

Supplementary Information

***De novo* Microbial Production of Picolinic Acid via a Short-Cut Biosynthetic Pathway**

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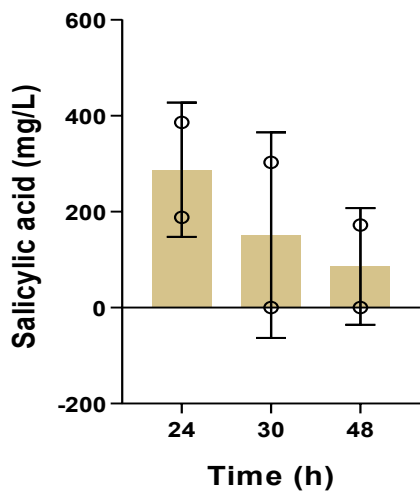


Fig. S1 Unconverted SA titer of strain PABW02.

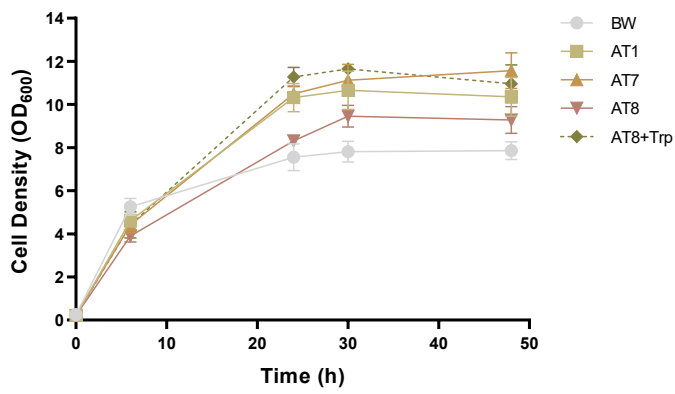


Fig. S2 OD₆₀₀ of glycerol strains

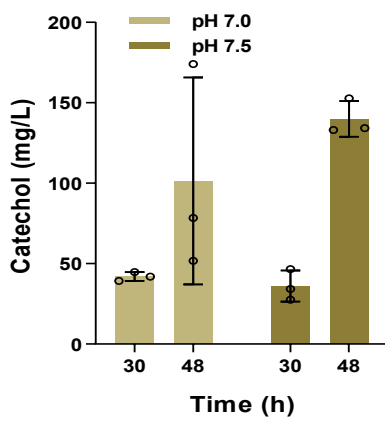


Fig. S3 Catechol accumulation in different pH medium.

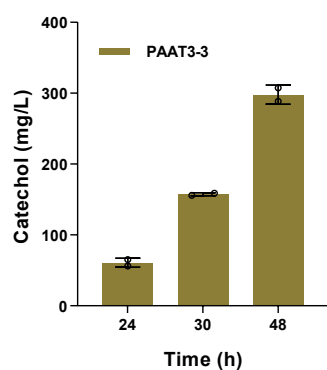


Fig. S4 Catechol accumulation of strain PAAT3-3.

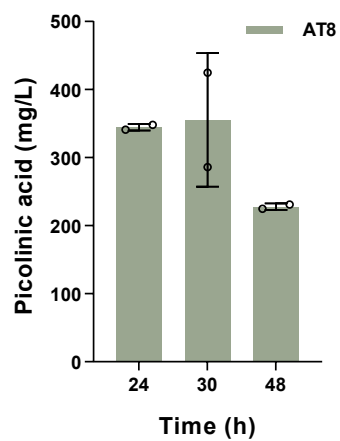


Fig. S5 Picolinic acid titer of strain PAAT8.

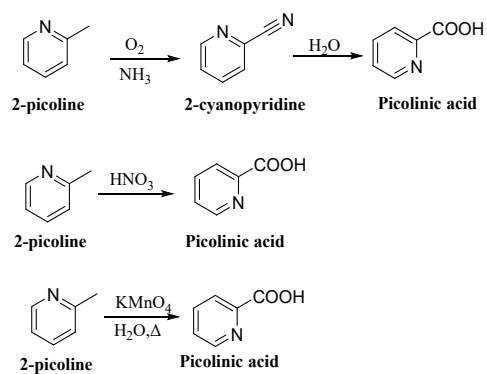


Fig. S6 Schematic of picolinic acid chemical synthesis routes.

Table S1. Plasmids and bacterial strains utilized in this study.

Strains/plasmids	Relevant characteristics	Sources
XL1-Blue	<i>recA1 endA1g yrA96 thi-1 hsdR17 supE44 relA1 lac</i> [F' <i>proAB lacI^qZΔM15 Tn10 (Ter^r)</i>]	Stratagene
BW25113 (F')	<i>rrnBT14 ΔlacZΔWJ16 hsdR514 ΔaraBADAH33 ΔrhaBADLD78</i> [F' <i>proAB lacI^qZΔM15 Tn10 (Ter^r)</i>]	¹
PABW01	BW25113 (F') containing pHA-CbzE	This study
PABW02	BW25113 (F') containing pLC-NahG	This study
AT1	BW25113Δ <i>pykAF</i>	This study
AT2	BW25113Δ <i>pykAFΔptsG</i>	This study
AT3	BW25113Δ <i>pykAFΔptsGΔtrpE</i>	This study
AT4	BW25113Δ <i>pykAFΔptsGΔtrpEΔedd</i>	This study
AT5	BW25113Δ <i>pykAFΔptsGΔmanXYZ</i>	This study
AT6	BW25113Δ <i>pykAFΔptsGΔmanXYZΔtrpE</i>	This study
AT7	BW25113Δ <i>pykAFΔgldA</i>	This study
AT8	BW25113Δ <i>pykAFΔgldAΔtrpE</i>	This study
PAAT3-1	AT3 containing pHA-EP, pMK-LPTG, and pLC-NahG-CbzE	This study
PAAT3-2	AT3 containing pHA-EP, pMK-LPTG-CbzE, and pLC-NahG	This study
PAAT3-3	AT3containing pHA-EP-CbzE, pMK-LPTG, and pLC-NahG	This study
PAAT8	AT8 containing pHA-EP, pMK-LPTG, and pLC-NahG-CbzE	This study
Plasmids	Description	
pCas	<i>lacI, araBAD, pCS101 ori, Kan^r, Exo, Beta, Gam, Cas9</i>	Addgene ²
pTargetF	J23119, <i>pMB1 ori, Sm^r, sgrNA</i>	Addgene ²
pHA-GFP	P _L lacO1, GFP gene, T1 terminator, <i>colE1 ori</i> , and Amp ^r	³
pMK-GFP	P _L lacO1, GFP gene, T1 terminator, <i>p15A ori</i> , and Kan ^r	¹
pLC-GFP	P _L lacO1, GFP gene, T1 terminator, <i>psc101 ori</i> , and Cm ^r	¹
pLC-NahG	pLC containing <i>nahG</i> from <i>Pseudomonas putida</i>	This study
pHA-CbzE	pHA containing <i>cbzE</i> from <i>Pseudomonas putida</i>	This study
pHA-EP	pHA containing <i>entC</i> from <i>E. coli</i> and <i>pchB</i> from <i>P. fluorescens</i> Migula	This study
pMK-LPTG	pMK containing <i>aroL, ppsA, tktA, aroG^{br}</i> from <i>E. coli</i>	¹
pHA-EP-CbzE	pHA containing <i>entC, pchB</i> and <i>cbzE</i>	This study
pMK-LPTG-CbzE	pMK containing P _L lacO1-LPTG and P _L lacO1-CbzE	This study
pLC-NahG-CbzE	pLC containing <i>nahG</i> and <i>cbzE</i>	This study
pTargetF-PykA	pTargetF containing an N20 sequence targeting <i>pykA</i> from <i>E. coli</i>	This study
pTargetF-PykF	pTargetF containing an N20 sequence targeting <i>pykF</i> from <i>E. coli</i>	This study
pTargetF-GldA	pTargetF containing an N20 sequence targeting <i>gldA</i> from <i>E. coli</i>	This study
pTargetF-TrpE	pTargetF containing an N20 sequence targeting <i>trpE</i> from <i>E. coli</i>	This study
pTargetF-PtsG	pTargetF containing an N20 sequence targeting <i>ptsG</i> from <i>E. coli</i>	This study
pTargetF-ManXYZ	pTargetF containing an N20 sequence targeting <i>manXYZ</i> from <i>E. coli</i>	This study
pTargetF-Edd	pTargetF containing an N20 sequence targeting <i>edd</i> from <i>E. coli</i>	This study

Table S2. List of primers used in this study.

Primers	Sequence (5'–3')	Description
cbzE-F(BamHI)	gggaaaGGATCCAGGAGATATACCATGAACAAA GGTATAATGCGCCCCG	For amplification of <i>cbzE</i> in construction of plasmid pHA-EP-CbzE
cbzE-R(ApaI)	gggaaaGGGCCCTCAGGTCAGCACGGTCATGAAT CGTTC	
pHA/MK-F (AvrII)	gggaaaCCTAGGaattgtgagcggataacaattgacattgtgagc	For amplification of <i>cbzE</i> in construction of plasmid pMK-LPTG-CbzE
pHA/MK-R (SacI)	gggaaaGAGCTCGCGGCGGATTTGTCCTACTCAG GAG	
cbzE-F(SalI)	gggaaaGTCGACAGGAGATATACCATGAACAAA GGTATAATGCGCCCC	For amplification of <i>cbzE</i> in construction of plasmid pLC-NahG-CbzE
cbzE-F(BamHI)	gggaaaGGATCCTCAGGTCAGCACGGTCATGAAT C	
pykA-Upstream-F	ATGTCCAGAAGGCTTCGCAG	For construction of <i>pykA</i> homologous arms
pykA-Upstream-R	CTTAATGTCTGCTTTGTCTTTTTCGGTCAG	
pykA-Downstream-F	CTGACCGAAAAAGACAAAGCAGACATTAAGG TATCAGCTCTGGTCTGCCAATTTTC	
pykA-Downstream-R	TTACTCTACCGTTAAAATACGCGTGG	
pykF-Upstream-F	ATGAAAAAGACCAAAATTGTTTGCACC	For construction of <i>pykF</i> homologous arms
pykF-Upstream-R	GTTACCTTCAATGGCGGTAACCTCG	
pykF-Downstream-F	GGTATGGAAGTTACCGCCATTGAAGGTAACAG TACGTAAATACTTCCCGGATGC	
pykF-Downstream-R	TTACAGGACGTGAACAGATGCG	
pykA-N20-sgRNA-F (SpeI)	gggaaaactagtAGCGTCAGTACCATCCAGAAgttttaga gctagaaatagcaagttaaaataaggctag	For amplification of <i>pykA</i> and <i>pykF</i> N20 fragments in construction of plasmids pTargetF-PyKA and pTargetF-PyKF
pykF-N20-sgRNA-F (SpeI)	gggaaaactagtTCACTGCGTCAGTACCGTTCGgttttagag ctagaaatagcaagttaaaataaggctag	
sgRNA-R (XhoI)	gggaaaactcagtagggataacagggtaatagatc	
ptsG-Upstream-F	ATGTTTAAGAATGCATTTGCTAACCTGC	For construction of <i>ptsG</i> homologous arm; amplification of <i>ptsG</i> N20 fragment in construction of pTargetF-PtsG
ptsG-Upstream-R	CAAAGAAGCCAAGATACTCAGGCAG	
ptsG-Downstream-F	AAGCTGCCTGAGTATCTTGCTTCTTTGCTGTA TTCTTCTGGGGATGCGTG	
ptsG-Downstream-R	TTAGTGGTTACGGATGTACTCATCC	
ptsG-N20-sgRNA-F (SpeI)	gggaaaactagtGGGTCACCCGCCATATAACGgttttaga gctagaaatagcaagttaaaataaggctag	
gldA-Upstream-F	ATGGACCGCATTATTCAATCACCG	For construction of <i>gldA</i> homologous arm; amplification of <i>gldA</i> N20 fragment in construction of pTargetF-GldA
gldA-Upstream-R	GTGTGACAATGACCATATTCGG	
gldA-Downstream-F	AAATAACCCGAATATGGTCATTGTCGACACCG TGATTGAAGCGAACACCTATTTG	
gldA-Downstream-R	TTATTTCCACTCTTGCAGGAAACG	
gldA-N20-sgRNA-F (SpeI)	gggaaaactagtGCACTTGCCGCCGCCATGGgttttaga gctagaaatagcaagttaaaataaggctag	
trpE-Upstream-F	ATGCAAACACAAAAACCGACTCTC	For construction of <i>trpE</i> homologous arm; amplification of <i>trpE</i> N20 fragment in construction of pTargetF-TrpE
trpE-Upstream-R	GCGTTTCAGCGAGATAAAAACAGAAATC	
trpE-Downstream-F	TAAGTGCCTGATTTCTGTTTTATCTCGCGTA CCGATCATAAAGAGCTGTCTGAAC	
trpE-Downstream-R	TCAGAAAGTCTCCTGTGCATGATG	
trpE-N20-sgRNA-F (SpeI)	gggaaaactagtAGCACGTAATAGGCCGCCAGgttttaga gctagaaatagcaagttaaaataaggctag	
manXYZ-Upstream-F	GTCCATTAAGAATTCCATGACAAGCTC	For construction of <i>manXYZ</i> homologous arm; amplification of <i>manXYZ</i> N20 fragment in construction of pTargetF- ManXYZ
manXYZ-Upstream-R	CTATAACAATAGCAATGGTCACTTGCTACC	
manXYZ-Downstream-F	GTAGCAAGTGACCATTGCTATTGTTATAGGTA TCGATATCGTTAAAGATATGGGTGGTG	
manXYZ-Downstream-R	CAATCAATCCGACGAAGATAACGCTATC	
manXYZ-N20-sgRNA-F (SpeI)	gggaaaactagtAGAAATGATAGAAGCCAGAGgttttag agctagaaatagcaagttaaaataaggctag	
edd-Upstream-F-1	ATGAATCCACAATTGTTACGCGTAACAAATC	For construction of <i>edd</i> homologous arm; amplification of <i>edd</i> N20 fragment in construction
edd-Upstream-R-1	GTTACCACCACTTTCTCATCGATCATC	
edd-Downstream-F-1	GATGATCGATGAGAAAGTGGTGGTGAACGAA	

	CAACCTTTCTCTCATCATGGTGGG	of pTargetF-EDD
edd-Downstream-R-1	TTAAAAAGTGATACAGGTTGCGCCCTG	
edd-N20-sgRNA-F V3 (SpeI)	gggaaaACTAGTGCATGAAGATGTCAATACGGGT TTAGAGCTAGAAATAGCAAGTAAAATAAGG CTAG	

Reference

1. C. Guo, N. N. T. Luu, M. M. Adwer, H. Hosseinzadeh, V. Balan, Y. Yan and Y. Lin, *Metabolic Engineering*, 2026, **94**, 24-34.
2. Y. Jiang, B. Chen, C. Duan, B. Sun, J. Yang and S. Yang, *Applied and environmental microbiology*, 2015, **81**, 2506-2514.
3. V. Nguyen, A. Tseng, C. Guo, M. Adwer and Y. Lin, *Enzyme and Microbial Technology*, 2025, **185**, 110590.