

Supplementary Information for

Functional characterization of Vib-PT, an aromatic prenyltransferase involved in the biosynthesis of vibrinalactone from *Stereum vibrans*

Na Bai^{1,3}, Guo-Hong Li^{1,3}, Shao-Liu Luo¹, Liangcheng Du², Qian-Yi Hu¹, Han-Ke Xu¹, Ke-Qin Zhang^{1,*}, Pei-Ji Zhao^{1,*}

¹State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, and Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University, Kunming 650091, PR China

²Department of Chemistry, University of Nebraska Lincoln, Lincoln, Nebraska 68588, USA

³These authors contributed equally to this work

*Correspondence:

Ke-Qin Zhang: E-mail: kqzhang1@ynu.edu.cn, Tel: 86-871-65034878

Pei-Ji Zhao: E-mail: pjzhao@ynu.edu.cn, Tel: 86-871-65031092

Na Bai and Guo-Hong Li contributed equally to this work. Author order was determined both alphabetically and in order of increasing seniority.

Table S1. Primer sequences used for PCR and qPCR

Name	Primer sequence (5'-3')
Alc_A-F	CCAAGCTTTGAAAAGCTGATTGTGAT
Alc_A-R	CGGGATCCGCTAATTAAGTACTGAGTAGA
PT-2UP-BamH1	ATGGATCCCGCTTCTCGGTCACTGTTATGGTC
PT-2DN-HpaI	ATGTTAAC TTGCGAACTCGTAGTGGTAAGGTA
PT-2iv-UP-sac1	AT GAGCTCTTGCGAACTCGTAGTGGTAAGGTA
PT-2iv-DN-xho1-Not1	ATCTCGAGGCGGCCGCGCTTCTCGGTCACTGTTAT GGTC
PT-FW F	CCACCATGGTAGATCTTTGCGAACTCGTAGTGGTAAGG
PT-FW R	AACACTAGTCAGATCTTTTAAAAGCTGATTGTGATAGTTC
PT-IV F	ATCCTCTAGAGTCGACCGCTTCTCGGTCACTGTT
PT-IV R	ATGCCTGCAGGTCGACAACAAGAGAGGTCCACAATGA
IN F	AATTCTGATGTGTAATTCTG
ALC R	GTAAACACTAGTCAGATCTTT
1HF	ATTCGGCTCCAACAATGTC
1IR	GTCCACAATGATAAAGAGGG
β -tubulin F	ATGCGTGAGATTGTCCATCT
β -tubulin R	CCATGGTTCCGGGCTCGAGA
PT F	CTATCCCTCCTTGGTCAACT
PT R	TTAGGAAGCTGCCTCTCTGC

Table S2. Strains and plasmids

Strain or Vector	Properties	Reference or Source
<i>E. coli</i> BL21(DE3)	F ⁻ , ompT, hsdS(rBB-mB-), gal, dcm (DE3)	Novagen
<i>Agrobacterium tumefaciens</i> AGL1	Genotype C58 RecA (rif R/carbR) Ti pTiBo542DT-DNA (strepR) Succinamopine.	Biomed
<i>Agrobacterium tumefaciens</i> LBA4404	Genotype Ach5 (RIF R) Ti pAL4404 (strepR) Octopine.	Biomed
<i>Agrobacterium tumefaciens</i> EHA105	Genotype C58 (rif R) Ti pEHA105 (pTiBo542DT-DNA) Succinamopine.	Biomed
<i>Agrobacterium tumefaciens</i> GV3101	Genotype C58 (rif R) Ti pMP90 (pTiC58DT-DNA) (gentR) Nopaline.	Biomed
<i>Stereum vibrans</i>	Wild-type, vibrallactone producer	Zhao et al., 2013
<i>Stereum vibrans</i> A2	<i>vib-PT</i> RNA silencing transformant by targeted PCR	This study
<i>Stereum vibrans</i> G7	<i>vib-PT</i> RNA silencing transformant by targeted PCR	This study
pUC18	pUC cloning vector	Addgene
pC-HYG-YR	Yeast recombinational cloning compatible <i>Agrobacterium tumefaciens</i> ternary vector containing a hygromycin selectable marker on transfer DNA	Addgene
pUCH2-8	pUCH2-8 containing the hygromycin B phosphotransferase gene, as the selectable marker for transformation of <i>Aspergillus</i>	Alexander et al., 1998
pMCB17apx	Promoter of alcohol dehydrogenase <i>alcA</i> (p)	Efimov, 2003
pUC18-RNAi	pUC18 harboring the second intron of the <i>Arabidopsis</i> small nuclear ribonuclear protein D1 (locus At 4g02840)	Kumar et al., 2012
pUNZ101	The promoter of <i>alcA</i> (p) was cloned into the expression vector pUCH2-8	This study
pUNZ103	A 1103-bp and reverse-complement of <i>vib-PT</i> cloned into the two ends of the intron of the pUC18-RNAi vector	This study
pUNZ104	The 2456-bp RNA silencing cassette digested with BamHI and NotI and cloned into pUNZ101	This study
pYUZ10	A 1479-bp element containing the <i>alcA</i> promoter and <i>vib-PT</i> forward sequence subcloned into the pC-HYG-YR vector	This study
pYUZ11	A 1357-bp element containing the intron and <i>Vib-PT</i> reverse-complemented sequence cloned into pYUZ10	This study

References

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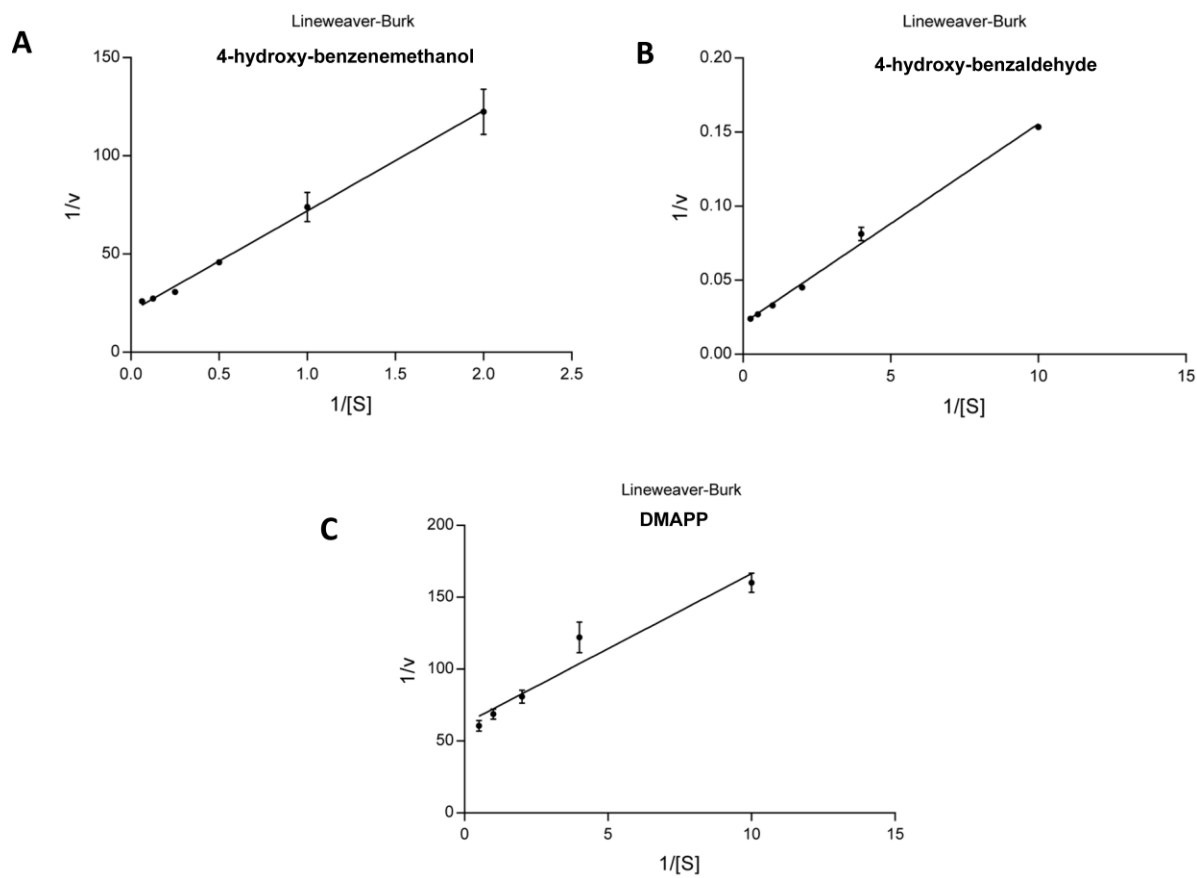


Figure S1. Michaelis-Menten plots of three substrates. (A) Lineweaver-Burk of 4-hydroxy-benzenemethanol. **(B)** Lineweaver-Burk of 4-hydroxy-benzaldehyde. **(C)** Lineweaver-Burk of DMAPP.

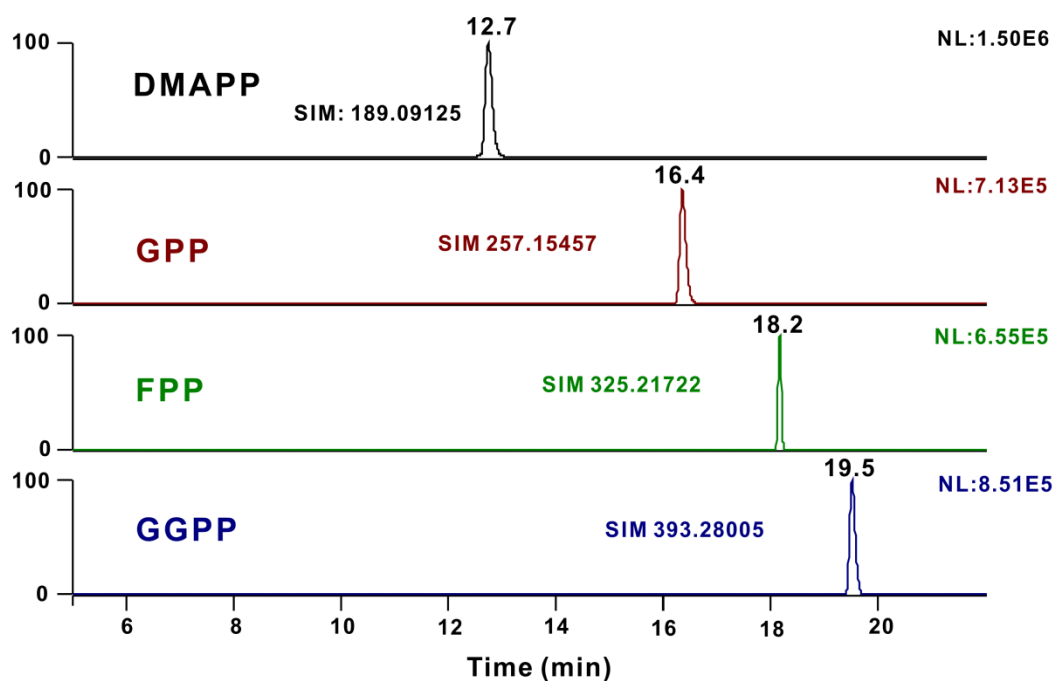


Figure S2. SIM chromatograms of the products by UPLC-HR-ESI-MS analysis. The donor specificity of Vib-PT was probed using DMAPP, GPP, FPP or GGPP and 4-hydroxy-benzaldehyde as an acceptor. **DMAPP** shows the specific ion of product for DMAPP and 4-hydroxy-benzaldehyde selected ion monitoring (SIM) peak at m/z 189.09125 [M - H]⁻; **GPP** shows the specific ion of the product for GPP and 4-hydroxy-benzaldehyde SIM peak at m/z m/z 257.15457 [M - H]⁻; **FPP** shows the specific ion of product for FPP and 4-hydroxy-benzaldehyde SIM peak at m/z 325.21722 [M - H]⁻; and **GGPP** shows the specific ion of the product for GGPP and 4-hydroxy-benzaldehyde SIM peak at m/z 393.28005 [M - H]⁻. The retention times of these products were 12.7, 16.4, 18.2 and 19.5 min, consistent with their polarity.

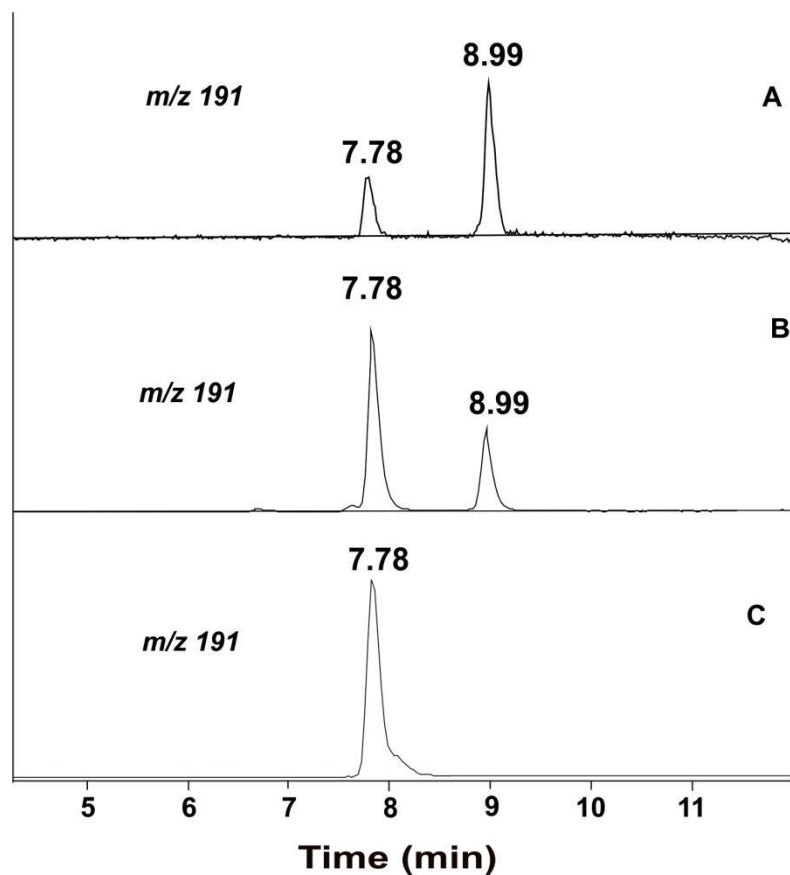


Figure S3. Chromatogram profiles for the products of 4-hydroxy-benzenemethanol by UPLC-MS analysis. (A) Extracted ion chromatographs of UPLC-MS analysis of the products for DMAPP and 4-hydroxy-benzenemethanol Vib-PT for 1 h. **(B)** Extracted ion chromatographs of UPLC-MS analysis of the products for DMAPP and 4-hydroxy-benzenemethanol Vib-PT for 20 h. **(C)** 4OPBM was fast converted into 4O3PBM under acidic (such as 0.1% formic acid) and nonenzymatic conditions.