Supplementary materials

# Insight into Metabolic Versatility of an Aromatic Compounds-Degrading Arthrobacter sp. YC-RL1

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Table S1| Typical *Arthrobacter* strains involved in xenobiotics degradation

| Strains | Substrates | | Genome availability (GenBank acc. no.) | References |
|---|---|---|---|---|
| | Substrates | Substrate type | | |
| *Arthrobacter* sp. IF1 | 4-Fluorophenol | Benzenoids and their derivatives | - | Ferreira et al., 2009 |
| *Arthrobacter* sp. JS443 | *p*-Nitrophenol | | - | Perry and Zylstra, 2007 |
| *Arthrobacter chlorophenolicus* A6 | 4-Chlorophenol | | + (NC_011886) | Nordin et al., 2005 |
| *Arthrobacter* sp. W1 | Phenols | | +JWMD01000000 | Wang et al., 2009 |
| *Arthrobacter* sp. SPG | 2-Nitrobenzoate | | - | Arora and Sharma, 2015 |
| *Arthrobacter sp.* PJ3 | Biphenyl | | - | Yang et al., 2007 |
| *Arthrobacter phenanthrenivorans* Sphe3 | Phenanthrene | | + (NC_015145) | Vandera et al., 2015 |
| *Arthrobacter oxydans* B4 | Benzo[α]pyrene | | - | Peng et al., 2012 |
| *Arthrobacter aurescens* M2012083 | Nicotine | Heterocyclic compounds | + (AKKK00000000) | Yao et al., 2015 |
| *Arthrobacter aurescens* TC1 | *s*-Triazine ring compounds | | + (NC_008711) | Strong et al., 2002 |
| *Arthrobacter* sp. TES6 | Atrazine | | - | Sebaï et al., 2011 |
| *Arthrobacter* sp. SPG23 | Diesel | Hydrocarbons | + JYCN01000000 | Romero et al., 2017 |
| *Arthrobacter* sp. DSM312 | Pentane | | - | Ionata et al., 2005 |

Table S2 | Bacterial strains, plasmids and primers

| Items | Relevant characteristics | References |
|---|---|---|
| **Strains** | | |
| *Arthrobacter* sp. YC-RL1 | CGMCC NO.10611 | Ren et al, 2016 |
| *Escherichia coli* DH5α | $F^-$, φ80, *lacZ*ΔM15, Δ(*lacZYA-argF*) U169 *endA1*, *recA1*, *hsdR17* (rk-, mk+) *supE44*, λ-, thi-1, *gyr*A96, *rel*A1, *pho*A | Promega |
| *Escherichia coli* BL21 (DE3) | $F^-$, *omp*T, *hsd*SB($rB^-$ $mB^-$), *gal*, *dcm* (DE3) | Promega |
| **Plasmids** | | |
| pMD19T | Cloning of target gene | TaKaRa |
| pET32a | Expression of target gene | Novagen |
| **Primers** | | |
| *bphC*-F | GCGCGGATCCATGACTCACATCCGTGGACTT | *BamH I** |
| *bphC*-R | GCGCCTCGAGGTTTGCCGCGGCGAC | *Xho I** |

*: The designed restriction site (the underline base pairs) in each primer.

Table S3 The detail information of chemicals involved in this study

| Chemicals | CAS No. | Provider | Purity |
|---|---|---|---|
| *p*-Nitrophenol | 100-02-7 | Sinopharm Chemical Reagent Beijing Co., Ltd (Beijing, China) | Analytical grade（99.2%） |
| Naphthalene | 91-20-3 | Shanghai Aladdin Bio-Chem Technology Co., Ltd (Shanghai, China) | Analytical grade（>97.0%） |
| Phenanthrene | 85-01-8 | Shanghai Aladdin Bio-Chem Technology Co., Ltd (Shanghai, China) | Analytical grade（>97.0%） |
| Biphenyl | 92-52-4 | J&K Scientific Ltd (Beijing, China) | Analytical grade（99.5%） |
| Bisphenol A | 80-05-7 | J&K Scientific Ltd (Beijing, China) | Analytical grade（99.0%） |
| *p*-Xylene | 106-42-3 | Fisher Scientific | HPLC grade（99.9%） |
| Methanol | 67-56-1 | Fisher Scientific | HPLC grade（99.9%） |
| Acetonitrile | 75-05-8 | Fisher Scientific | HPLC grade（99.9%） |
| Salicylic acid | 69-72-7 | Sinopharm Chemical Reagent Beijing Co., Ltd (Beijing, China) | Analytical grade（99.3%） |
| Gentisic acid | 490-79-9 | J&K Scientific Ltd (Beijing, China) | Analytical grade（99.0%） |
| Benzoate | 65-85-0 | Sinopharm Chemical Reagent Beijing Co., Ltd (Beijing, China) | Analytical grade（99.0%） |
| Protocatechuate | 95-50-3 | J&K Scientific Ltd (Beijing, China) | Analytical grade（99.0%） |
| DEHP* | 75-09-2 | Sinopharm Chemical Reagent Beijing Co., Ltd (Beijing, China) | Analytical grade（99.0%） |
| Dichloromethane | 141-78-6 | Sinopharm Chemical Reagent Beijing Co., Ltd (Beijing, China) | Analytical grade（99.5%） |

DEHP*: di 2-ethyl hexyl phthalate

Table S4 | Genomic features of the six subject *Arthrobacter* strains

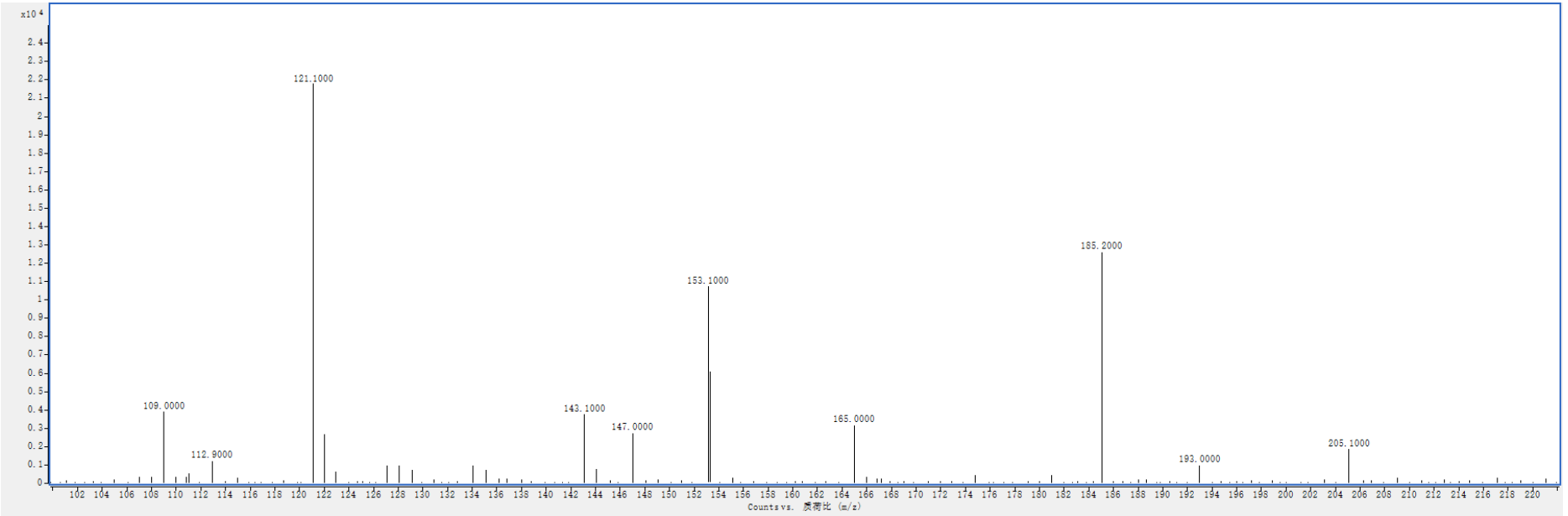
| Genomic features | *Arthrobacter* strain | | | | | |
|---|---|---|---|---|---|---|
| | YC-RL1 | A6 | FB24 | Sphe3 | TC1 | RE117 |
| Genome size (bp) | 4,018,639 | 4,980,870 | 5,070,478 | 4,535,320 | 5,226,648 | 3,918,192 |
| G+C content (mol %) | 64.0 | 66.0 | 65.4 | 65.4 | 62.4 | 59.3 |
| Accession number | NZ_CP013297.1 | NC_011886.1 | NC_008541.1 | NC_015145.1 | NC_008712.1 | NC_014550.1 |
| Protein coding genes | 3,720 | 4,590 | 4,523 | 4,131 | 4,523 | 3,436 |
| No. of genes with function prediction | 3,579 | 3,095 | 3,256 | 2,922 | 3,256 | 2,378 |
| No. of tRNAs | 67 | 88 | 51 | 50 | 51 | 64 |
| No. of rRNAs | 19 | 15 | 15 | 12 | 15 | 18 |

Table S5 | Ring-cleavage related dioxygenases identified in strain YC-RL1

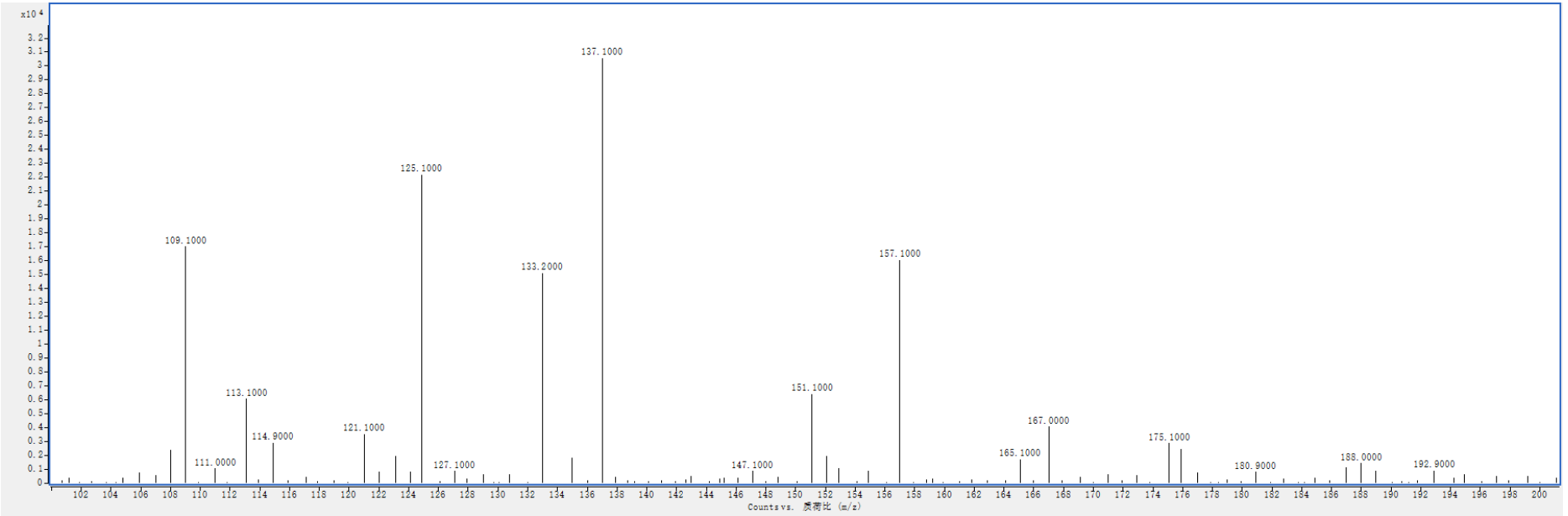
| Location | Accession number | Gene annotation |
|---|---|---|
| Chromosome | WP_047118329 | Catechol 2,3-dioxygenase |
| Chromosome | WP_047120477 | 2,3-dihydroxybiphenyl 1,2-dioxygenase |
| Chromosome | WP_047118329 | Tryptophan 2,3-dioxygenase |
| Chromosome | WP_047118013 | Protocatechuate 3,4-dioxygenase beta chain |
| Chromosome | WP_047118014 | Protocatechuate 3,4-dioxygenase alpha chain |
| Plasmid02 | WP_060617062 | Hydroxyquinol 1,2-dioxygenase |
| Chromosome | WP_047120102 | 4-hydroxyphenylpyruvate dioxygenase |
| Chromosome | WP_047120099 | 3,4-dioxygenase subunit beta |

Dioxygenases involved in the ring-cleavage were marked with gray background.

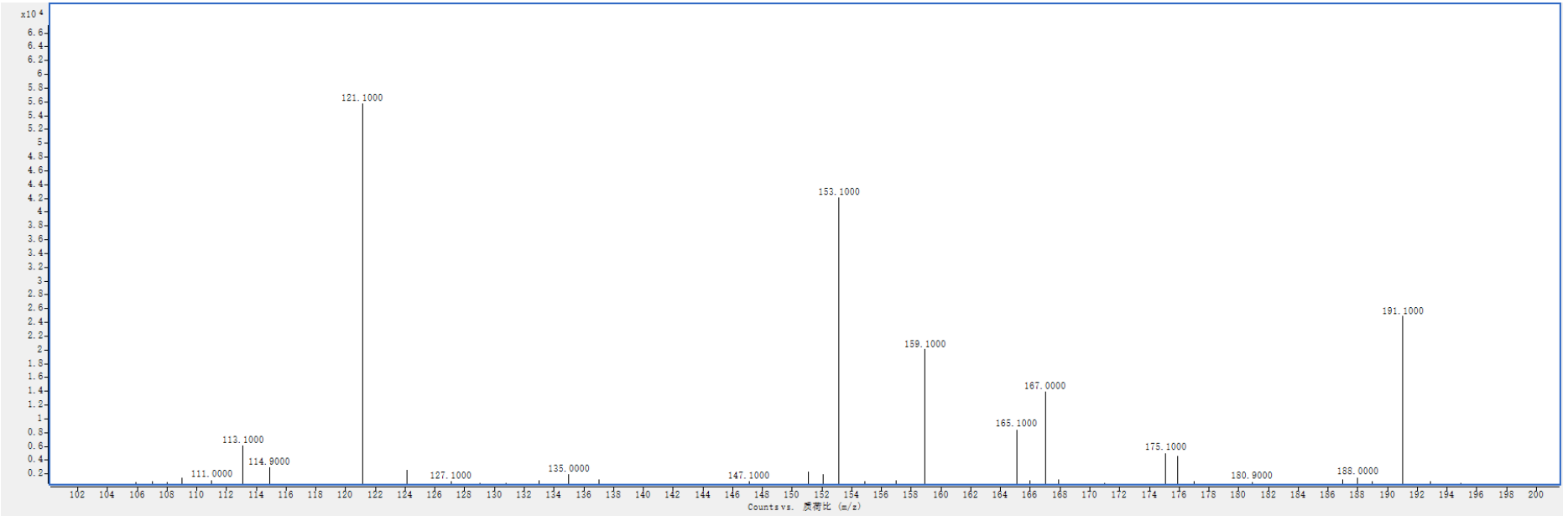
(I) Metabolic intermediates of BP at 24 h



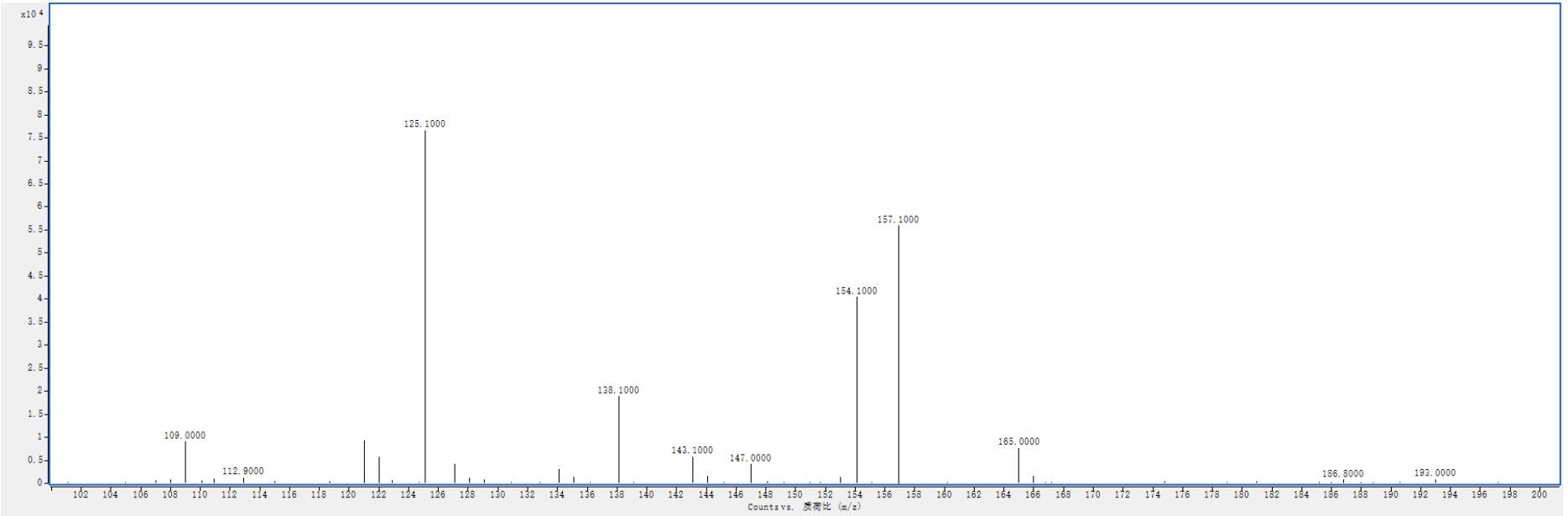
(II) Metabolic intermediates of Bisphenol A at 36 h



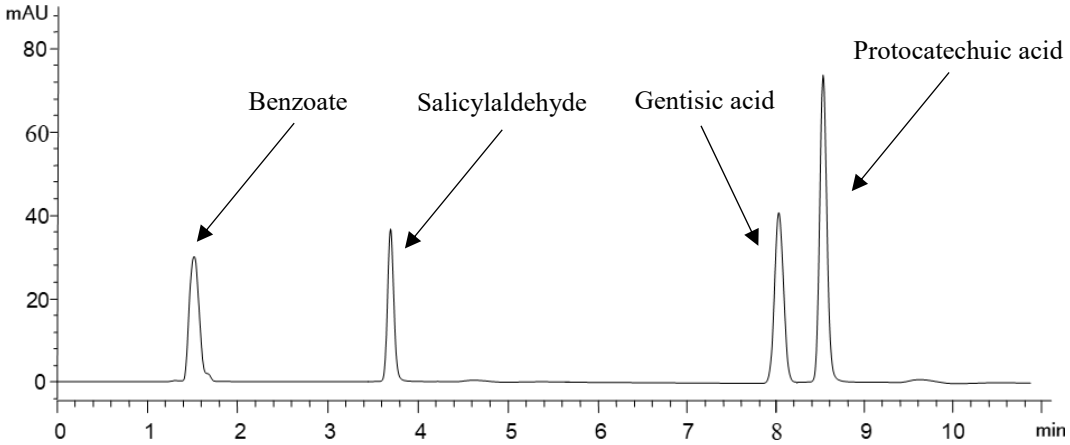
(III) Metabolic intermediates of naphthalene at 36 h



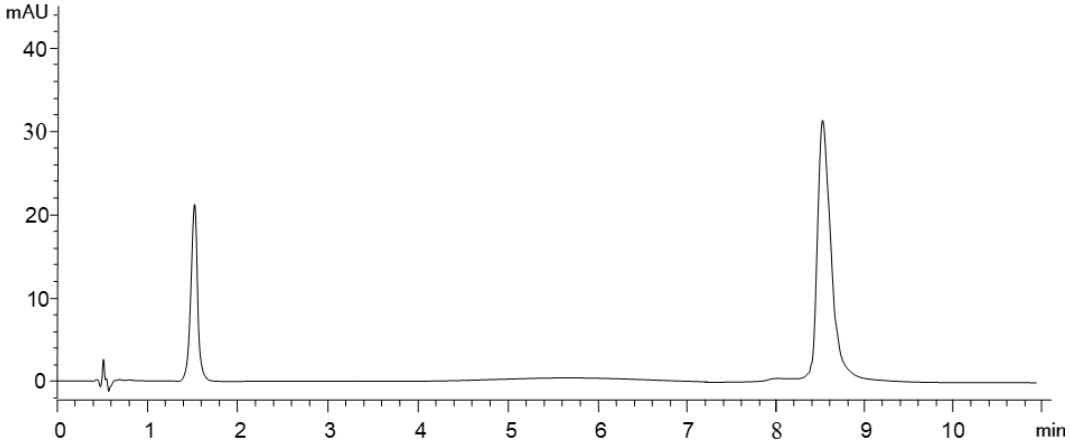
(IV) Metabolic intermediates of *p*-nitrophenol at 24h



(V)HPLC analysis of benzoate, protocatechuate, salicylaldehyde and gentisic acid (standard chemicals)




(VI) HPLC analysis of biphenyl metabolic intermediates



(VII) HPLC analysis of naphthalene metabolic intermediates

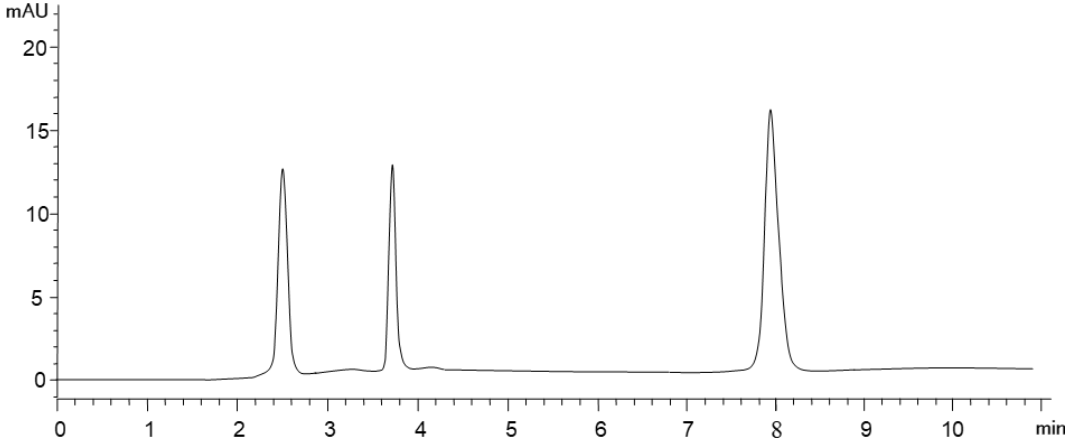



FIGURE S1 | Detection of metabolic intermediates by HPLC and HPLC-MS

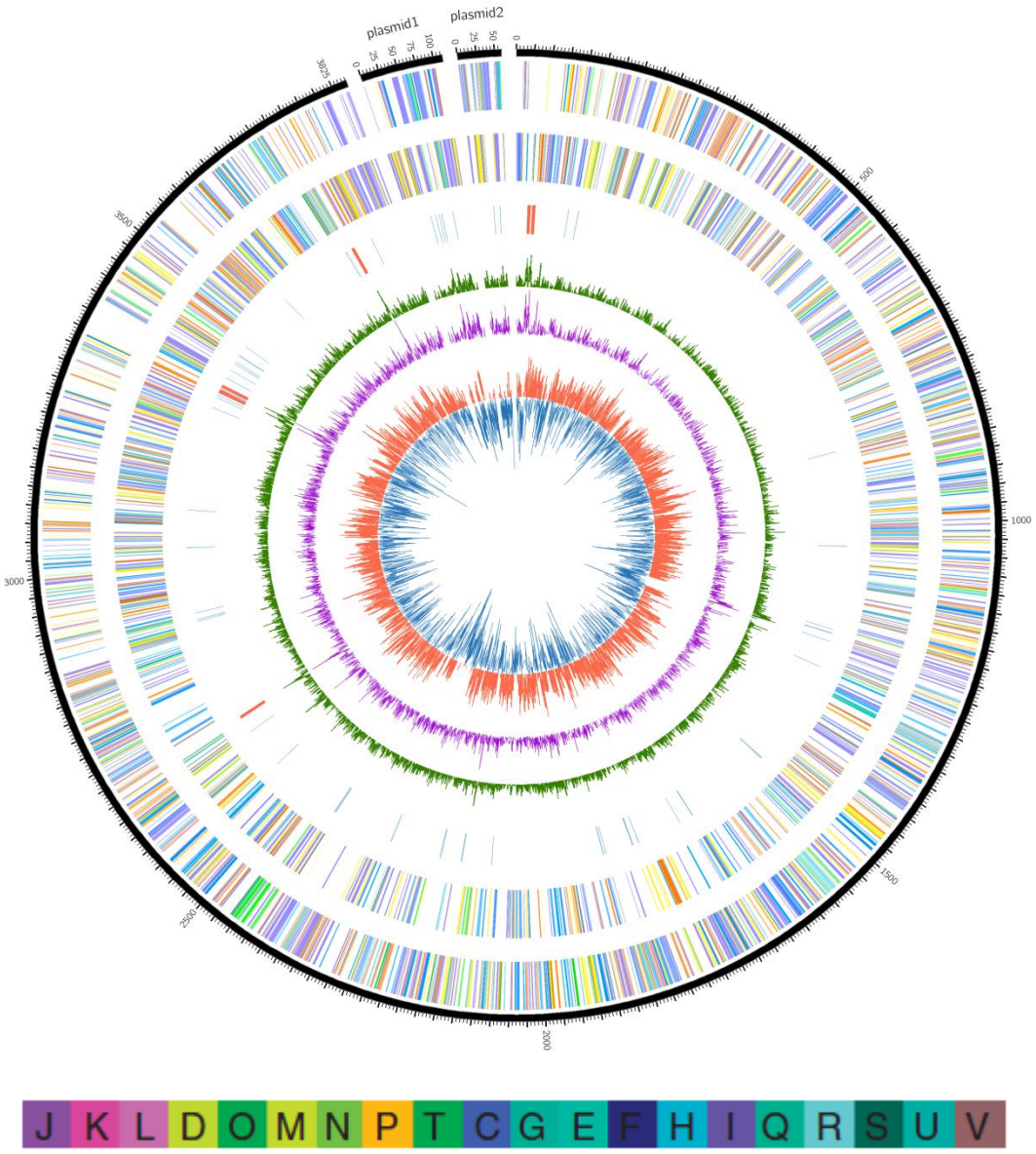



FIGURE S2 | Circular representations of strain YC-RL1 chromosome and plasmids displaying relevant genome features. From the outer to the inner concentric circle: circle 1 (outermost circle) represents the scale; circles 2 and 3, the predicted protein-coding sequences (CDS) on the forward (outer wheel) and the reverse (inner wheel) strands colored according to the assigned COG classes; circle 4 includes tRNA (blue) and rRNA (red) positions; circles 5 and 6 represent the GC skew (-) and skew (+) respectively; circles 7 and 8, G+C content showing deviations from the average (red mean higher than the average, blue means lower than the average). The bar of color indicates the COG function groups: C, energy production and conversion; D, cell cycle control, mitosis and meiosis; 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; K, transcription; L, replication, recombination and repair; M, cell wall/membrane biogenesis; N, cell motility; O, post-translational 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 and secretion; V, defense mechanisms.

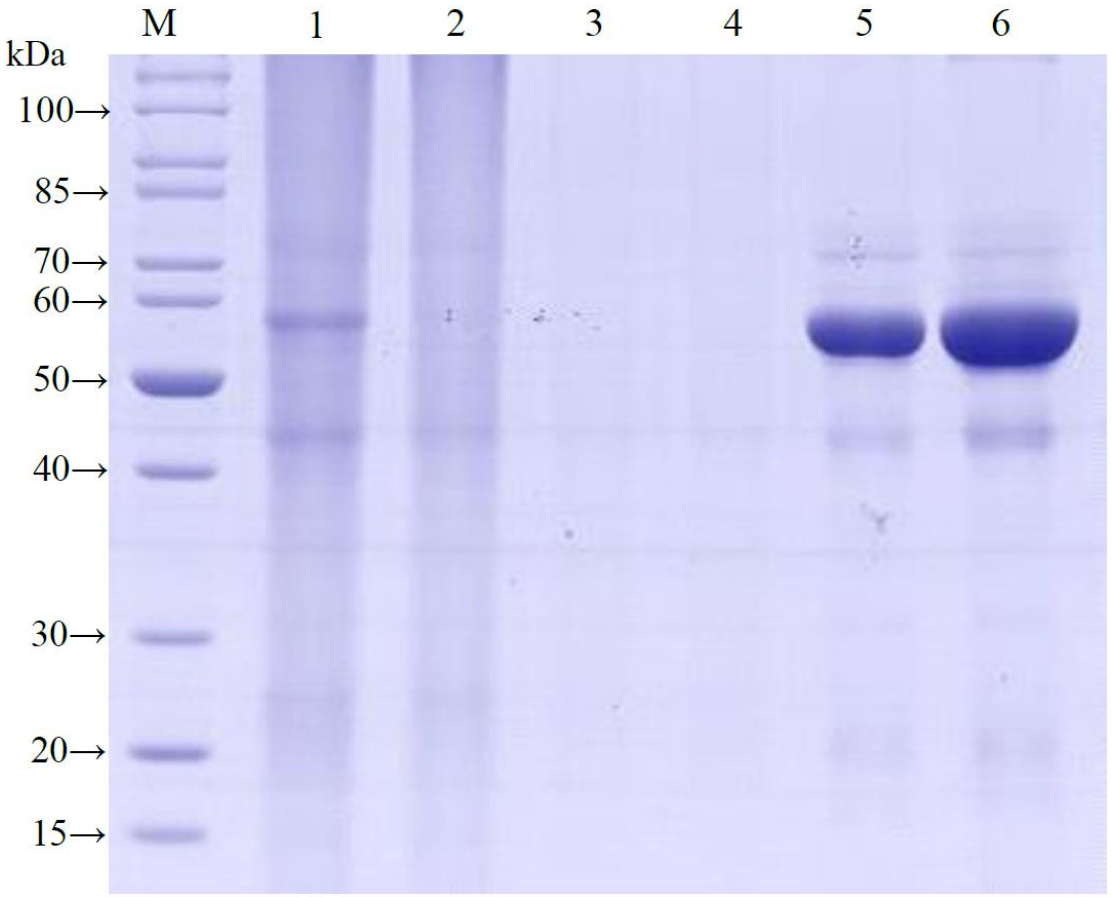



FIGURE S3| SDS-PAGE analysis of the purified BphC

(M = Protein maker, 1 = Induced E. coli BL21 cells with recombinant plasmid, 2 = Induced E. coli BL21 cells without recombinant plasmid; 3 = Purification of induced E. coli BL21 cells without recombinant plasmid, 4 = Purification of induced E. coli BL21 cells with pET32a(+) plasmid only, 5 and 6 = Purified BphC.)

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