

New Phytologist Supporting Information

Article title: Targeted plant hologenome editing for plant trait enhancement Authors: Mohammadhossein Ravanbakhsh, George A. Kowalchuk, Alexandre Jousset Article acceptance date: 05 August 2020

The following Supporting Information is available for this article:

Fig. S1 The concentration of ethylene, Fe, Zn, and Cu in the shoot of *Arabidopsis thaliana* Col-0 (a) and eto1 (b) inoculated with the bacterial treatment. Treatments were: inoculation with a non-inoculated control (Ctrl), wildtype *Pseudomonas putida* UW4 (WT), and ACC deaminase-deficient mutant (AcdS⁻). The differences between treatments were analyzed by one way ANOVAs complemented with Tukey's range test (p<0.05). Error bars show ± SE.





Fig. S2 (a) Correlation between ethylene level and phosphorus (P) concentration in the shoot of *Arabidopsis thaliana* in non-bacterized Col-0 (light red circles) or the ethylene overproducer *eto1* (dark red circles), and Col-0 plants bacterized with Pseudomonas putida UW4 (light blue cycle) or its ACC deaminase-deficient mutant (AcdS-) (closed blue cycle). Two separate regressions were calculated to highlight the similarity between the effects of mutations in the plant (red line) and bacterial genome (blue line). We split the results between mutations in the plant and bacterial genomes. b) effect of mutations in the plant genome: non-bacterized Col-0 and ethylene-overproducer genotype *eto1*. (c) effect of mutations in the bacterial genome: *A. thaliana* Col-0 inoculated with *Pseudomonas putida* UW4 (wildtype; WT) or its ACC deaminase-deficient mutant (AcdS-). Error bars show ± SE. Columns with asterisks are significantly different according to a t-test. **; P< 0.001).



Fig. S3 Shoot dry weight of *Arabidopsis thaliana* Col-0 (a) and *eto1* (b) inoculated with the bacterial treatment. Treatments were: inoculation with a non-inoculated control (Ctrl), wildtype *Pseudomonas putida* UW4 (WT), and ACC deaminase-deficient mutant (AcdS⁻). The differences between treatments were analyzed by one way ANOVAs complemented with Tukey's range test (p<0.05). Error bars show \pm SE.





Gradients	Concentration (per liter medium	Proposed stock solution
KH ₂ PO ₄	4 g	
Na ₂ HPO ₄	6 g	
Gluconic acid	2 g	
Citric acid	2 g	
(NH4)2SO4	2 g	N source: don't add it for isolation or enumeration of ACC deaminase-producing bacteria. Instead, add 3 mM ACC form stock solution of 0.5M (aliquot in 50-100 μl microtubes and keep at -20°C).
MgSO ₄	0.2 g	Add ~830 μL (from 1M stock solution of MgSO4,7H2O) to 1 liter DF salts^2
FeSO ₄ ·7H ₂ O	1 mg FeSO₄·7H₂O	Add 100 μL (from 10 g/L FeSO4·7H2O stock solution) to 1 liter DF salts medium ²
Glucose	2 g/L	Add 10 mL (from 0.2 g/mL filter-sterilized stock solution) to 1 liter medium 1
Trace elements	Below table	(Add 100 μL from stock solution (below table) to 1 liter DF salts^2

Table S1 DF minimum medium composition.

Trace elements stock solution							
Ingredient	Amount (per 100 mL)						
H ₃ BO ₃	10 mg						
MnSO ₄ ·H ₂ O	11.19 mg						
ZnSO ₄ ·7H ₂ O	124.6 mg						
CuSO₄·5H₂O	78.22 mg						
MoO ₃	10 mg						

1. Filter-sterilize Glucose and keep it in 4 $^{\circ}C$.

2. Autoclave MgSO4, FeSO4,7H2O, and trace elements stock solution separately at 121°C for 20 min, then keep it at 4 °C.

Table S2 Two-way ANOVA table summarizing the interactive effects of plant genotypes (Col-0 versus ethylene overproducer (*eto1*)) and bacterial inoculation (wildtype *Pseudomonas putida* UW4 (wildtype) versus ACC deaminase-deficient mutant (AcdS-)) on selected nutrient concentration (μ g.g⁻¹) and ethylene level (pl g⁻¹ FW h⁻¹) in plants.

Treatments		ET		Fe		Zn		Cu	
	df	F	Р	F	Р	F	Р	F	Р
Plants	1	49.39	p<0.0001	14.58	p<0.0001	22.62	p<0.0001	11.63	0.001
Bacteria	2	16.42	p<0.0001	12.26	p<0.0001	26.78	p<0.0001	29.14	p<0.0001
Plants×Bacteria	2	0.79	0.46	0.24	0.786	2.76	0.075	1.37	0.265
Error	42								