Supplementary materials

Table 1: Conversion rates of aromatics in single, binary and ternary mixtures under different cultivation conditions. The corresponding generation times are given in the last column.
B: benzene T: toluene FB: fluorobenzene CB: chlorobenzene

Cultivation substrate / test substrate(s)	Substrate specific transformation rate [mg C/(L·h·OD)]				Transformation rate total [mg C/L·h·OD)]	Generation time g [h]
	B	T	FB	CB		
B / B	121.4	-	-	-	121.4	6.8
B / T	-	178.2	-	-	178.2	5.0
B / FB	-	-	81.2	-	81.2	14.2
B / B + T	56.2	63.2	-	-	119.4	6.6
B / B + FB	24.5	-	51.0	-	75.4	12.1
B / B + FB + T	24.9	41.8	20.2	-	86.9	8.7
T / B	53.4	-	-	-	53.4	8.3
T / T	-	89.6	-	-	89.6	4.8
T / FB	-	-	40.7	-	40.7	16.4
T / CB	-	-	-	51.4	51.4	6.4
T / B + FB	38.8	-	26.7	-	65.5	5.5
T / B + T	29.4	40.9	-	-	70.3	5.6
T / FB + T	-	41.1	32.7	-	73.8	8.5
T / T + CB	-	25.0	-	20.2	45.1	12.6
T / B + FB + T	25.0	41.5	21.1	-	87.5	8.9
T / B + T + CB	61.8	77.4	-	50.1	189.4	13.5
FB / FB + T	-	50.0	31.7	-	81.7	6.2
FB / B + FB + T	25.7	31.9	21.5	-	79.1	7.4

Table 2: Specific oxygen uptake activities for the initial enzyme(s) of cells of FLU100 pre-grown on fluorobenzene, toluene or benzene. The value in parenthesis represents the number of independent batch cultures tested twice. The first value describes the average value of oxygen uptake while the second one describes the variation. The absolute activity for fluorobenzene grown cells was 465 ± 77.2 units, 623.8 ± 243.7 units for toluene and 629.4 ± 190.1 units for benzene. One unit of oxygenase activity was defined as the conversion of 1 µg $O_2 \cdot L^{-1} \cdot min^{-1} \cdot OD^{-1}$.

	Specific activity fluorobenzene (7) [%]	Specific activity toluene (11) [%]	Specific activity benzene (7) [%]
Mono-halogen aromatics			
fluorobenzene	62.5 ± 11.2	24.3 ± 5.8	28.3 ± 10.6
chlorobenzene	89.9 ± 10.4	63.8 ± 12.2	125.4 ± 27.6
bromobenzene	92.7 ± 12.6	60.6 ± 6.1	119.0 ± 18.0
2-chlorotoluene	-1.5 ± 3.1	-1.3 ± 2.2	2.9 ± 1.1
Methyl aromatics			
benzene	59.2 ± 21.9	43.6 ± 9.6	42.8 ± 13.5
toluene	100.0 ± 4.2	100.0 ± 7.1	100.0 ± 6.6
styrene	27.0 ± 8.2	28.7 ± 8.4	43.8 ± 10.0
cumene	21.9 ± 10.6	15.8 ± 7.0	9.2 ± 5.7
p-cymene	-3.7 ± 4.3	-2.3 ± 3.0	-4.0 ± 6.9
o-xylene	-2.3 ± 2.9	0.2 ± 0.5	-2.7 ± 3.5
m-xylene	-1.4 ± 1.8	-0.5 ± 1.3	-0.3 ± 5.3
p-xylene	2.0 ± 1.8	-0.9 ± 1.9	1.9 ± 0.4
Side chain oxidation			
benzyalcohol	7.5 ± 2.7	7.3 ± 2.9	8.8 ± 4.5
benzaldehyde	105.0 ± 25.5	43.8 ± 11.2	34.2 ± 7.2
benzoate	1.3 ± 3.4	-1.2 ± 2.9	5.6 ± 3.0
o-toluate	1.8 ± 2.4	-2.8 ± 0.7	-2.0 ± 1.5
m-toluate	-0.1 ± 0.9	2.7 ± 2.8	-2.0 ± 2.4
p-toluate	2.9 ± 1.9	4.0 ± 2.2	-6.3 ± 6.8
Phenols			
phenol	11.4 ± 3.5	16.0 ± 9.8	14.2 ± 3.4
o-cresol	5.5 ± 3.9	12.6 ± 5.5	10.2 ± 3.3
m-cresol	18.2 ± 4.5	19.9 ± 9.0	19.8 ± 5.9
p-cresol	6.2 ± 4.5	6.3 ± 5.9	12.3 ± 3.4
2-fluorophenol	4.8 ± 2.4	5.1 ± 4.6	5.3 ± 1.1
3-fluorophenol	13.6 ± 4.6	12.0 ± 3.3	10.8 ± 1.4
4-fluorophenol	9.3 ± 4.1	18.7 ± 4.4	19.0 ± 5.9
2-chlorophenol	0.8 ± 2.3	2.7 ± 2.4	3.1 ± 0.8
3-chlorophenol	15.5 ± 4.3	16.8 ± 5.3	18.6 ± 6.9
4-chlorophenol	14.1 ± 3.1	12.1 ± 4.5	20.1 ± 4.0
Others			
glucose	12.8 ± 4.6		-1.7 ± 0.8

Table 3: Specific oxygen uptake activity of the (chloro)catechol-1,2-dioxygenase of strain FLU100 after growth on fluorobenzene (FB), toluene (T) or benzene (B). The value in parenthesis represents the number of independent batch cultures tested twice. The first value describes the average oxygen uptake while the second one describes the variation. The absolute activity of fluorobenzene grown cells was 2090.5 ± 395.1 units, 189.4 ± 124.5 units for toluene and 88.7 ± 29.0 units for benzene. One unit of oxygenase activity was defined as the conversion of 1 µg $O_2 \cdot L^{-1} \cdot min^{-1} \cdot OD^{-1}$.
B: benzene T: toluene FB: fluorobenzene

	Specific activity FB with reference T (7)	Specific activity FB with reference catechol (7)	Specific activity T with reference T (7)	Specific activity T with reference catechol (7)	Specific activity B with reference T (7)	Specific activity B with reference catechol (7)
Catechols						
catechol	465.7 ± 128.0	100.0 ± 5.1	30.5 ± 10.5	100.0 ± 9.6	16.0 ± 5.5	100 ± 12.3
3-methylcatechol	598.6 ± 128.6	136.1 ± 16.3	43.2 ± 14.6	145.2 ± 32.9	80.5 ± 25.4	571.6 ± 247.2
4-methylcatechol	504.8 ± 87.9	119.8 ± 25.6	20.9 ± 13.4	68.1 ± 34.2	33.8 ± 8.2	232.9 ± 91.1
3-methoxycatechol	400.4 ± 34.5	108.7 ± 29.7	17.8 ± 9.3	50.0 ± 23.2	13.5 ± 3.7	91.2 ± 36.4
3-fluorocatechol	123.4 ± 11.5	33.3 ± 8.6	4.2 ± 5.5	8.4 ± 13.8	1.5 ± 3.4	8.6 ± 21.4
4-fluorocatechol	376.4 ± 99.7	83.8 ± 4.3	28.1 ± 7.0	97.0 ± 43.9	31.8 ± 12.7	195.5 ± 51.1
3-chlorocatechol	275.8 ± 32.0	65.9 ± 18.3	19.7 ± 10.2	63.7 ± 20.5	33.5 ± 9.7	235.5 ± 83.1
4-chlorocatechol	95.1 ± 11.2	23.9 ± 10.2	13.0 ± 5.6	43.2 ± 20.1	23.4 ± 9.9	151.0 ± 72.9
2,3-dihydroxybenzoate	-3.3 ± 0.7	-0.9 ± 0.2	-0.7 ± 1.9	-2.7 ± 6.5	-1.6 ± 1.5	-7.6 ± 6.8
3,4-dihydroxybenzoate	9.4 ± 2.3	3.1 ± 1.4	11.7 ± 6.3	38.0 ± 22.4	16.4 ± 5.4	125.8 ± 74.7

Table 4: Identification of 3-methylcatechol (native form) and 2-methoxy-3-methylphenol (methylated form) as intermediate of toluene degradation of strain FLU100 by GC-MS analyses. The fragment with the highest intensity is normalised to 100 % and other fragments are given as relative intensities.

Identity	Fragment [m/z]	Intensity [%]	Description
3-methylcatechol	124	100.0	M^+
	123	35.0	M^+ - H
	105	7.4	M^+ - H - H_2O
	95	4.0	M^+ - HCO
	78	24.0	M^+ - HCO - OH
	77	11.2	M^+ - CH_3 – OH – OH - H
2-methoxy-3-methylphenol	138	100.0	M^+
	123	94.2	M^+ - CH_3
	95	6.8	M^+ - CH_3 - HCO
	77	12.7	M^+ - OCH_3 – OH – CH

Table 5: Conversion rates (in $mmol \cdot L^{-1} \cdot h^{-1} \cdot OD_{546}^{-1}$) and generation times (in h) of cells of FLU100 pre-grown on toluene or glucose as reference.
-: not analysed n.d. not detectable

	Toluene without CAP	with CAP	generation time [h]	Glucose without CAP	with CAP	Generation time [h]
3-methylcatechol	0.18	-	8.6	-	-	-
acetate	0.80	n. d.	9.5	0.69	n. d.	19.1
pyruvate	1.48	n. d.	10.1	-	-	-
succinate	3.33	n. d.	2.7	-	-	-
methyl succinate	n. d.	n. d.	30.9	-	-	-
adipate	0.62	0.01	4.5	-	-	-
β-hydroxy butyrate	0.79	n. d.	7.3	0.47	0.11	13.6
γ-hydroxy butyrate	n. d.	n. d.	51.2	0.03	0.03	> 100
2-methyl butyrate	n. d.	n. d.	> 100	n. d.	n. d.	98.6
γ-hydroxy butyrolactone	n. d.	0.06	> 100	n. d.	0.01	> 100
malonate	n. d.	n. d.	> 100	n. d.	n. d.	> 100
3-oxoglutarate	0.37	0.48	10.5	0.18	0.04	5.1
2-ML	0.41	0.04	5.4	-	-	-

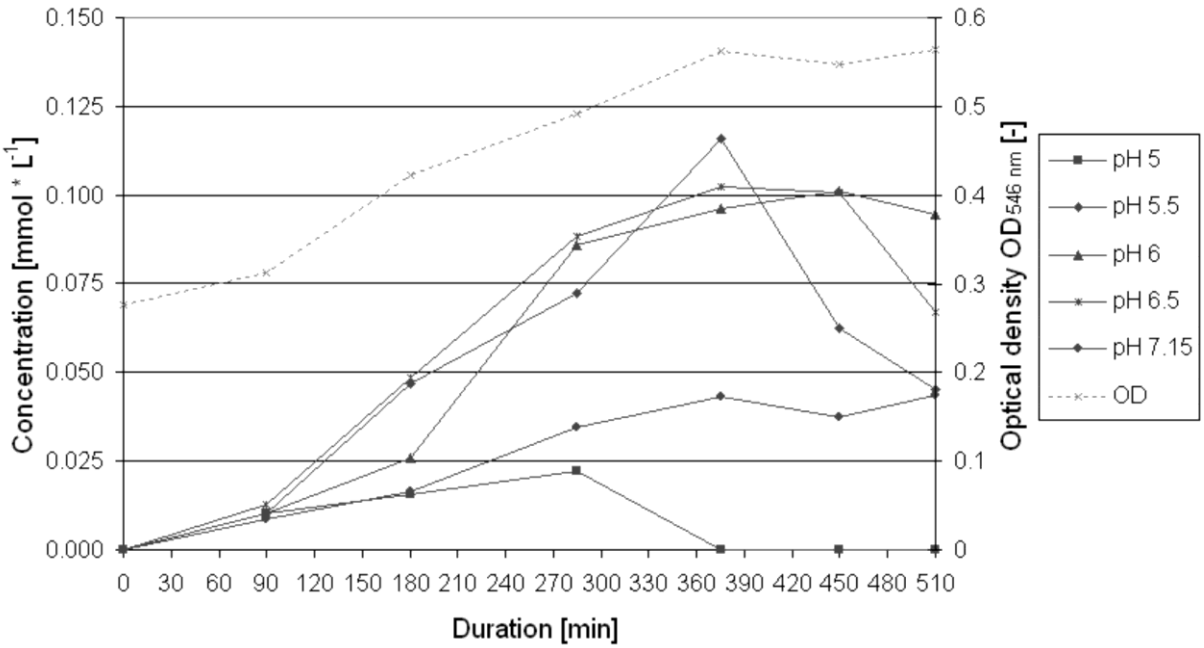


Fig 1: Influence of the pH value of the medium on the concentration of 2-methylmuconic acid as intermediate during conversion of 1.55 mmol toluene·L^{-1} by strain FLU100 pre-grown on toluene. The chart for pH 5.0 fell out of the series due to reduced conversion rates of toluene as substrate.

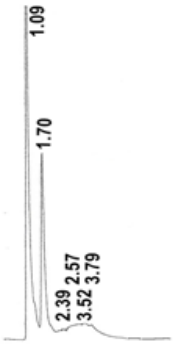
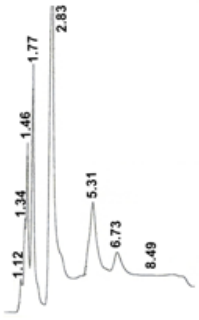
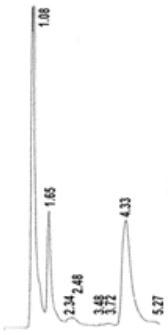
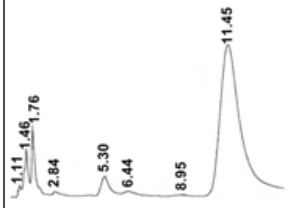
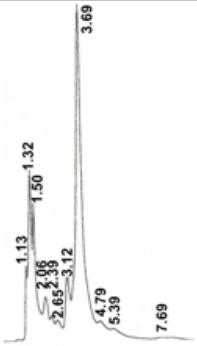
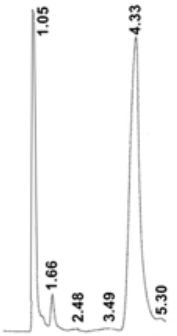
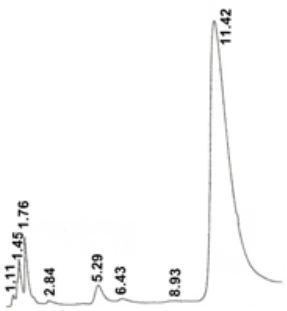
substrate	benzene	toluene	fluorobenzene
Corresponding ‚diendiol'	cyclohexa-3,5-diene-1,2-diol	3-methylcyclohexa-3,5-diene-1,2-diol	3-fluorocyclohexa-3,5-diene-1,2-diol
HPLC chromatogram with corresponding ‚diendiol'			
HPLC chromatogram after acidification (pH 1-2) and denaturation (80°C, 20')			
spiking substance	phenol	2-methylphenol	2-fluorophenol
HPLC chromatogram after spiking			
Wavelength spectrum ‚diendiol'			
Retention time ‚diendiol'	1.65 min	2.83 min	1.75 min

Fig 2: Charakterisation and identification of formed ‘diendiol’ structures of benzene, toluene and fluorobenzene by mutant strain FLU100 P2R5 via HPLC analyses (column: ProntoSIL™ SC-04 Eurobond C18 column, 125 mm · 4 mm, i.d. 5 µm; solvent: H_2O : CH_3OH : H_3PO_4 (85 w/v%) = 74.9 % : 25 % : 0.1 %). The flow rate was maintained at 1 mL · min^{-1}.