

Features of diclofenac biodegradation by *Rhodococcus ruber* IEGM 346

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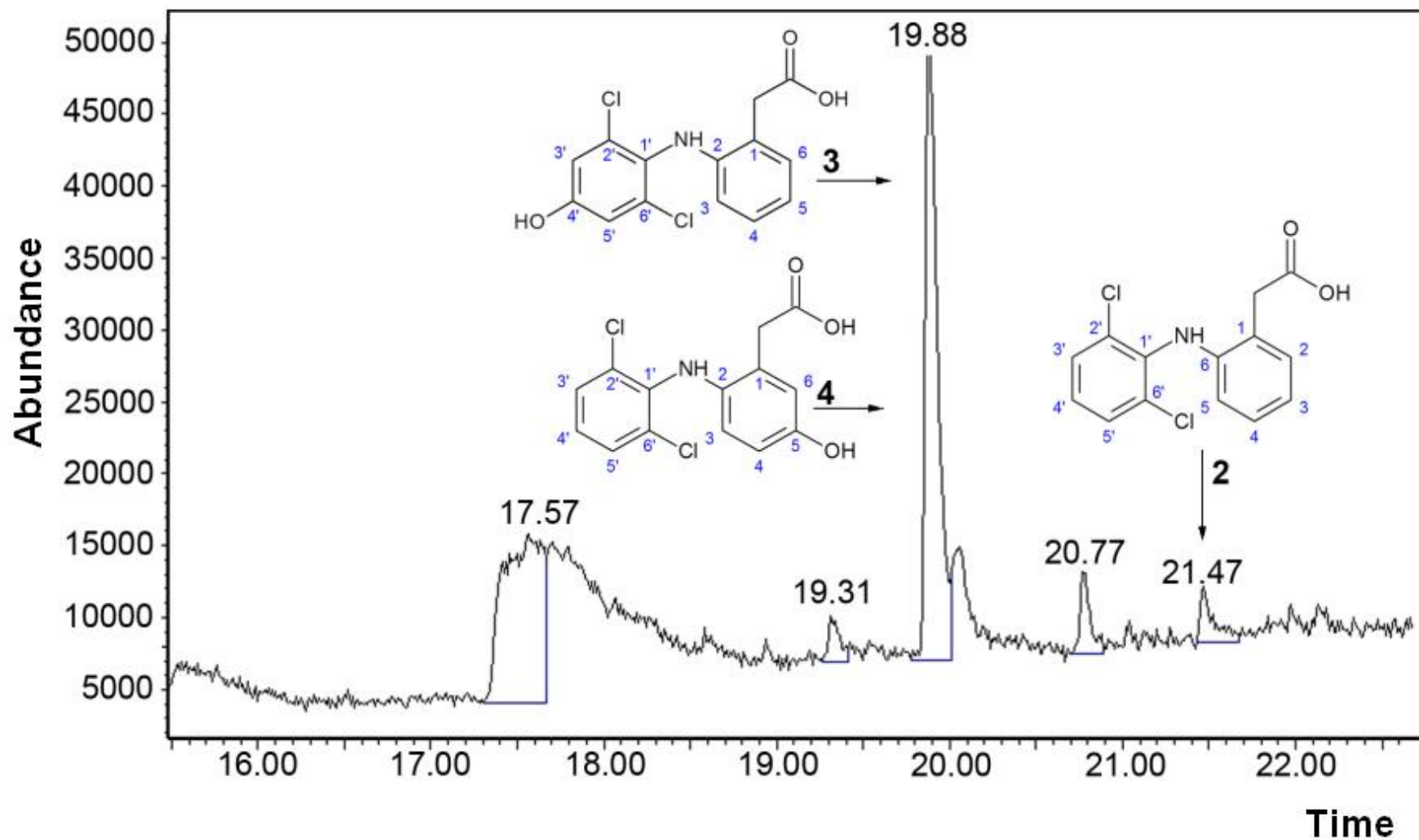
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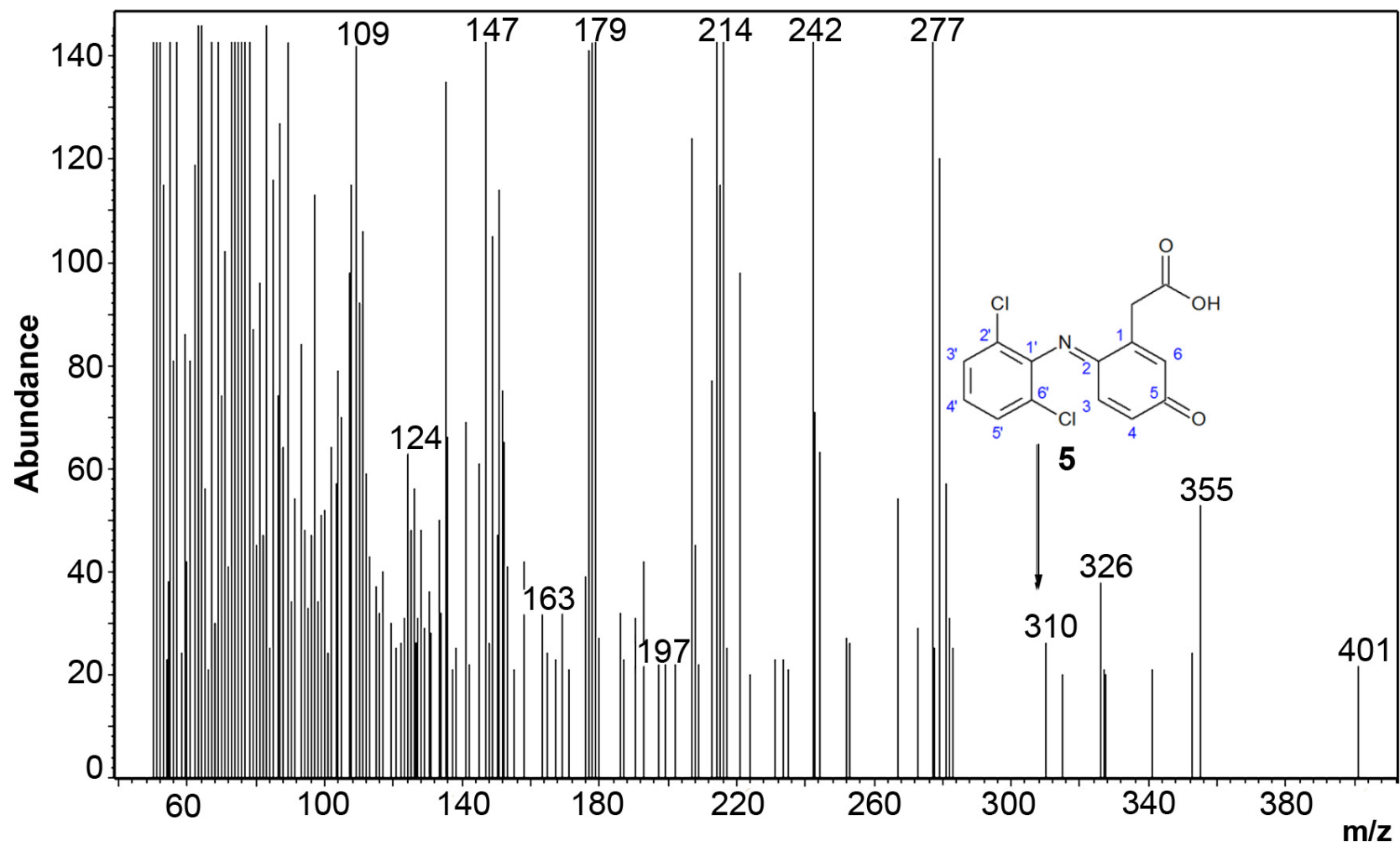
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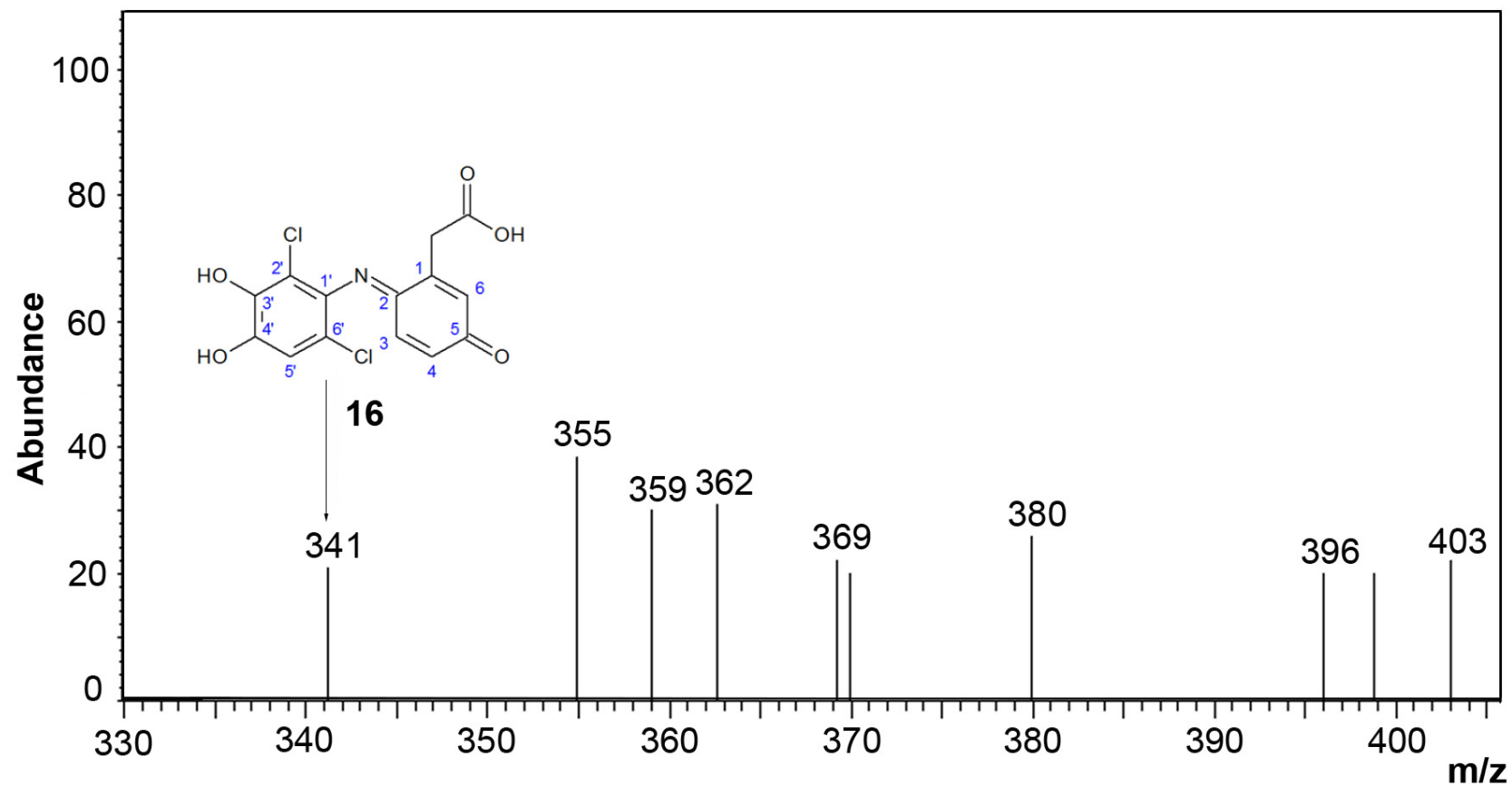
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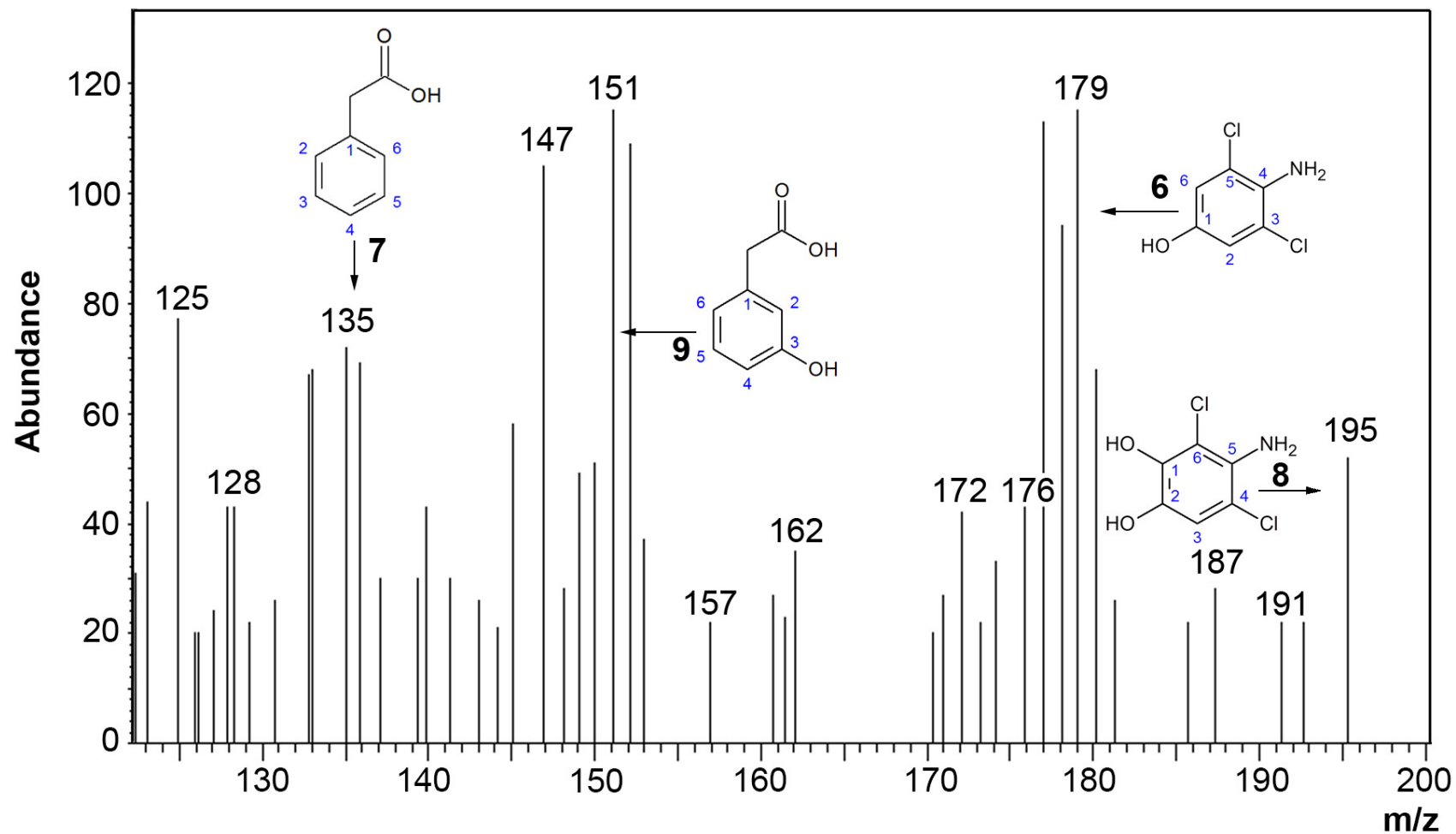
Supplementary Figure S1. Chromatogram of DCF metabolites detected in the post-fermentation medium of rhodococci at the beginning of the biodegradation process. **2** – 2-[2-(2',6'-dichloroanilino)phenyl]acetic acid; **3** – 2-[2-(2',6'-dichloro-4'-hydroxyanilino)phenyl]acetic acid; **4** – 2-[2-(2',6'-dichloroanilino)-5-hydroxyphenyl]acetic acid.



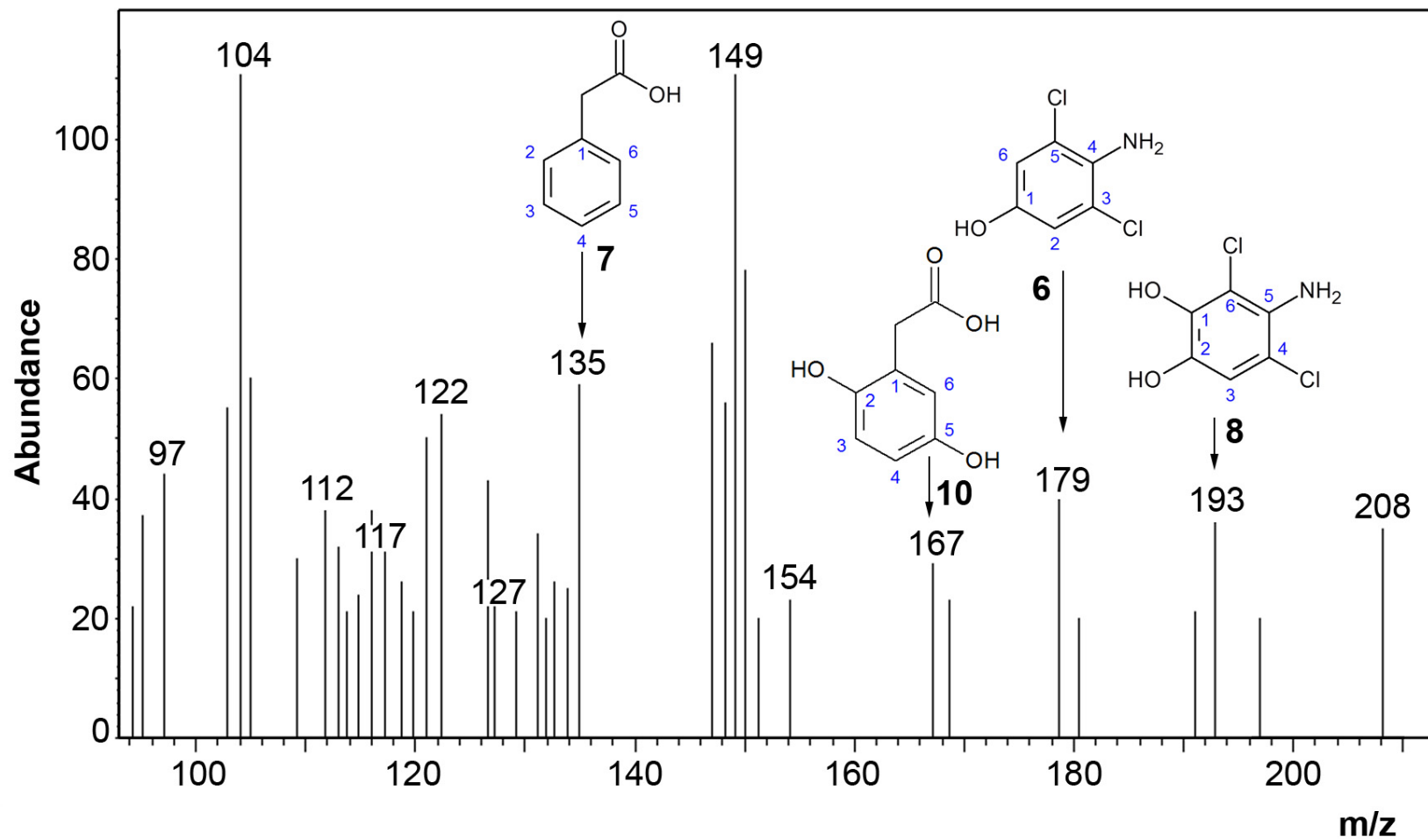
Supplementary Figure S2. Mass-spectrum of **(5)** 2-(1-(5-oxo-cyclohexa-1,3-dienyl-2-(2',6'-dichloro-phenylimino)acetic acid, $m/z=310.0$.



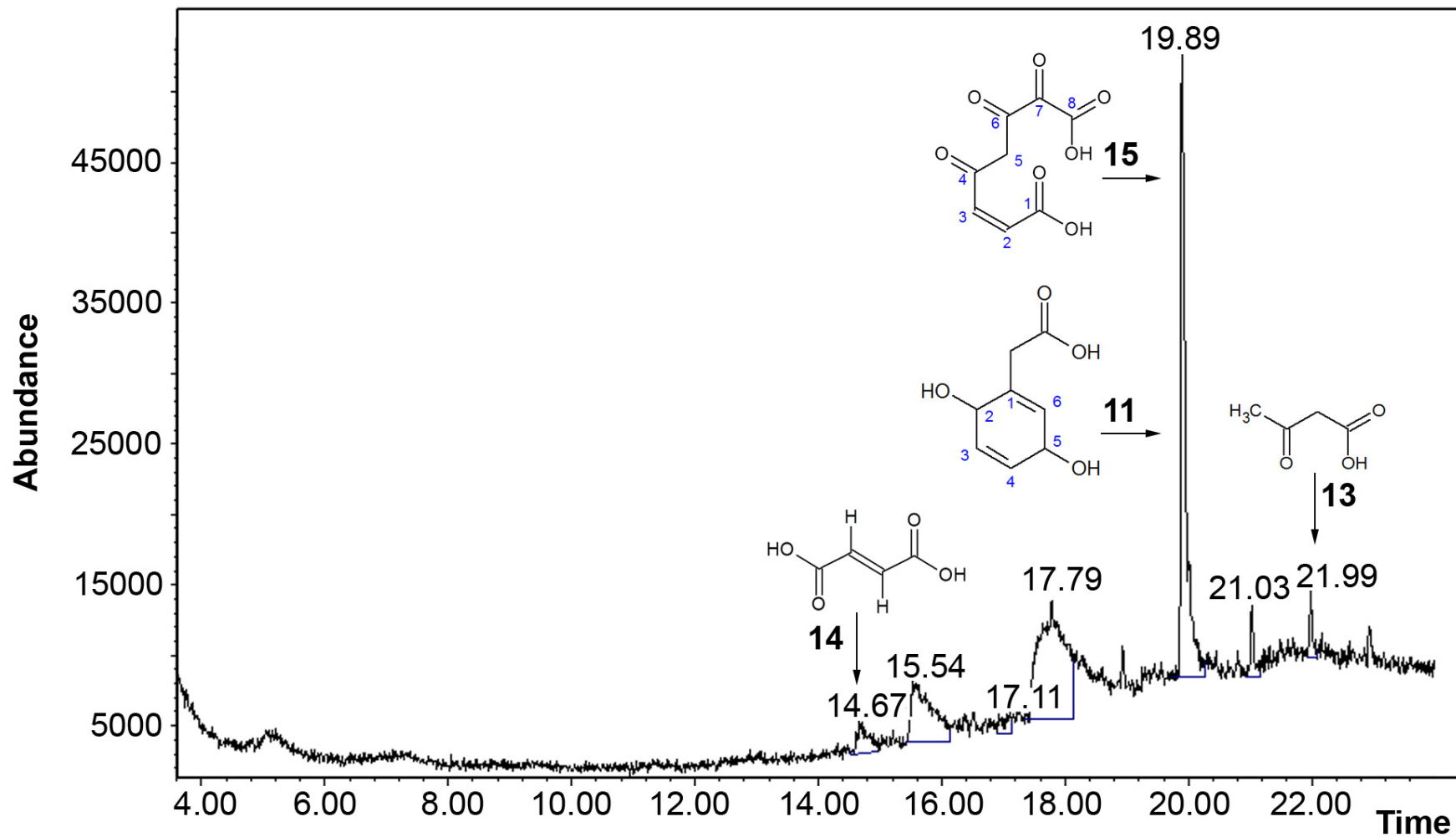
Supplementary Figure S3. Mass-spectrum of (**16**) 2-[1-(5-oxocyclohexa-1,3-dienyl-2-(3',4'-dihydroxy-2',6'-dichlorophenyl)imino)acetic acid, $m/z=340.0$.



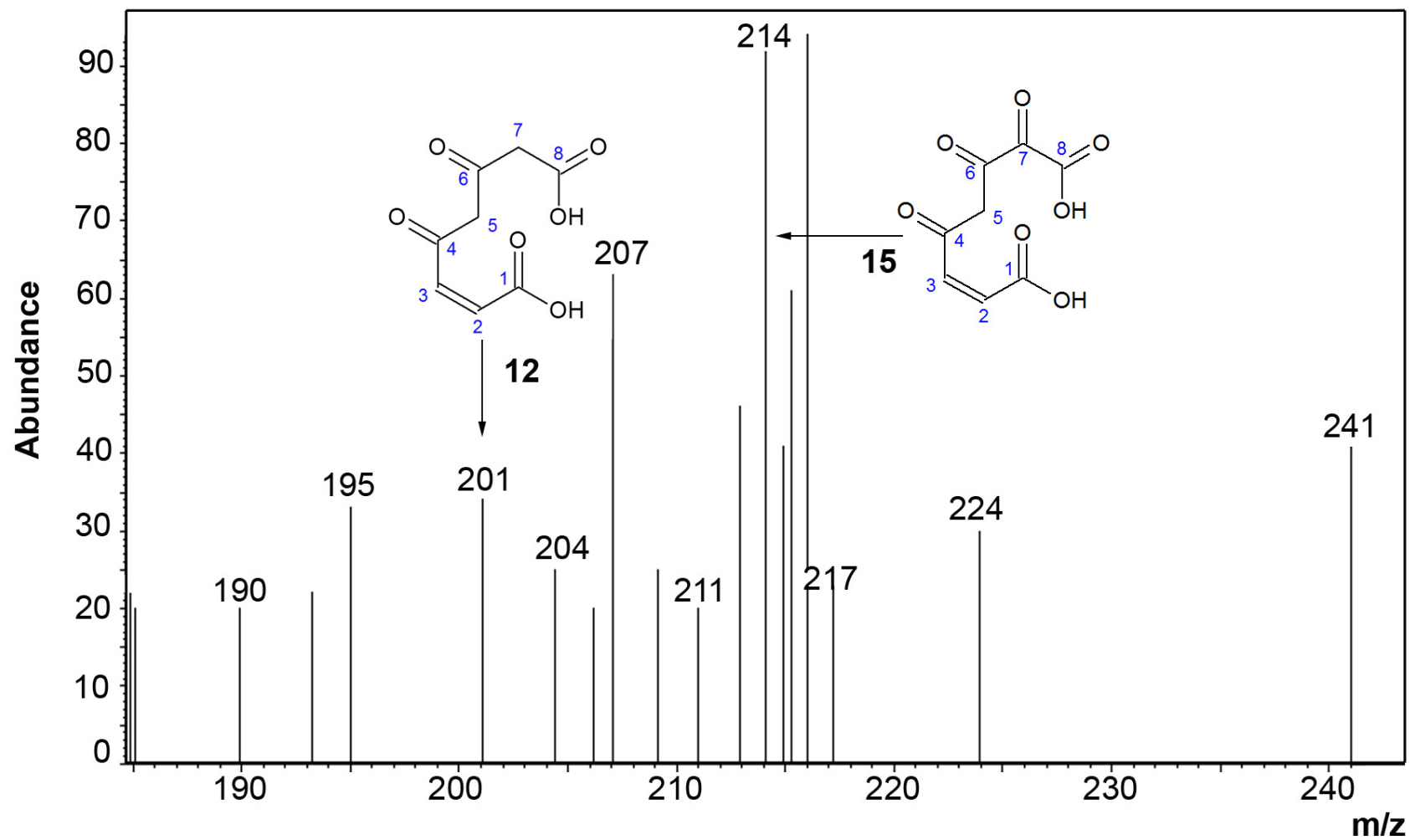
Supplementary Figure S4. Mass-spectra of **(6)** 4-amino-3,5-dichlorophenol, $m/z=178.0$; **(7)** phenylacetic acid, $m/z=136$; **(8)** 5-amino-4,6-dichlorobenzene-1,2-diol, $m/z=194.0$; **(9)** 3-hydroxyphenylacetic acid, $m/z=152.0$.



Supplementary Figure S5. Mass-spectra of **(6)** 4-amino-3,5-dichlorophenol, $m/z=178.0$; **(7)** phenylacetic acid, $m/z=136$; **(8)** 5-amino-4,6-dichlorobenzene-1,2-diol, $m/z=194.0$; **(9)** 3-hydroxyphenylacetic acid, $m/z=152.0$; **(10)** 2,5-dihydroxyphenylacetic acid (homogentisic acid), $m/z=167.0$.

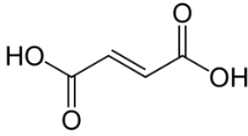
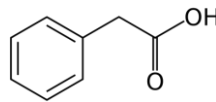
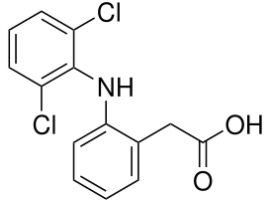


Supplementary Figure S6. Chromatogram of DCF metabolites detected in the post-fermentation medium of rhodococci at the end of the biodegradation process. **11** – 2-(*p*-benzoquinone-2)acetic acid, **13** – 3-oxobutanoic acid (acetoacetic acid), **14** – *trans*-butenedioic acid (fumaric acid), **15** – 4,6,7-trioxooct-2-enedioic acid.



Supplementary Figure S7. Mass-spectra of **(12)** 4,6-dioxo-oct-2-*trans*-enedioic acid (fumarylacetoacetic acid), $m/z=200.0$; **(15)** 4,6,7-trioxooct-2-enedioic acid, $m/z=214.0$.

Supplementary Table S1. R_f values of DCF and its biodegradation products in culture liquids of *R. ruber* IEGM 346.

No.	Test substance	Chemical structure	R _f value
1	Fumaric acid	 <p>Trans isomer</p>	0.83
2	Phenylacetic acid		0.64
3	DCF		0.65
4	Culture fluid containing 50% of residual DCF		0.83 0.65 0.64
5	The culture fluid containing only DCF metabolites (0% diclofenac)		0.83

Note. Each value of R_f is the average of three variables.

Identification of DCF biodegradation products using TLC in the system of benzene: 95% ethanol: glacial acetic acid (45:8:4, v/v/v) showed that fumaric acid was present in sample No. 5 (culture fluid containing only DCF metabolites).