

## Supplemental Material

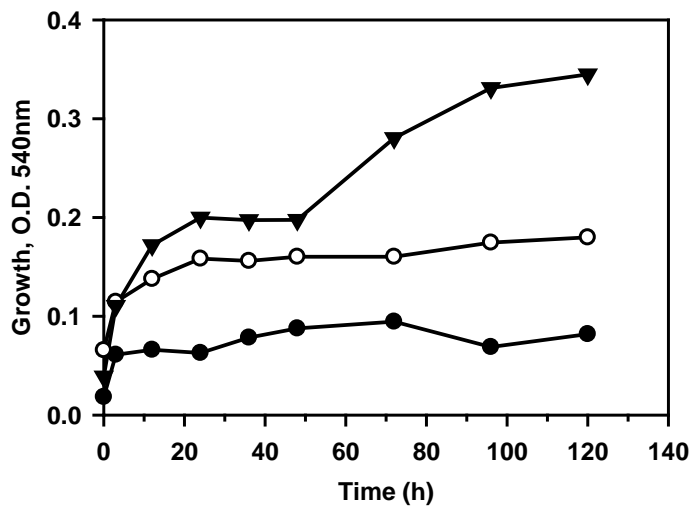
### AEM03326-16

#### Insight into the Carbon-Source Dependent Inducible Veratrylalcohol and Ferulic acid Metabolism in *Pseudomonas putida* CSV86

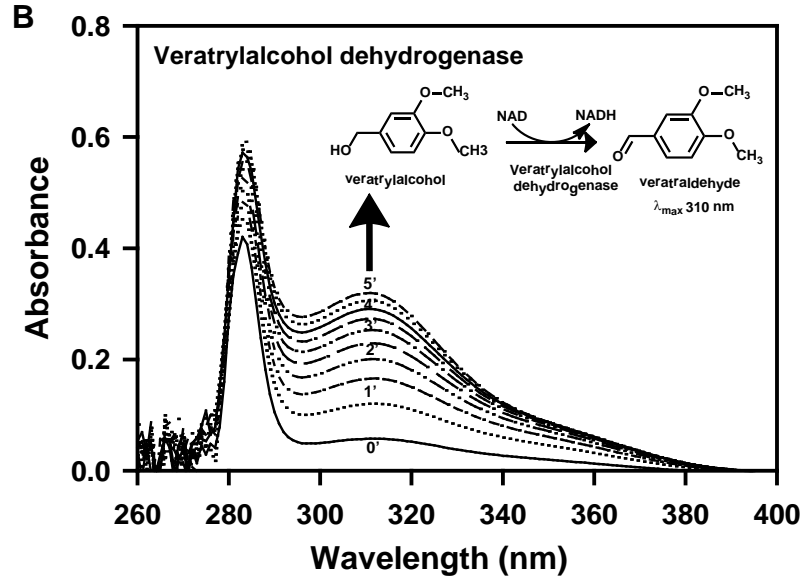
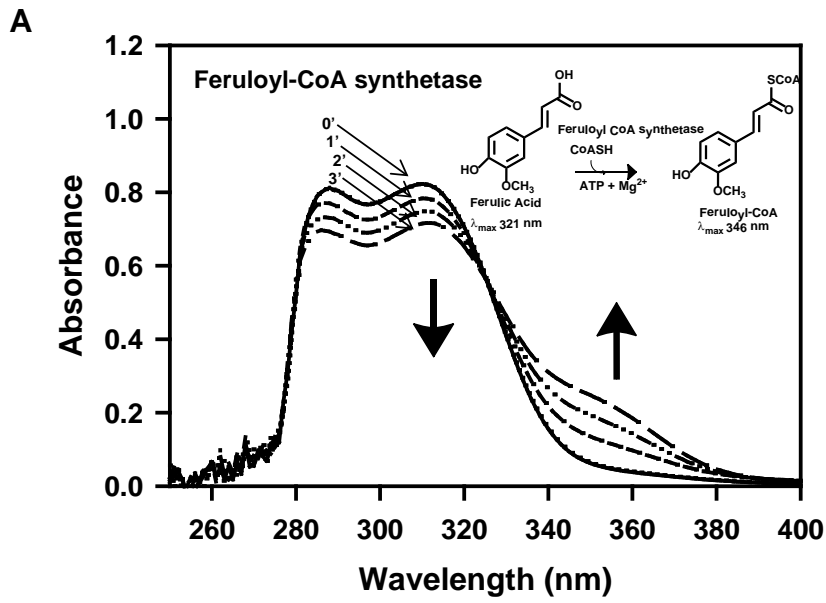
Karishma Mohan and Prashant S. Phale✉

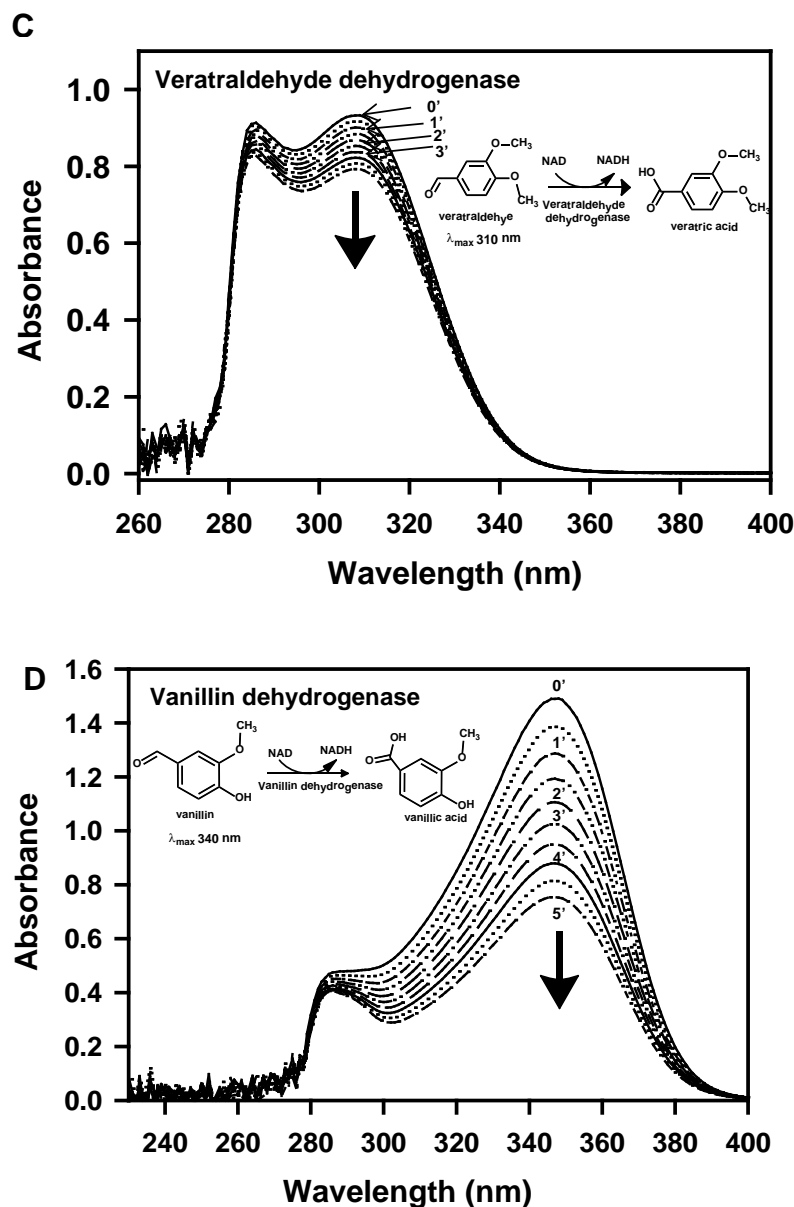
Department of Biosciences and Bioengineering, Indian Institute of Technology-Bombay, Powai,  
Mumbai, 400 076, India.

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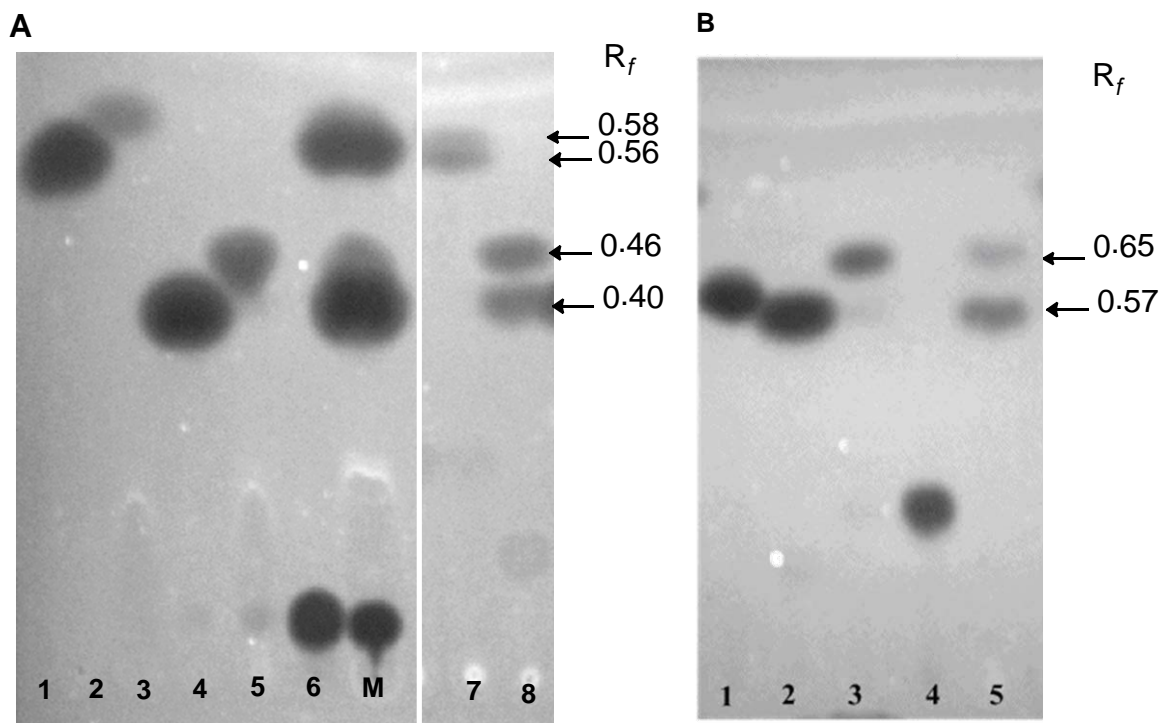


**Fig S1** Growth profile of *P. putida* CSV86 on different concentration of lignin as a sole carbon sources. The concentration of Lignin sulphonate used was: 0.05% (●), 0.1% (○), and 0.2% (▼). Culture grown on 0.1% naphthalene was used as an inoculum.

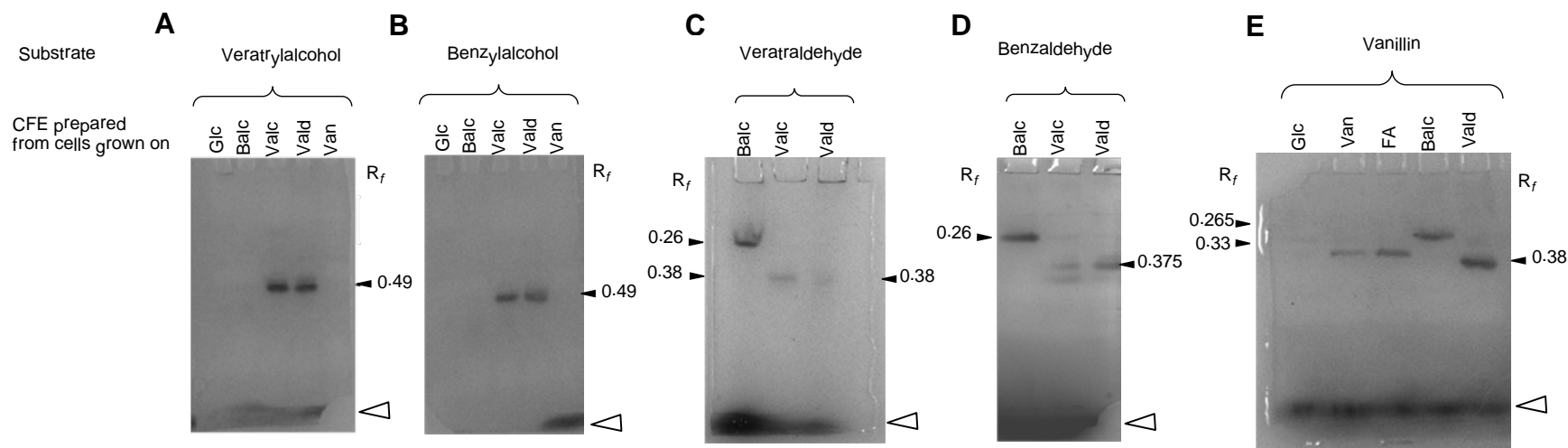




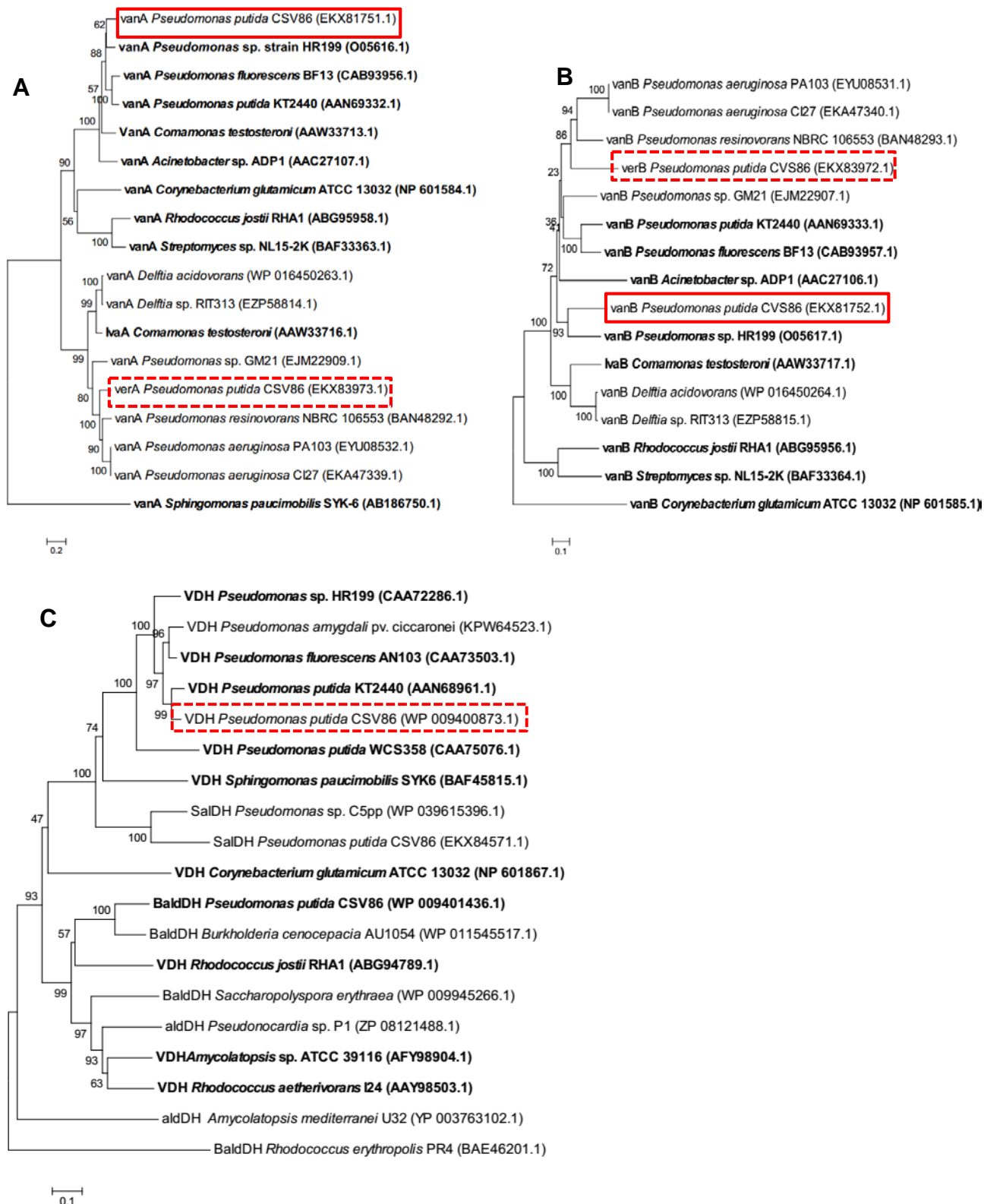
**Fig S2** Time-dependent spectral scan of various enzyme reactions in the cell-free extract of *P. putida* CSV86. Enzyme reactions were scanned from 200 to 400 nm with 30 seconds or one minute interval for: (A) Feruloyl-CoA synthetase (using cells grown on ferulic acid, no spectral changes were observed in the absence of either ATP or Co-A), (B) Veratryl alcohol dehydrogenase (using cells grown on veratryl alcohol), (C) Veratraldehyde dehydrogenase (using cells grown on veratryl alcohol), and (D) Vanillin dehydrogenase (using cells grown on ferulic acid). Arrows indicate the appearance of product (up arrow) and disappearance of the substrate (down arrow), respectively. The reactions are depicted in inset. See materials and method for assay details.



**Fig S3** TLC analysis of of bulk enzyme reaction of VDH, ValDDH and FCS from the CFE of *P. putida* CSV86. (A) The authentic compounds and enzyme reaction products were separated using solvent system A [Hexane: Chloroform: Acetic acid, 8:2:2 (v/v)],  $R_f$  values are: Lane 1, veratraldehyde (0.55); 2, veratric acid (0.59); 3, vanillin (0.4); 4, vanillic acid (0.44); 5, protocatechuic acid (0.06); 7, extract of enzyme reaction for ValDDH from cell grown on veratraldehyde; 8, extract of enzyme reaction for VDH from cells grown on vanillin. Lane M is a mixture of all authentic compounds. (B) The solvent system B [Hexane: Chloroform: Acetic acid, 7:3:2 (v/v)] was used to separate the authentic compounds and enzyme reaction products, the  $R_f$  values are: lane 1, ferulic acid (0.58); 2, vanillin (0.56); 3, vanillic acid (0.65); 4, protocatechuic acid (0.23); 5, extract of enzyme reaction for FCS from cells grown on ferulic acid.



**Fig S4** In-gel activity (zymogram) for various aromatic alcohol- and aldehyde-dehydrogenases from CFE of *P. putida* CSV86 cells grown on different aromatic compounds. Activity staining was performed using  $\text{NAD}^+$  as a co-factor and substrates (100  $\mu\text{M}$ ) like veratrylalcohol (panel A), benzylalcohol (panel B), veratraldehyde (panel C), benzaldehyde (panel D) or vanillin (panel E). Each lane contained equal amount (100  $\mu\text{g}$ ) of total protein from CFE prepared from cells grown on: glucose (Glc), benzylalcohol (Balc), veratrylalcohol (Valc), veratraldehyde (Vald), vanillin (Van) and ferulic acid (FA). Open arrow head indicates the dye front.



**Fig S5** Rooted phylogenetic neighbor-joining tree for *O*-demethylase oxygenase (A), reductase (B) and vanillin dehydrogenase (C) components identified in *Pseudomonas putida* CSV86.

Multiple-sequence alignment for proteins was performed using ClustalW. The phylogenetic tree was constructed using MEGA 6 (44) with a bootstrap consensus of 500 replicates. Protein accession number (NCBI database) is given in the parenthesis. Enzymes characterized at the functional level are shown in boldface type. Boot strap confidence limits (expressed as percentage) are shown at nodes.

**Table S1** List of primers used in current study.

Name	Primer	Sequence (5'----- 3')	Expected amplicon length (bp)
<b>A] Primers for co-transcription studies</b>			
i) <i>fer</i> locus			
FO1	FO F1 FO R1	GTCAACGAAAGCGTGCCATT CCAGCTTGACGTTGAATCCG	650
FO2	FO F2 VDH DR	GACTCCGAGTTCGGCTTGTC AGGGATCCGGCGAAGTCCTGGGACAGCAAGG	800
FO3	FO F3 FO R2	CGAGGATTTCAAGCTTTCGT CCCAGAGAAAATCCCTGAACC	900
FO4	FA 4 F FO R3	CGAGGCGCAGGCGGCACAGGTGC CGAGGACCAGATGAAGGATG	896
ii) <i>ver</i> locus			
VrO1	V10 F1 V101 NR	GCTCTGGACGCGTGATTGAAG TAAGTCGGCGGTTTCATAGG	712
VrO2	V10 F2 V10 R1	GGGGACGTCTACCACTATGA CCCATGTCAACGTAGCCAT	1096
VrO3	V103 NF V10 R2	TCGGCATTCCGGGCCTGTACGTCA GCGTACTCACCTGCAATGAC	914
VrO4	V104F V104R	GGACTIONACTGATAGGTCTGC AGTTCCGACCGAGAGCTTCG	862
iii) <i>van</i> locus			
VnO	V20 NF V20 NR	GCTTCATGGAGAACATCCTGCC TGTCCAGCGCGAACAGGTTGC	791
<b>B] Primers for q-PCR</b>			
verA	Ver F Ver R	GTTCAATGGTGCAGGACAGTG AGATACTCGCCGCTACTGTGG	186
vanA	Van F Van R	TAACCTGCATTGGGCTGACA ATGTTCTCCATGAAGCGACTG	216
rpoD	rpoD RT F rpoD RT R	GACAGTGACGACGAAGACGA TCACGACCATGCTTCTTGAG	155



**Table S2** Identification and annotation of Phenylpropanoid metabolic genes in *Pseudomonas putida* CSV86.

Gene	Predicted function*	Size (bp)	Protein Homolog <sup>#</sup>	Coverage / Identity (%)	E-value
<b>A] Ferulic acid metabolism (<i>fer</i> locus, contig 115)</b>					
<i>marR</i>	Transcriptional regulator, MarR family (EKX84209.1)	471	MarR family transcriptional regulator [ <i>Pseudomonas putida</i> ] (WP_064304187.1)	100/99	5e-104
			<b>Transcriptional regulator [<i>Pseudomonas fluorescens</i> BF13] (CAD60265.1)</b>	<b>89/83</b>	<b>2e-78</b>
			<b>MarR family transcriptional regulator [<i>Sphingobium</i> sp. SYK-6] (BAK67179.1)</b>	<b>80/34</b>	<b>5e-19</b>
<i>ech</i>	<i>p</i> -Hydroxycinnamoyl CoA hydratase/lyase (EKX84210.1)	831	<i>p</i> -Hydroxycinnamoyl CoA hydratase/lyase [ <i>Pseudomonas putida</i> ] (WP_046785643.1)	100/98	0.0
			<i>p</i> -Hydroxycinnamoyl-CoA hydratase-lyase [ <i>Pseudomonas putida</i> KT2440] (AAN68962.1)	100/95	0.0
			<b>Enoyl-CoA hydratase [<i>Pseudomonas</i> sp. HR199] (CAA72285.1)</b>	<b>88/91</b>	<b>4e-170</b>
<i>vdh</i>	Vanillin dehydrogenase* (EKX84211.1)	1449	Salicylaldehyde dehydrogenase [ <i>Pseudomonas putida</i> strain CBF10-2] (OAI86349.1)	100/99	0.0
			<b>Vanillin dehydrogenase [<i>Pseudomonas putida</i> KT2440] (AAN68961.1)</b>	<b>100/90</b>	<b>0.0</b>
			<b>Vanillin dehydrogenase [<i>Pseudomonas</i> sp. HR199] (CAA72286.1)</b>	<b>100/80</b>	<b>0.0</b>

<i>fcs</i>	Feruloyl-CoA synthase* (EKX84212.1)	1884	Feruloyl-CoA synthase [ <i>Pseudomonas putida</i> strain CBF10-2] (OAI86350.1)	100/99	0.0
			<b>Feruloyl-CoA synthetase [<i>Pseudomonas</i> sp. HR199] (CAB60226.1)</b>	<b>93/76</b>	<b>0.0</b>
			<b>Feruloyl-CoA synthetase [<i>Rhodococcus aetherivorans</i> I24] (AA Y98502.1)</b>	<b>67/21</b>	<b>7e-12</b>
<i>kct</i>	Acetyl-CoA acetyltransferase (EKX84213.1)	1233	Acetyl-CoA acetyltransferase [ <i>Pseudomonas putida</i> CBF10-2] (OAI86351.1)	100/97	0.0
			Acetyl-CoA acetyltransferase [ <i>Pseudomonas putida</i> SQ1](KHL76142.1)	99/85	0.0
			Acetyl-CoA acetyltransferase [ <i>Pseudomonas putida</i> ] (WP_046785641.1)	99/85	0.0
<i>acdH</i>	Acyl-CoA dehydrogenase domain-containing (EKX84214.1)	1734	Acyl-CoA dehydrogenase [ <i>Pseudomonas putida</i> CBF10-2](OAI86352.1)	100/98	0.0
			Acyl-CoA dehydrogenase [ <i>Pseudomonas</i> sp. NBRC 111134] (WP_060517258.1)	99/78	0.0
			Acyl-CoA dehydrogenase [ <i>Pseudomonas putida</i> SQ1] (KHL76143.1)	100/76	0.0
<b>B] <u>Veratric acid metabolism (<i>ver</i> locus, contig 119)</u></b>					
<i>marR</i>	Transcriptional regulator, MarR family (EKX83971.1)	603	MarR family transcriptional regulator [ <i>Pseudomonas putida</i> CBF10-2] (OAI92365.1)	77/99	5e-106
			MarR family transcriptional regulator [ <i>Pseudomonas</i> sp. URHB0015] (WP_027618844.1)	75/84	1e-88

			MarR family transcriptional regulator [ <i>Comamonas testosteroni</i> strain BR60] (AAW33714.1)	77/26	2e-06
<i>verB</i>	Veratric acid <i>O</i> -demethylase oxidoreductase* (EKX83972.1)	954	Vanillate <i>O</i> -demethylase oxidoreductase [ <i>Pseudomonas putida</i> ] (WP_064303028.1)	100/98	0.0
			Vanillate <i>O</i> -demethylase oxidoreductase [ <i>Pseudomonas</i> sp. URHB0015] (WP_027618843.1)	100/79	0.0
			<b>Ferredoxin-NADP<sup>+</sup> reductase [<i>Comamonas testosteroni</i>] (AAW33717.1)</b>	<b>99/49</b>	<b>3.00E-109</b>
<i>verA</i>	Veratric acid <i>O</i> -demethylase oxygenase subunit* (EKX83973.1)	1077	(2Fe-2S) ferredoxin [ <i>Pseudomonas putida</i> ] (WP_064303029.1)	100/99	0.0
			(2Fe-2S) ferredoxin [ <i>Pseudomonas</i> sp. ES3-33] (WP_045058486.1)	100/86	0.0
			<b>4-<i>O</i>-demethylase oxygenase subunit [<i>Comamonas testosteroni</i>] (AAW33716.1)</b>	<b>94/68</b>	<b>0.0</b>
<i>tdh</i>	Formaldehyde dehydrogenase, glutathione-independent (EKX83974.1)	1200	Formaldehyde dehydrogenase, glutathione-independent [ <i>Pseudomonas putida</i> ] (WP_064303030.1)	100/99	0.0
			Formaldehyde dehydrogenase, glutathione-independent [ <i>Pseudomonas nitroreducens</i> ] (WP_017521542.1)	100/98	0.0
			Glutathione-independent formaldehyde dehydrogenase [ <i>Pseudomonas denitrificans</i> ATCC 13867] (AGI26785.1)	100/98	0.0
<i>vanK</i>	MFS transporter (EKX83975.1)	1335	MFS transporter [ <i>Pseudomonas putida</i> ] (WP_064303031.1)	100/99	0.0
			MFS transporter [ <i>Pseudomonas</i> sp. URHB0015] (WP_027618841.1)	100/83	0.0

**VanK, MFS transporter [*Acinetobacter* sp. ADP1] (AAC27108.1)** **97/48** **8e-143**

<i>oprD</i>	Porin (EKX83976.1)	1278	Porin [ <i>Pseudomonas putida</i> ] (WP_064303032.1)	100/99	0.0
			Porin [ <i>Pseudomonas</i> sp. URHB0015] (WP_051550534.1)	98/82	0.0
			porin [ <i>Pseudomonas putida</i> MC4] (KEX91953.1)	97/76	0.0

**C] Vanillic acid metabolism (*van* locus, contig 220)**

<i>gntR</i>	GntR family transcriptional regulator (EKX81750.1)	678	GntR family transcriptional regulator [ <i>Pseudomonas putida</i> ] (WP_037062423.1)	100/99	8e-160
			VanR, transcriptional regulator [ <i>Comamonas testosteroni</i> ] (AAW33712.1)	92/42	1e-50
			<b>VanR, transcriptional regulator [<i>Acinetobacter</i> sp. ADP1] (AAC27105.1)</b>	<b>90/45</b>	<b>8e-68</b>
<i>vanA</i>	Vanillic acid <i>O</i> -demethylase oxygenase subunit* (EKX81751.1)	1053	Rieske (2Fe-2S) protein [ <i>Pseudomonas putida</i> ] (WP_064303990.1)	100/99	0.0
			<b>Vanillate <i>O</i>-demethylase alpha subunit [<i>Pseudomonas</i> sp. Strain HR199 ] (O05616.1)</b>	<b>100/80</b>	<b>0.0</b>
			<b>Vanillate <i>O</i>-demethylase oxygenase subunit [<i>Pseudomonas putida</i> KT2440] (AAN69332.1)</b>	<b>100/76</b>	<b>0.0</b>
<i>vanB</i>	Vanillic acid <i>O</i> -demethylase oxidoreductase* (EKX81752.1)	924	Vanillate <i>O</i> -demethylase oxidoreductase [ <i>Pseudomonas putida</i> ] (WP_064303991.1)	100/99	0.0
			<b>Vanillate <i>O</i>-demethylase beta subunit [<i>Pseudomonas</i> sp.]</b>	100/62	3.00E-

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<b>Strain HR199 ] (O05617.7)</b>		136
<b>Vanillate O-demethylase beta subunit [<i>Pseudomonas fluorescens</i> BF13 ] (CAB93957.1)</b>	100/59	5.00E-128

**D] Protocatechuate metabolism (contig 99)**

<i>pcaH</i>	Protocatechuate 3,4-dioxygenase, beta subunit* (EKX84869.1)	720	Protocatechuate 3,4-dioxygenase beta subunit [ <i>Pseudomonas japonica</i> ] (WP_042124863.1)	100/98	1e-174
			<b>Protocatechuate 3,4-dioxygenase beta subunit [<i>Pseudomonas putida</i>] (2PCD_M)</b>	<b>98/89</b>	<b>3e-167</b>
			<b>Protocatechuate 3,4-dioxygenase beta subunit [<i>Pseudomonas</i> sp. Strain HR199] (CAB43484.1)</b>	<b>100/89</b>	<b>5e-171</b>
<i>pcaG</i>	Protocatechuate 3,4-dioxygenase, alpha subunit* (EKX84870.1)	606	Protocatechuate 3,4-dioxygenase alpha subunit [ <i>Pseudomonas japonica</i> ] (WP_042124861.1)	100/93	3e-137
			<b>Protocatechuate 3,4-dioxygenase alpha subunit [<i>Pseudomonas putida</i>] (2PCD_A)</b>	<b>99/88</b>	<b>1e-137</b>
			<b>Protocatechuate 3,4-dioxygenase alpha subunit [<i>Pseudomonas</i> sp. Strain HR199] (CAB43485.1)</b>	<b>100/82</b>	<b>2e-131</b>

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\* indicates activity demonstrated in strain CSV86

Bold faced indicate functionally characterized protein