

**SUPPLEMENTAL MATERIAL for:**

**A plant-responsive bacterial signaling system senses an ethanolamine derivative**

Bruna G. Coutinho, Emily Mevers, Amy L. Schaefer, Dale A. Pelletier, Caroline S. Harwood, Jon Clardy and E. Peter Greenberg

Table S1. Compounds present in the Biolog plates PM1-8 that were able to induce *pipA* expression in GM79  $\Delta pipAaapA$  (pP<sub>*pipA*</sub>-*gfp*)

Compound	Average fold change relative to negative control from two independent experiments
Ala-Asp	8.9
Ala-Lys	4.8
Ala-Thr	3.3
D-Ala-D-Ala	9.2
D-Ala-Gly	3.8
<b>Ethanolamine</b>	<b>48.2</b>
Ethylamine	4.4
Gly-Asp	8.5
Gly-D-Asp	7.4
Gly-D-Ser	7.7
Gly-D-Thr	8.4
Gly-Gly-Phe	2.6
Gly-Lys	17.2
Gly-Met	4.9
Gly-Ser	6.8
Gly-Thr	12.0
His-Gly	4.7
His-His	2.6
L-Cysteine	4.1
L-Lyxose	4.5
Ser-Glu	4.6
Ser-Phe	11.4
Ser-Pro	8.3
Sulfate	2.7
Taurocholic acid	3.2
Thr-Ser	2.5

Table S2. Strains and plasmids used in this study

Strain or plasmid	Relevant genotype/phenotype	Ref.
<b>Strains</b>		
<i>Pseudomonas sp.</i>		
GM79	Wild-type isolated from <i>Populus deltoides</i> root endosphere	(1, 2)
79Δ <i>aapF</i>	Transporter PBP gene ( <i>aapF/PMI36_04617</i> ) in-frame deletion in GM79	this work
79Δ <i>pipR</i>	<i>pipR</i> ( <i>PMI36_04623</i> ) in-frame deletion in GM79	(3)
79Δ <i>aapB</i>	Transporter TMD gene ( <i>aapB/PMI36_04621</i> ) in-frame deletion in GM79	(3)
79Δ <i>pipAaapA</i>	Peptidase-encoding genes <i>pipA</i> ( <i>PMI36_04624</i> ) <i>aapA</i> ( <i>PMI36_04622</i> ) double deletion in GM79	(3)
<i>E. coli</i>		
M15	F-, Φ80Δ <i>lacM15</i> , <i>thi</i> , <i>lac</i> -, <i>mtl</i> -, <i>recA</i> +	Qiagen
S17-1	<i>recA</i> , <i>thi</i> , <i>pro</i> , RP4-2-Tc::Mu-Km::Tn7	(4)
<b>Plasmids</b>		
pQE30	N-terminal His-protein expression vector, Ap <sup>R</sup>	Qiagen
pRep4	<i>lacI</i> -containing vector, Km <sup>R</sup>	Qiagen
pQE <i>aapF</i>	Cytoplasmic N-terminal His <sub>6</sub> -AapF expression plasmid, Ap <sup>R</sup>	This work
pPROBE-NT	Broad host vector containing promoterless <i>gfp</i> reporter, Km <sup>R</sup>	(5)
pP <sub><i>pipA</i></sub> - <i>gfp</i>	<i>pipA</i> promoter region cloned into <i>gfp</i> -reporter pPROBE-NT, Km <sup>R</sup>	(3)
pEX19Gm	Suicide vector, <i>sacB</i> , Gm <sup>R</sup>	(6)
pMMB67EH-TetRA	IPTG-inducible, broad host expression plasmid derived from pMMB67EH (7), Tc <sup>R</sup>	(3)
pMMA <i>aapF</i>	Transporter SBP gene ( <i>aapF/PMI36_04617</i> ) cloned into pMMB67EH-TetRA, Tc <sup>R</sup>	this work

PBP, periplasmic binding protein; TMD, transmembrane domain; Ap, ampicillin; Km, kanamycin; Gm, gentamicin; IPTG, isopropyl β-D-1-thiogalactopyranoside; Tc, tetracycline.

Table S3. Primers used in this study

Name	DNA sequence (5'-3')	Description
aapFmut_1F	ACTAG <u>AATT</u> CCCCGTGTCGATGAACTGCTGACT	79ΔaapF construction
aapFmut_1R	CGGCGAGCAATTTCAAATGCCTGG	79ΔaapF construction
aapFmut_2F	CATTTGAAATTGCTCGCCGCTACATGACCCGCTACAAGAAC GACAA	79ΔaapF construction
aapFmut_2R	CATGGATCCTCACCTCGTTACAGGCGTTGTGG	79ΔaapF construction
aapFcomp_F	AATGAATTCCAAGAAGGAGTTTGACCATGAGATCCAGG	79ΔaapF complementation
aapFcomp_R	CATAAGCTTTCAGTTCTTCACCGTTTCCTCAAGAGAG	79ΔaapF complementation
CytoHisAapF_F	CCGCGCATGCGCGGGTGTACTCACCATCGG	His <sub>6</sub> -AapF cytoplasmic expression in <i>E. coli</i>
CytoHisAapF_R	GGCAAGCTTTCAGTTCTTCACCGTTTCCTCAAGAGAGAA	His <sub>6</sub> -AapF cytoplasmic expression in <i>E. coli</i>

Restriction enzyme sites are underlined.

## SUPPLEMENT REFERENCES

1. Brown SD, *et al.* (2012) Twenty-one genome sequences from *Pseudomonas* species and 19 genome sequences from diverse bacteria isolated from the rhizosphere and endosphere of *Populus deltoides*. *J. Bacteriol.* 194(21):5991-5993.
2. Timm CM, *et al.* (2015) Metabolic functions of *Pseudomonas fluorescens* strains from *Populus deltoides* depend on rhizosphere or endosphere isolation compartment. *Front. Microbiol.* 6(1118).
3. Schaefer AL, *et al.* (2016) A LuxR homolog in a cottonwood tree endophyte that activates gene expression in response to a plant signal or specific peptides. *mBio* 7(4):e01101-01116.
4. Simon R, Prierer U, & Puhler A (1983) A broad host range mobilization system for *in vivo* genetic engineering: transposon mutagenesis in Gram-negative bacteria. *Nat. Biotech.* 1(9):784-791.
5. Miller WG, Leveau JH, & Lindow SE (2000) Improved *gfp* and *inaZ* broad-host-range promoter-probe vectors. *Mol. Plant Microbe Interact.* 13(11):1243-1250.
6. Hoang TT, Karkhoff-Schweizer RR, Kutchma AJ, & Schweizer HtP (1998) A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked *Pseudomonas aeruginosa* mutants. *Gene* 212(1):77-86.
7. Furste JP, *et al.* (1986) Molecular cloning of the plasmid RP4 primase region in a multi-host-range *tacP* expression vector. *Gene* 48(1):119-131.