Supplemental Material

Blue valorization of lignin-derived monomers via reprogramming marine bacterium *Roseovarius nubinhibens*

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Table S1. Plasmids used in this study.

Name	Description	Source
pQLL-mCherry	*pBR322* ori, *bla*, mCherry	Beijing Liuhe BGI.
pBBR1MCS-5	*pBBR1* ori, *pBBR1* Rep, Gm^R	Lab stock
pTemplate	*pUC* ori, *bla*, gRNA scaffold	Lab stock [1]
pCasEnv-lacI	pBBR1MCS-5, *lacI*-P_{trc}, *cas9*	Lab stock [2]
pWY06	pWY, gRNA06	Lab stock [2]
pgRNA-pcaG	pTemplate, gRNA07	Lab stock [2]
pgRNA-pcaC	pTemplate, gRNA09	This study
pmCherry	pBBR1MCS-5, *lacI*-P_{trc}, mCherry	This study
pWYi01	pBBR1MCS-5, *lacI*-P_{trc}, *dcas9*, gRNA09	This study
pWYi02	pBBR1MCS-5, *lacI*-P_{trc}, *dcas9*, gRNA06	This study
pWYi-M03	pWYi02, gRNA09	This study
pWYi-M04	pWYi02, gRNA07	This study

Table S2. CRISPRi-mediated PCA production by *R. nubinhibens* at 36 h. [a]

Target	Plasmid	IPTG (mM)	4HB Consumption (mM)	PCA Titer (mM)	Yield (%, moL/moL)
/	pBBR1MCS-5	0.25	7.52 ± 0.39	0.33 ± 0.07	4.38 ± 1.11
		0.5	9.69 ± 0.91	0.46 ± 0.43	5.00 ± 4.69
pcaC	pWYi01	0.25	6.61 ± 0.43	0.39 ± 0.02	5.90 ± 0.12
		0.5	8.28 ± 0.58	0.97 ± 0.03	11.74 ± 1.16
pcaH	pWYi02	0.25	5.77 ± 0.76	0.54 ± 0.05	9.44 ± 0.30
		0.5	7.26 ± 0.65	1.39 ± 0.06	19.27 ± 1.93

[a] Data are the averages and standard deviations from two independent biological experiments. IPTG, Isopropyl-β-D-thiogalactopyranoside; 4HB, 4-hydroxybenzoate; PCA, protocatechuate.

Table S3. Multiplex CRISPRi-mediated PCA production by *R. nubinhibens* at 36 h. [a]

Target	Plasmid	4HB Consumption (mM)	PCA Titer (mM)	Yield (%, moL/moL)
/	pBBR1MCS-5	9.69 ± 0.91	0.46 ± 0.43	5.00 ± 4.69
pcaC and *pcaH*	pWYi-M03	7.41 ± 0.14	1 49 ± 0.12	20.15 ± 1.72
pcaH and *pcaG*	pWYi-M04	5.88 ± 1.51	1.42 ± 0.22	24.47 ± 2.30

[a] Data are the averages and standard deviations from three independent biological experiments.
IPTG, Isopropyl-β-D-thiogalactopyranoside; 4HB, 4-hydroxybenzoate; PCA, protocatechuate.

Table S4. Primers used in this study.

Primer	Sequence
Primers for In-Fusion DNA assembly	
XIA-WY-109	TTTCACACAGGAAACAGACCATGGTGAGCAAGGGCGAGGA
XIA-WY-110	CGCTTACAATTTCCATTCGCCTACTTGTACAGCTCGTCCA
XIA-WY-111	TCCTCGCCCTTGCTCACCATGGTCTGTTTCCTGTGTGAAA
XIA-WY-112	TGGACGAGCTGTACAAGTAGGCGAATGGAAATTGTAAGCG
XIA-WY-093	TTGACTACCGGAAGCAGTGTTCTAGATTGTAAAACGACGGCCAGTC
XIA-WY-094	CATTTGAGAAGCACACGGTCACAGGAAACAGCTATGACCG
XIA-WY-095	GACTGGCCGTCGTTTTACAATCTAGAACACTGCTTCCGGTAGTCAA
XIA-WY-096	CGGTCATAGCTGTTTCCTGTGACCGTGTGCTTCTCAAATG
XIA-WY-201	GATCATTTATTCTGCCTCCCTCGAACCACGCAATGCGTCT
XIA-WY-202	CACCGTTTTTATCAGGCTCTTTGAGTGAGCTGATACCGCT
XIA-WY-203	AGACGCATTGCGTGGTTCGAGGGAGGCAGAATAAATGATC
XIA-WY-204	AGCGGTATCAGCTCACTCAAAGAGCCTGATAAAAACGGTG
Primers for inverse PCR	
XIA-WY-155	TTTCAGACGCTGATCACCGAGTTTTAGAGCTAGAAATAGC
XIA-WY-156	TCGGTGATCAGCGTCTGAAAGCTAGCATTATACCTAGGAC
XIA-WY-199	TAGAGATCGCTAACTGGTTGGGACTGGTTGCATAACCATG
XIA-WY-200	CAACCAGTTAGCGATCTCTAGTCACCTCCTAGCTGACTCA
Primers for colony PCR	
XIA-WY-044	TTAGGTGGCGGTACTTGGGT
XIA-WY-045	GCAGTCGCCCTAAAACAAAG
Primers for RT-qPCR	
XIA-WXY-001	CCTGATCTAGCCATGCCG
XIA-WXY-002	CGTATTACCGCGGCTGCT
XIA-WY-222	TTTCGAGGAAATCCCCATGC
XIA-WY-223	TTCCGCATAGGTTTCCTTGG
XIA-WY-224	ATGACCAGGGCTATTACGTC
XIA-WY-225	CGCCCTCGAAATAACATTGG
XIA-WY-226	CATCGGTCTCAACACAAGGC
XIA-WY-227	GGATGTCGAAACGGTAGACG

Table S5. gRNA sequences used in this study.

gRNA	Target	Strand [a]	PAM	Protospacer
gRNA06	*pcaH*	C	CGG	CAAAGCGCCAGCGAAATCAC
gRNA07	*pcaG*	C	AGG	GCAACGTCTCGACTACCTCA
gRNA09	*pcaC*	C	AGG	TTTCAGACGCTGATCACCGA

[a] C stands for coding strand and N stands for non-coding strand.

Table S6. Sequences of synthesized mCherry gene used in this study.

Gene	Sequence [a]
mCherry	CGGTTCTGGCAAATATTCTGAAATGAGCTGTTGACAATTAATCATCC GGCTCGTATAATGTGTGGAATTTCACACAGGAAACAGACCATGGTG AGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATG CGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTC GAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCA GACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGC CTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTAC GTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTC CCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGG CGGCGTGGTGACCGTGACCCAGGACTCCTCCCTCCAGGACGGCG AGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGA CGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTC CGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCA AGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAG GTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGC GCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGG ACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACT CCACCGGCGGCATGGACGAGCTGTACAAGTAG [b]

[a] Synthesized gene was carried by pQLL plasmid with Amp^R.

[b] The promoter sequence was shown in Blue and the coding sequence of mCherry was shown in Red.

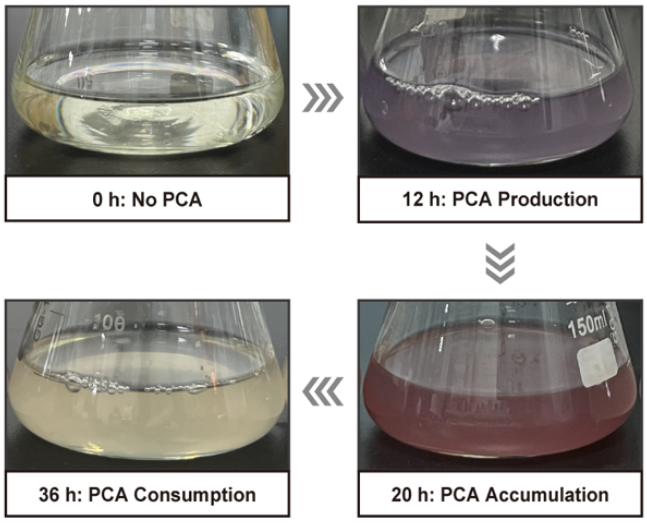

Fig. S1. The color variation of the wild-type *R. nubinhibens*. During the cultivation, 4HB was added into the basal medium as the sole carbon source.

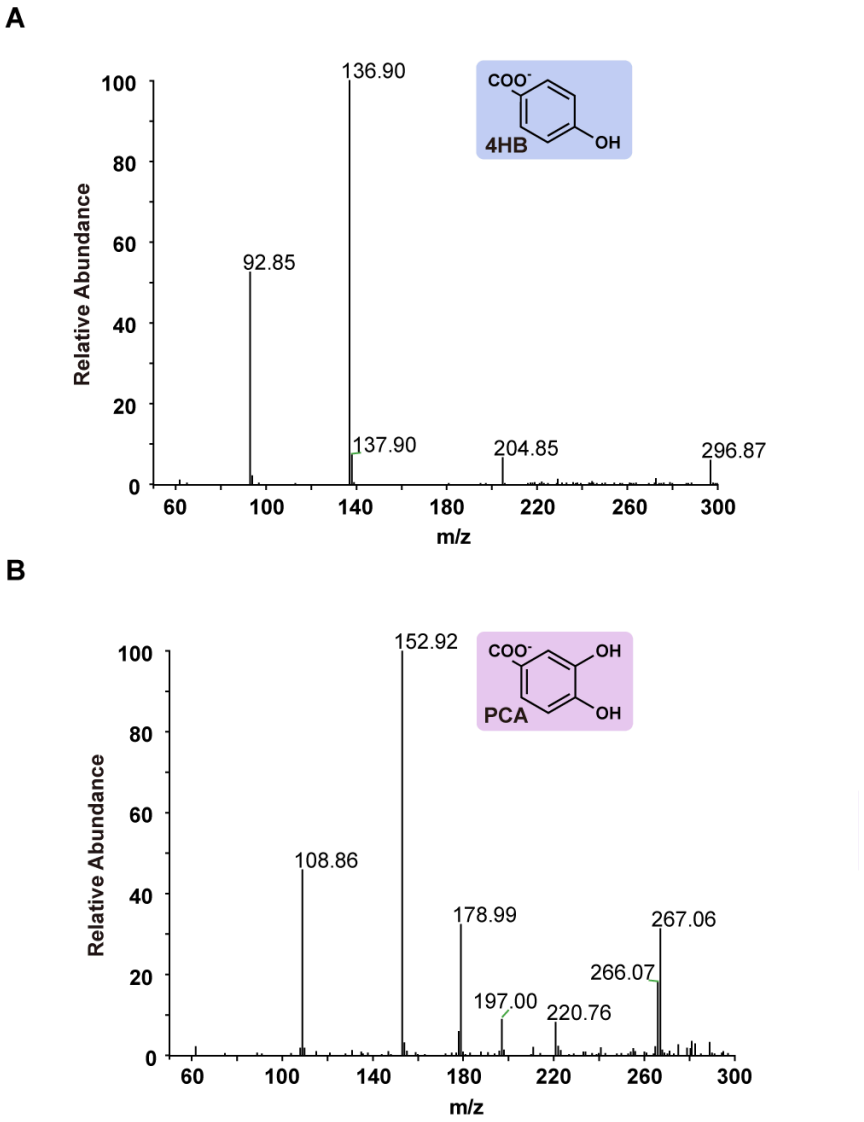

Fig. S2. MS analysis of standards. (A) MS analysis of the 4HB standard. **(B)** MS analysis of the PCA standard.

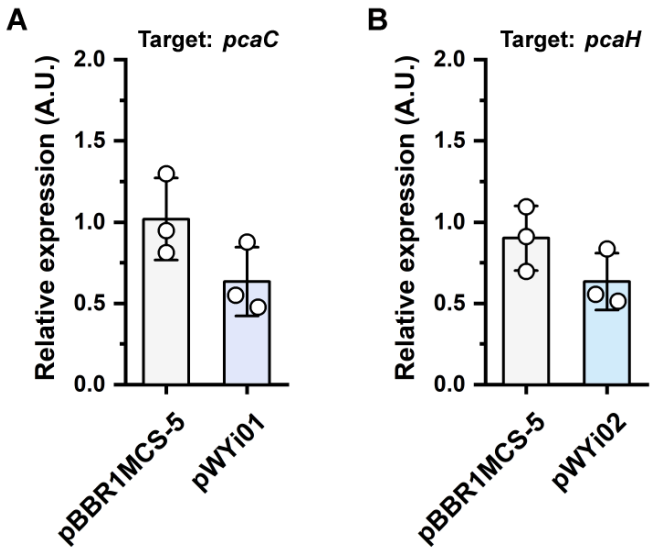

Fig. S3. Relative expression of target genes. (A) Relative expression of *pcaC* in the strain with pWYi01. **(B)** Relative expression of *pcaH* in the strain with pWYi01. Experiments were carried in triplicate and the error bars represented the standard deviations of the means of three biological replicates.

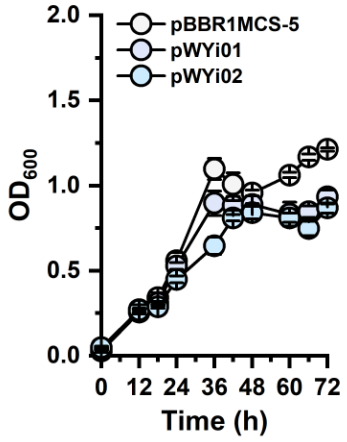

Fig. S4. Growth profile. OD_{600} of the strain with pBBR1MCS-5, pWYi01 and pWYi02 with 0.5 mM IPTG induction. Experiments were carried in triplicate and the error bars represented the standard deviations of the means of three biological replicates.

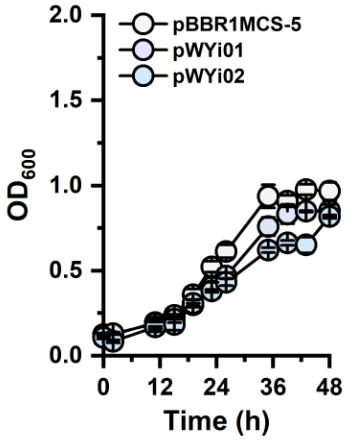

Fig. S5. Growth profile. OD_{600} of the strain with pBBR1MCS-5, pWYi01 and pWYi02 with 0.25 mM IPTG induction. Experiments were carried in triplicate and the error bars represented the standard deviations of the means of three biological replicates.

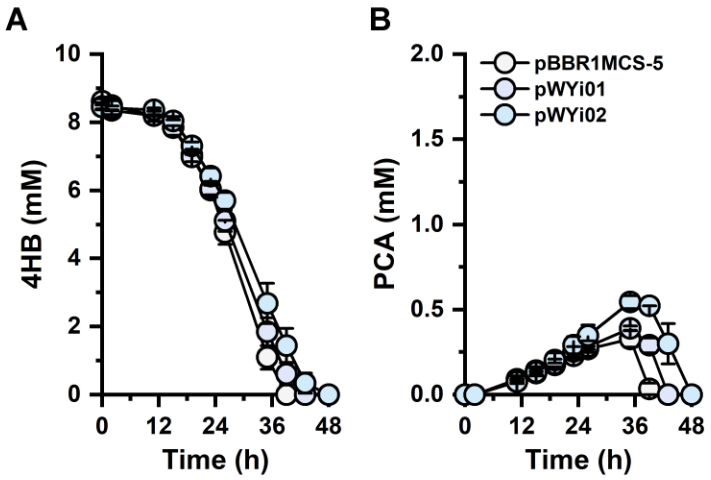

Fig. S6. PCA production in strains with pWYi. (A) Utilization of 4HB, **(B)**Titer of PCA with pBBR1MCS-5, pWYi01 and pWYi02 with 0.25 mM IPTG induction. Experiments were carried out in duplicate and the error bars represented the standard deviations of the means of two biological replicates.

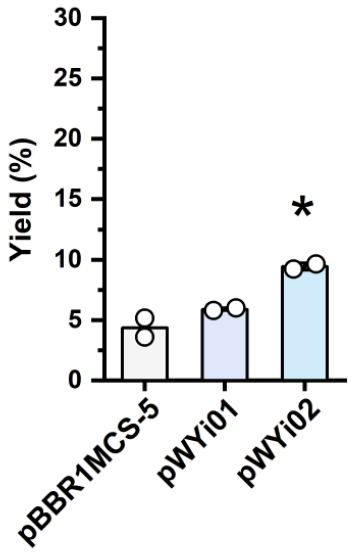

Fig. S7. Molar yield of PCA in strains with pWYi. Yield (%) of PCA with pBBR1MCS-5, pWYi01 and pWYi02 with 0.25 mM IPTG induction. Experiments were carried out in duplicate and the error bars represented the standard deviations of the means of two biological replicates. The differences were statistically evaluated by t-test (*, $p<0.05$).

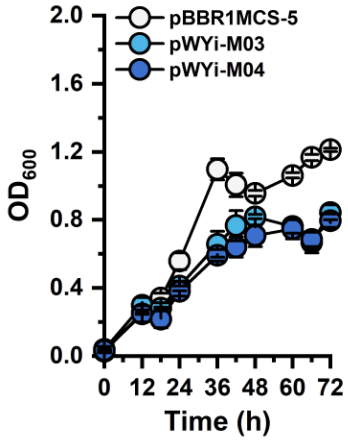

Fig. S8. Growth profile. OD_{600} of the strain with pBBR1MCS-5, pWYi-M03 and pWYi-M04 with 0.5 mM IPTG induction. Experiments were carried in triplicate and the error bars represented the standard deviations of the means of three biological replicates.

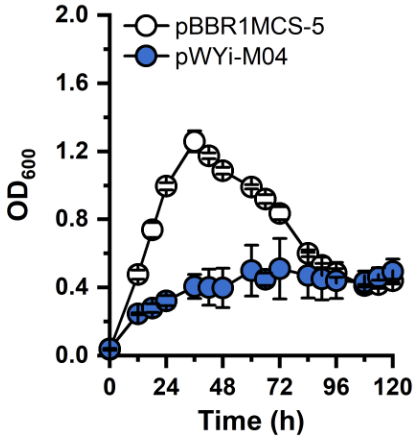

Fig. S9. Growth profile. OD_{600} of the strain with pBBR1MCS-5 and pWYi-M04 in real seawater media. Experiments were carried in triplicate and the error bars represented the standard deviations of the means of three biological replicates.

References

(1) Li, X.; Bao, N.; Yan, Z.; Yuan, X. Z.; Wang, S. G.; Xia, P. F. Degradation of antibiotic resistance genes by VADER with CRISPR-Cas immunity. *Appl Environ Microbiol*. **2023**, *89*, e00053-00023.

(2) Wei, Y.; Feng, L. J.; Yuan, X. Z.; Wang, S. G.; Xia, P. F. Developing a base editing system for marine *Roseobacter* clade bacteria. *ACS Synth Biol*. **2023**, *12*, 2178-2186.