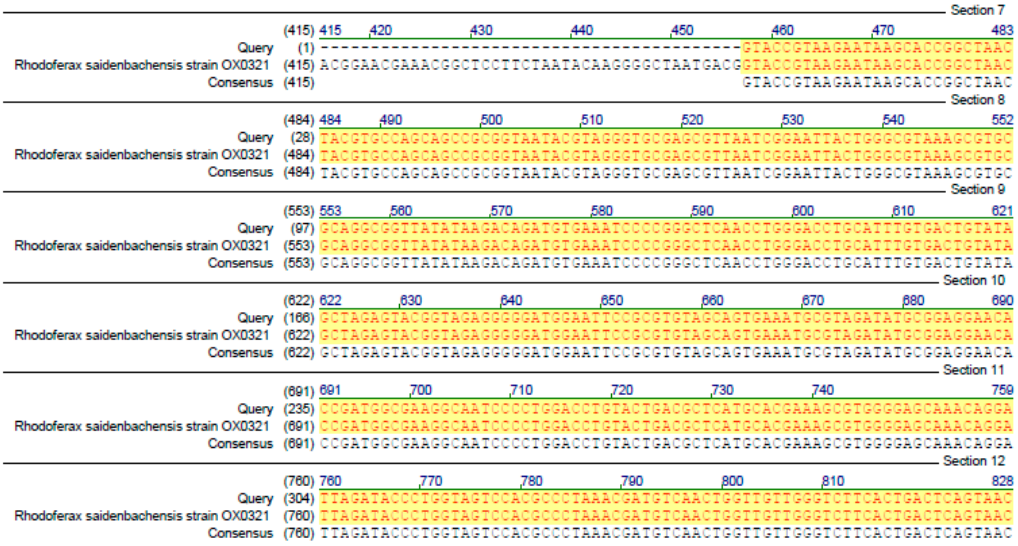

Figure S1. The Alignment Analysis of *16S rRNA* Gene Fragment (1300–1400 bp) Amplified in Samples Enriched with MCPA and Siringic Acid (Query Sequence) and Nucleotide Sequence of *Rhodoferax Saidenbachensis* OX00321.

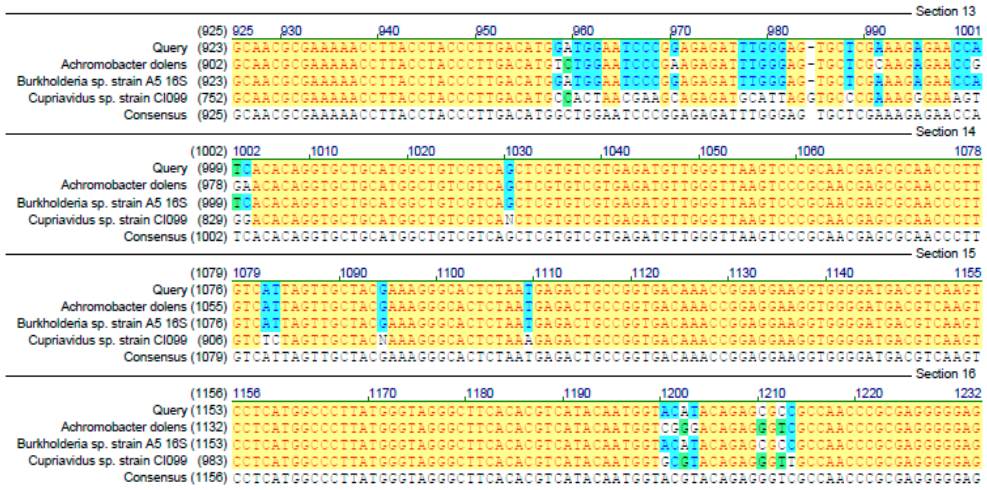

Figure S2. The Alignment Analysis of *16s rRNA* Gene Fragment (1300–1400 bp) Amplified in Samples Enriched with Mcpa and Syringic Acid (Query Sequence) and Nucleotide Sequence of *Achromobacter Dolens*, *Burkholderia* sp. Strain a5 and *Cupriavidus* sp. Strain ci099.

References

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