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Supplementary Information

Bioconversion of a lignin-derived biphenyl dimer into the strategic building block 5-carboxyvanillic acid in *Pseudomonas putida* KT2440

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Supplementary Figures

Figure S1. Schematic representation of plasmids pIZDDVA_K, pIZDDVA and p254DDVT. Generated with SnapGene 7.2.

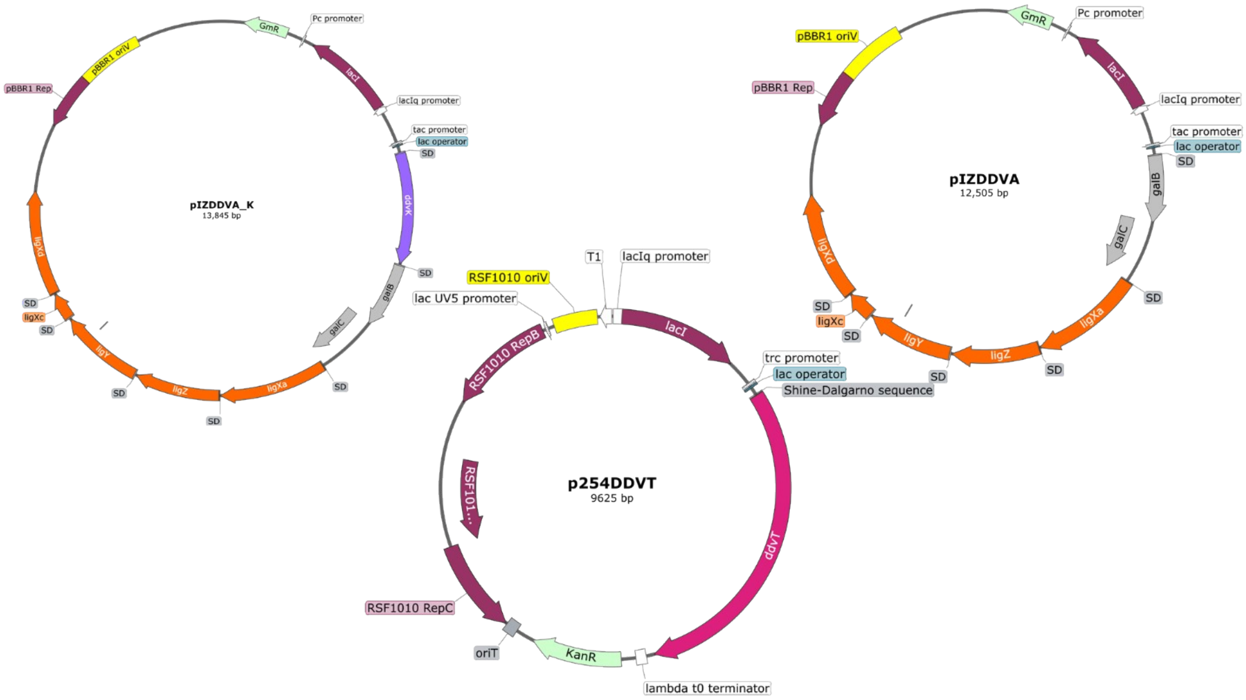
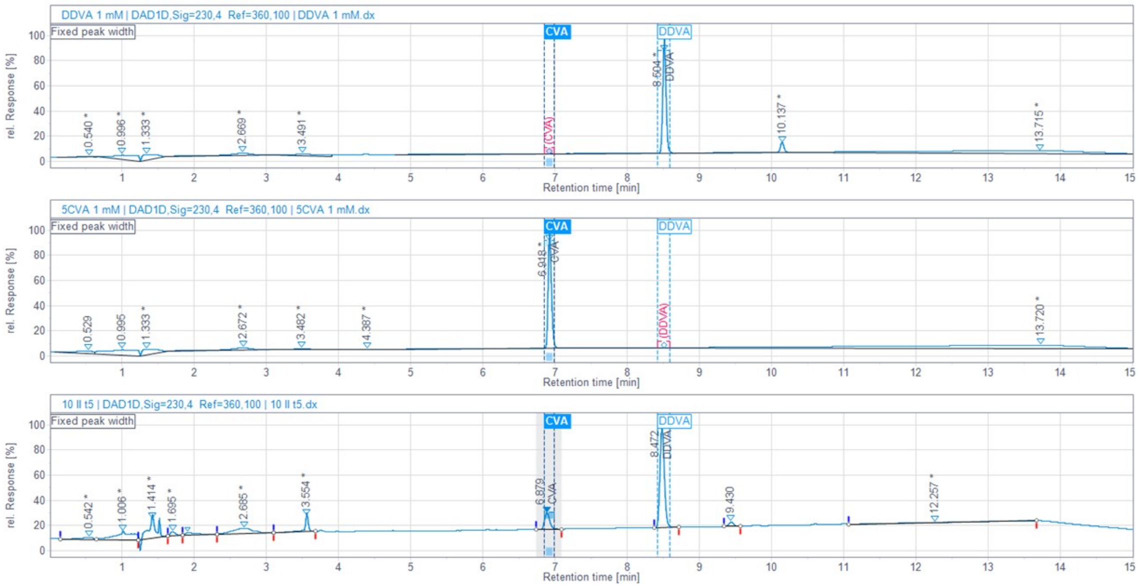
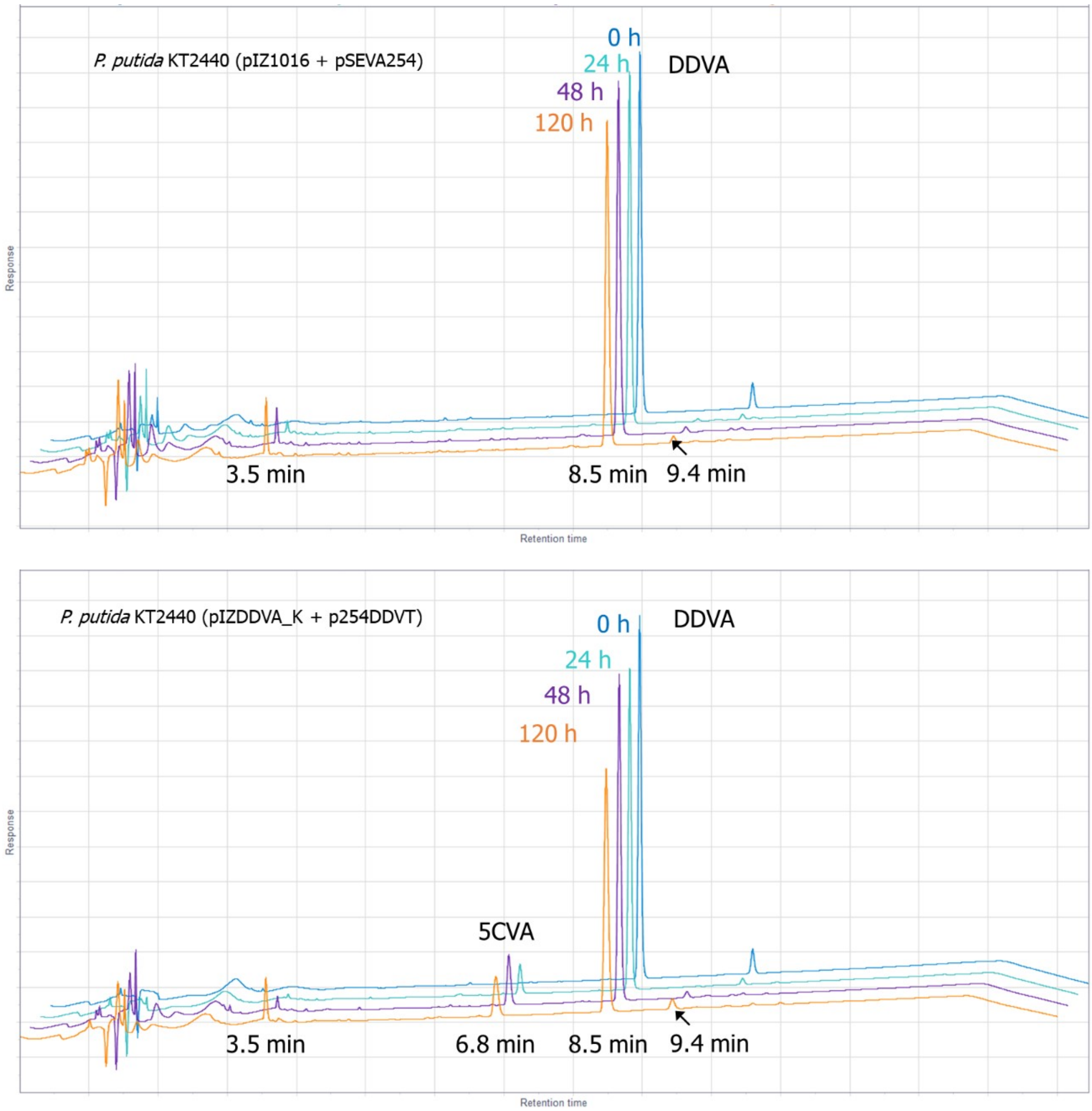


Figure S2. LC/MS representative chromatograms. (A) HPLC Chromatograms showing DAD signals at 230 nm. From top to bottom: signals corresponding to authentic DDVA, authentic 5CVA and sample taken from *P. putida* KT2440 (pIZDDVA_K + p254DDVT) resting cells incubated during 5 days in the presence of 1 mM DDVA at pH 7.5. (B) HPLC chromatograms showing evolution of DAD signals at 230 nm over time of samples taken from resting cells experiments (1 mM DDVA, pH 7.5) with *P. putida* KT2440 bearing either empty plasmids pIZ1016 and pSEVA254 (top) or pIZDDVA_K and p254DDVT (bottom). (C) MS spectrum corresponding to peaks at retention times 3.5 (top), 6.8 (middle) and 9.4 (bottom) minutes from the sample shown in panel B (down). Expected *m/z* values in this MS method for DDVA pathway intermediaries and final product are: OH-DDVA, 321; DCHM-HOPDA, 350.23; 5CVA, 213.16.

A

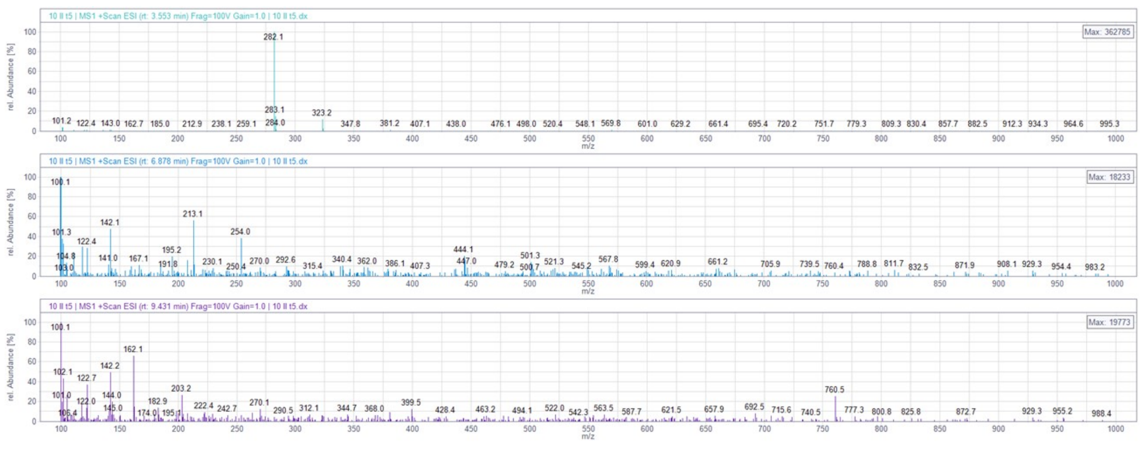


B



P. putida KT2440 (pIZ1016 + pSEVA254)
0 h
24 h
48 h
120 h
DDVA
3.5 min
8.5 min
9.4 min
Response
Retention time
P. putida KT2440 (pIZDDVA_K + p254DDVT)
0 h
24 h
48 h
120 h
DDVA
5CVA
3.5 min
6.8 min
8.5 min
9.4 min
Response
Retention time

C



Max: 362785
Max: 18233
Max: 19773
rel. Abundance [%]
m/z

Figure S3 Identification of a consensus TonB box in *P. putida* and functional analysis of a chimeric DdvT* transporter. (A) Amino acid sequence alignment of 11 putative TonB boxes of TonB-dependent transporters from *P. putida* KT2440 and the BtuB receptor from *P. aeruginosa* with the TonB box region of the DdvT transporter from *S. lignivorans* SYK6 (highlighted in purple). Colors indicate similarity (dark pink indicates similar residues in all sequences, light pink indicates similar residues in more than 80% sequences, and white indicates similar residues in less than 80% sequences) considering a Blossum62 score matrix. Logo image was modified from the obtained using WebLogo 3 tool.[55] (B) 5CVA production profile from 1 mM DDVA using resting cells of *P. putida* KT2440 containing plasmid pIZDDVA_K and plasmid p254DDVTB (encodes the chimeric DdvT* transporter). Samples were taken at 0, 4, 24, 48, 72, and 120 hours. DDVA consumption and 5CVA production are indicated with discontinuous and continuous lines, respectively. All the resting cells experiments were performed in triplicates. Error bars represent standard deviation of the triplicates.

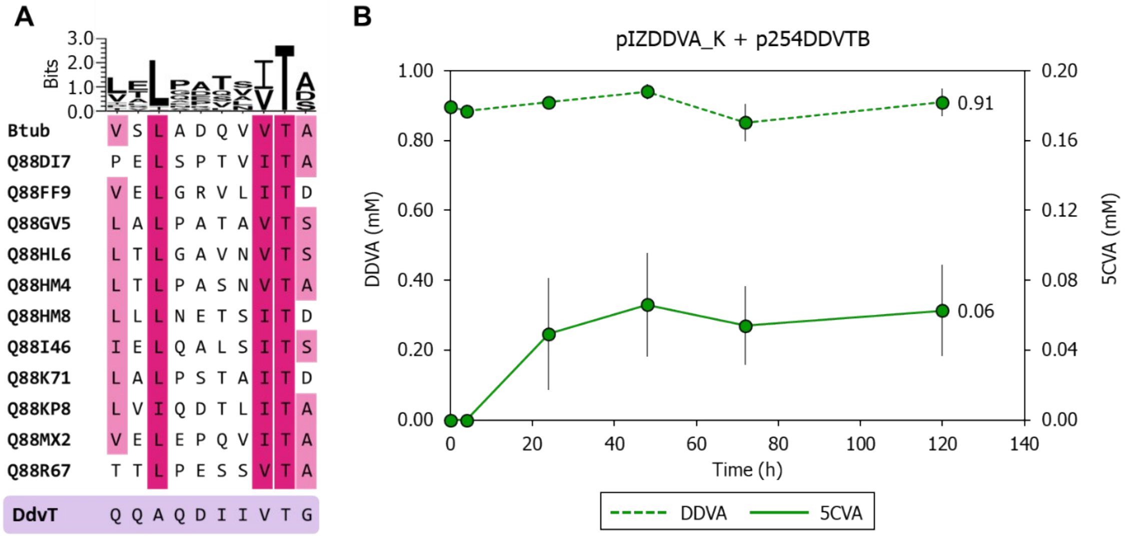
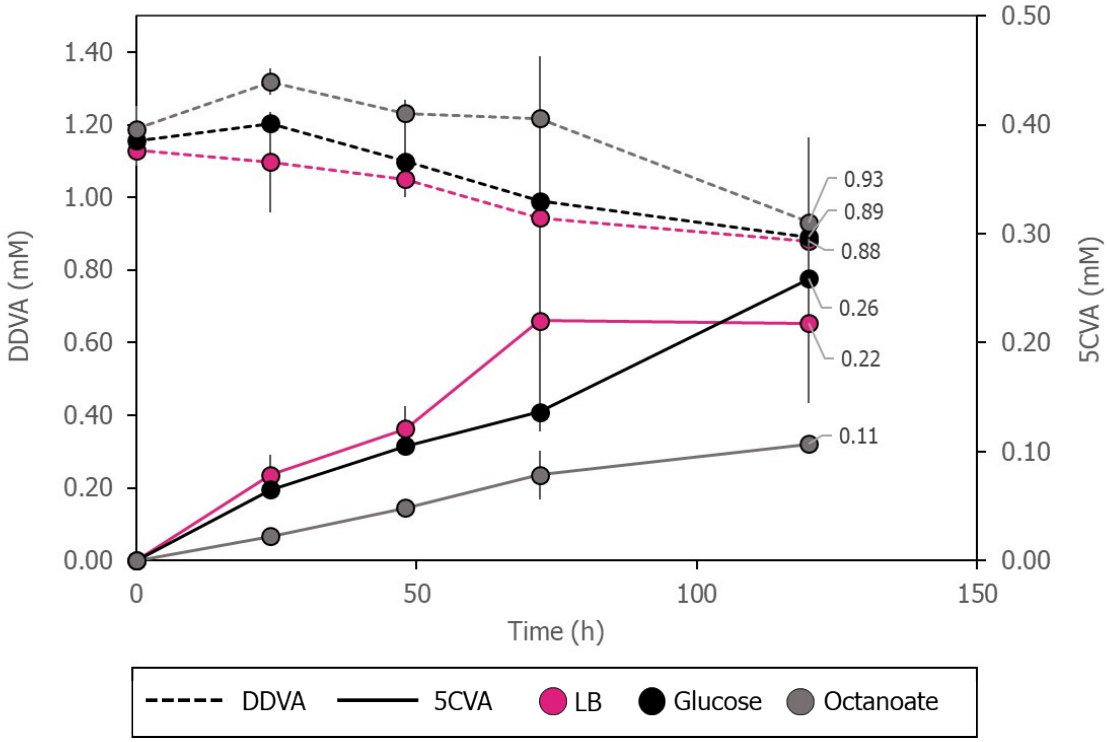


Figure S4. 5CVA production over time with *P. putida* KT2440 (pIZDDVA_K, p254DDVT) cells. Cells were grown in LB medium, M63 medium with 20 mM glucose, or M63 medium with 15 mM octanoate, and then collected (OD_{600} 24) for biotransformation of 1 mM DDVA in resting cells conditions.



Supplementary Tables

Table S1. Oligonucleotides used in this study.

Primer	Sequence (5'-3')
5 ligX	CGGCCGCGCGAATTCGAGCTCGGTACCCTAAGGAGGTGATCTAATGCTGTCCGCAGACCAGAACGACAAGCTG
3 ligX	TATCCTCTCCCAACCTCCTCTTCGGCACCGCATTAGATCACCTCCTTATCACGCGTCGACATAGCCCTTGTTATCCTG
5 ligY	TTCGTGGCCTGGCAATAAGCGAACGCGTTTTAAGGAGGTGATCTAATGATCATCGACTGCCATGGTCACGTCAGCGCTCCGGTGGA
3 ligY	ATGCCTGCAGGTCGACTCTAGAGGATCCCCTCAAACGTCCAAATTAAACACCTTCCGCGCATTATC
5 ligZ	CAGGATAACAAGGGCTATGTCGACGCGTGATAAGGAGGTGATCTAATGCGGTGCCGAAGAGGAGGTTGGGAGAGGATA
3 ligZ	TCCACCGGAGCGCTGACGTGACCATGGCAGTCGATGATCATTAGATCACCTCCTTAAAACGCGTTCGCTTATTGCCAGGCCACGAA
ligXc_Fw	ATATATCTAGA*AAGGAGGTGATCTA*ATGGCGCAGCTGAAGGTCG
ligXc_Rv	ATATAAAGCTTTCAATCCTCCGGGGCGATGGT
ligXd_Fw	ATATAAAGCTT*AAGGAGGTGATCTA*ATGCCGCATTTTGATTGCCTTA
ligXd_Rv	ATATAACTAGTTCATCCCTGCGCGGCGGTCA
ligXa tune Fw KpnI	CGGGGTACCCTAAGGAGGTGATCTAATGCTGTCCGCAG
ligXa tune Rv NheI NdeI	AAGCAAGCTAGCCATATGATCACCTCCTTATCACGCGTCGACAT
ligZ tune Fw NdeI	ATATATCATATGGCCGAAATCGTGCTGGGT
ligZ tune Rv XhoI	TCCGCTCGAGCGTTCGCTTATTGCCAGGCCACGAAGC
ligY tune Fw NheI XhoI	AAGCAAGCTAGCCTCGAGAAGGAGGTGATCTAATGATCATCGACTGCCATGG
ligY tune Rv BamHI	CCGGGATCCCCTCAAACGTCCAAATTAAACAC
MFS Fw AvrII	ATATCCTAGGAAGGAGGTGATCTAATGGAGAGGATGACGATGGCA
MFS Rv EcoRI	TATATGAATTCTCAGTCCGGGATTTTGGCGG
galBC Fw AvrII EcoRI	TATCCTAGGGAATTCAAGGAGGTGATCTAATGACATCCTGCGCCCACCC
galBC Rv SacI	ATAGAGCTCTCAGCCTTCCAGGTCGGTGA
XmaI lig frag Fw	ATATATCCCGGGAAGGAGGTGATCTAATGGAGAG
SpeI lig frag Rv	ATATATACTAGTTCATCCCTGCGCGGCGGTC
oCDCS7	GGGTGGGCGCAGGATGTCATTAGATCACCTCCTTCCCGGGC
oCDCS8	ATGACATCCTGCGCCCACCCC
ddvT_Fw_SD_XbaI	TATATTCTAGATGACCTAAGGAGGTAAATAATGGCTCGCGGAACGTACTTG
ddvT Rv NheI	TATATGCTAGCTCAGAAGCCGAACCGCAC
LELPATVITA_Rv	CGCGGTGATGACGGTGGCCGGCAGCTCCAGCGCCGTCATTTCCTGAGC
LELPATVITA_Fw	CTGGAGCTGCCGGCCACCGTCATCACCGCGTCGCGCGTGGCCCGAAGC
ddvT_Rv_HindIII	TGACTGAAGCTTTCAGAAGCCGAACCGCAC