The Arabidopsis LHT1 amino acid transporter contributes to *Pseudomonas simiae*-mediated plant-growth promotion by modulating bacterial metabolism in the rhizosphere.

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ABSTRACT

The root microbiome structure ensures optimal plant-host health and fitness, and it is, at least in part, defined by the plant genotype. It is well-documented that root-secreted amino acids promote microbial chemotaxis and growth in the rhizosphere. However, whether the plant-mediated re-uptake of amino acids contributes to maintaining optimal levels of amino acids in the root exudates, and in turn, microbial growth and metabolism, remains to be established. Here we show that LHT1, an amino acid inward transporter expressed in *Arabidopsis thaliana* roots, limits the growth of the plant-growth-promoting bacteria *Pseudomonas simiae* WCS417r (*Ps* WCS417r). Amino acid profiling of the *lht1* mutant root exudates showed increased levels of glutamine, among other amino acids. Interestingly, *lht1* exudates or Gln-supplemented wild-type exudates enhance *Ps* WCS417r growth. However, despite promoting bacterial growth and robust root colonization, *lht1* exudates and Gln-supplemented wild-type exudates inhibited plant growth in a *Ps* WCS417r-dependent manner. Transcriptional analysis of defense and growth marker genes revealed that plant growth inhibition was not linked to the elicitation of plant defense, but likely to the impact of *Ps* WCS417r amino acids metabolism on auxin signaling. These data suggest that an excess of amino acids in the rhizosphere impacts *Ps* WCS417r metabolism which in turn inhibits plant growth. Together, these results unveil that LHT1 regulates the amino acid-mediated interaction between plants and *Ps* WCS417r and suggest a complex relationship between root-exuded amino acids, root colonization by beneficial bacteria, bacterial metabolism, and plant growth promotion.

Keywords: Arabidopsis, LHT1, rhizosphere, amino acid homeostasis, beneficial microbes, plant fitness

35

36 **INTRODUCTION**

37 Crop agriculture has been fueled by an over-reliance on chemical fertilizers that have
38 environmentally damaging consequences. Proposed alternative approaches to enhancing plant
39 growth include the use of plant growth-promoting bacteria (PGPB) such as Pseudomonas simiae
40 WCS417r, formerly known as *Pseudomonas fluorescens* WCS417r (1). In addition to factors that
41 impact soil quality, metabolites exuded by roots have a major impact on the establishment of root
42 associations with PGPB. Plants exude nutrient-rich fluids through their roots. These fluids, known
43 as root exudates, influence the rhizosphere by inhibiting the growth of harmful microbes while
44 promoting the growth of beneficial ones. Amino acids (AAs) are among the most represented
45 metabolites in the root exudates of the model plant *Arabidopsis thaliana* (2–4). Microbes in the
46 rhizosphere use AAs, or their derivatives, as food sources and perhaps to communicate with other
47 microbes (*e.g.,* quorum sensing) (5–7) In addition, microbes use AA concentration gradients to
48 navigate the soil toward roots. For example, in the colonization of tomato root tips, wild-type
49 *Pseudomonas fluorescens* Pf0-1 outcompetes a triple-mutant derivative strain with impaired
50 chemotaxis towards AAs (8). Microbes also use rhizospheric AAs to communicate with the plant.
51 For instance, some microbial products trigger the release of AAs from plant roots (9), creating a
52 self-reinforcing microbe-plant interaction. Microbes can also convert AAs into bioactive
53 compounds with opposing effects on plant physiology. For example, microbes can convert AAs
54 into plant growth-promoting substances such as the auxin hormone indole-3-acetic acid (IAA)
55 (10). In contrast, on the other side of the spectrum, microbes have the capacity to metabolize AAs
56 present in the rhizosphere (*e.g.*, glutamine) into derivatives that can inhibit plant growth (*e.g.*,
57 ammonium) (11, 12).

58 On the plant side, the uptake of inorganic and organic nitrogen (including AAs) through the plant
59 roots has been thoroughly studied, and AA-importers mediating this uptake have been reported
60 (13–17). There are at least 61 transporters with confirmed or putative AA uptake activity encoded
61 in the genome of *Arabidopsis thaliana* (18–20). However, to our knowledge, only three of these
62 transporters localize to the plasma membrane of root cortex cells and can take up AAs at the low
63 concentrations at which they are present in the soil. These three AA-importers are LHT1 of the
64 LHT family (Lysine-Histidine-like Transporters), AAP5 of the AAP family (Amino Acid Permeases),
65 and ProT2 of the ProT family (Proline Transporters) (21–25). These AA-importers have broad and
66 overlapping specificities. However, they also show increased affinity for AAs that share molecular
67 geometries and charges (21, 26, 27). Notwithstanding their detailed characterization, it is still
68 unclear to which extent AA-importers expressed in the root cortex modulate the concentration of

69 plant-derived AAs in the rhizosphere and how their activities contribute to building associations
70 with beneficial bacteria. Answering this question is important as the concentration and balance of
71 different AAs in the rhizosphere may impact the growth of belowground microbes, and hence, the
72 growth of plants themselves. For example, colonization of cucumber roots by *Bacillus*
73 *amyloliquefaciens* SQR9 enhances tryptophan secretion from the roots and promotes IAA
74 biosynthesis by *B. a.* SQR9, which in turn boosts plant growth (10). Amino acids also serve as
75 chemoattractants for different soil microbes such as *Pseudomonas fluorescens* Pf0-1 (8),
76 *Ralstonia pseudosolanacearum* (28), and *Sinorhizobium meliloti* (29). To address if and how AA
77 importers contribute to maintaining the amino-acidic composition of the root exudates, the
78 composition and concentration of individual AAs were assessed in root exudates of Arabidopsis
79 wild-type and loss-of-function mutants for the amino acid transporters AAP5, ProT2, and LHT1.
80 Data obtained from AAs profiling, root colonization, plant growth assessment, and Arabidopsis
81 gene expression indicate that LHT1 modulates the concentration of AAs in the root exudates,
82 which in turn controls the growth and metabolism of *P.s. WCS417r* and its ability to promote plant
83 growth.
84
85 **RESULTS**
86 **The amino acid transporter LHT1 modulates amino acid content in Arabidopsis root**
87 **exudates**
88 Plants can modulate amino acid (AA) content in the rhizosphere by controlling the expression and
89 activity of exporters that exude AAs out of the roots or importers that take up AAs present in the
90 soil (30). However, it is less clear to which extent plants use AA-importers to take up AAs that the
91 plants themselves exude into the rhizosphere. This putative retrieval of AAs from the exudates
92 could contribute to modulating AA content in the rhizosphere, and hence, the growth of PGPB.
93 To establish whether plants control the accumulation of plant-derived amino acids in the
94 rhizosphere through the activity of AA importers, loss-of-function mutants for AA transporters with
95 confirmed uptake activity and expressed in the root (21–25) were tested. The loss-of-function
96 mutants *aap5, prot2*, and *lht1* were chosen because: (i) they represent three families of AA
97 importers, (ii) are expressed in the cortex of the root (23, 25, 27), and (iii) LHT1 and AAP5, at
98 least, can take up AAs at the concentrations present in the soil (24).
99 The experimental strategy was based on the method described by Haney *et al* (31) with some
100 modifications (**Fig. S1A**). Briefly, Arabidopsis seedlings were initially grown in full-strength (1x)
101 MS medium with sucrose (0.5%) for 12 days and then transferred to half-strength (0.5x) MS
102 without sucrose for 3 days. To enable the collection of root exudates for further analysis, the

103 seedlings were grown with the roots submerged in liquid culture and separated from the shoots
104 by a sterilized polytetrafluoroethylene (PTFE) mesh that floats at the surface of the liquid. After
105 15 days of growth, the root exudates were collected, and the total AA content was assessed using
106 as previously described (32). Two *aap5* and one *prot2* mutant lines showed no differences in total
107 AA content in the root exudates when compared to Col-0 wild-type plants (**Fig. S1B**). By contrast,
108 the root exudates of the *lht1* mutants showed increased levels of AAs when compared to wildtype
109 (**Fig. 1A**). Since LHT1 localizes to the plasma membrane of the rhizodermis (21) where it acts as
110 an AA importer (22) and the culture media was not supplemented with AAs, the AAs that
111 accumulate in the *lht1* root exudates must be of plant origin. Hence, the changes in the
112 composition of the *lht1* exudates are most likely due to deficient rhizospheric AA reuptake by
113 LHT1. Together, the results indicate that the loss of the AA importer LHT1 can alter the AA profile
114 of root exudates and that not all AA importers expressed in the root serve the function of
115 maintaining the AA concentrations of the exudates. Even though the biochemical activities and
116 localization of LHT1 have been known to be compatible with regulating the rhizosphere's AA
117 composition through re-uptake, this specific function had not been previously reported.
118 Liquid chromatography coupled to mass spectrometry (LC-MS) analysis was used to define the
119 specific AA changes in the root exudates of the *lht1* plant. This analysis revealed that glutamine
120 (Gln) was the most abundant amino acid that had significantly elevated concentrations in the root
121 exudates of *lht1* compared to those of wild-type plants (**Fig. 1B**). Nonetheless, in line with the
122 broad AA-substrate specificity of LHT1 (21), the analysis revealed that several other AAs were
123 enriched in *lht1* root exudates, including alanine, asparagine, aspartic acid, leucine, isoleucine,
124 methionine, phenylalanine, proline, serine, threonine, and valine when compared to the root
125 exudates of wild-type plants (**Fig. 1C**). The rationale to assign LHT1 a role in AA re-uptake from
126 the root exudates is based on its thoroughly established importer function (33). Since LHT1 moves
127 AAs only in an inward direction and our root exudate assays were performed on AA-free media,
128 the most parsimonious model to explain the overaccumulation of AAs in the *lht1*-root exudates is
129 that LHT1 modulates the levels of AAs in the exudates through the re-uptake of previously exuded
130 amino acids.
131
132 ***lht1* root exudates enhance *Ps* WCS417r growth**
133 Plant-Growth-Promoting Bacteria (PGPB), such as *Ps* WCS417r (formerly *Pseudomonas*
134 *fluorescens* WCS417r), colonize the surface of Arabidopsis roots and promote plant growth (1).
135 Since the growth of PGPB depends on the biophysical properties and composition of the
136 rhizosphere, which are modulated by the plant itself, testing whether the changes in *lht1* root

Figure 1

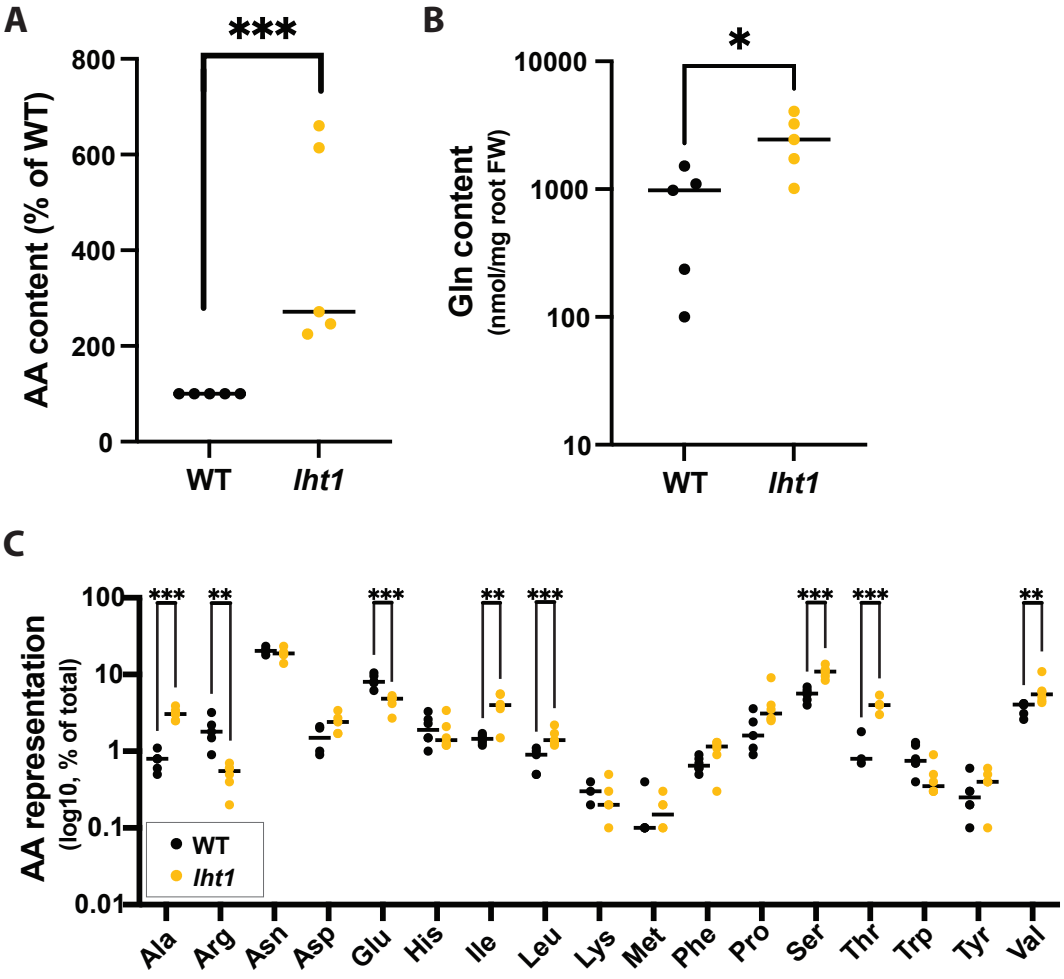

Figure 1. LHT1 modulates amino acid content of Arabidopsis root exudates. Across all panels in this figure, unless otherwise stated, data are from at least 3 independent biological replicates. In each biological replicate, at least 5 seedlings/plants or their products (e.g., exudates) were analyzed. Each dot in the scattered dot plots shows the median of the 5 or more seedlings/plants, and the median of all replicates is depicted as a bar. A two-sided Student's t-test was performed for statistical comparison of two means, or a Welch's t-test for two means with unequal variances, when relevant. P values are represented as *≤0.05, **≤0.01, and ***≤0.005. Further experimental and analysis details are described in the Methods section. (A) lht1-mutant root exudates have higher AA content than wild-type (WT) exudates. Exudates obtained from 15-day-old lht1 or WT seedlings grown on 1x MS liquid medium containing 0.5% sucrose were filter-sterilized, and their total AA content was assessed using the L-Amino Acid Quantitation Colorimetric/ Fluorometric Kit (see Methods). Each data point represents the median of 6 wells in each of 5 independent biological replicates. Data originally scored as nM of total L-AAs per mg of fresh root tissue are presented as a percentage of WT to be able to compare independent biological replicates in the same graph. (B) lht1-mutant root exudates have higher glutamine (Gln) content than wild-type exudates. Root exudates obtained as in (A) were treated with formic acid and profiled via LC-MS. Data analysis was performed on 5 independent samples per condition. (C) lht1-mutant root exudates show imbalanced AA content. Data obtained as in (B) is shown as a percentage for total AAs in lht1 and WT exudates. Glycine data was omitted because unplanted MS medium contained background levels of glycine (2 mg/L). LC-MS analysis was performed on 6 independent samples per condition.

137 exudates affected the growth of *Ps* WCS417r would provide important information to define which
138 plant-made AAs could favor bacterial and plant growth. To this end, the growth of *Ps* WCS417r
139 in wild-type and *lht1* root exudates, as well as in unplanted MS media (MS medium not conditioned
140 by plants) as a control, was longitudinally measured using absorbance OD_{600nm} over a 24 h period
141 with intermittent shaking in a microtiter-plate reader. In line with their higher AA content, *lht1* root
142 exudates supported more bacterial growth than wild-type root exudates (**Fig. 2A**). Considering
143 that wild-type and *lht1* root exudates may differ in the content and abundance of metabolites other
144 than AAs, testing the effect of AA abundance on PGPB growth would at least address if AAs that
145 are over-represented in *lht1* exudates could augment bacterial growth. Supplementation of wild-
146 type exudates with Gln was sufficient to boost *Ps* WCS417r growth (**Fig. 2B**), suggesting that
147 plant-derived Gln is a limiting factor for *Ps* WCS417r growth in the Arabidopsis rhizosphere.
148 Similarly, supplementing wild-type root exudates with serine (Ser), another AA that accumulates
149 in the *lht1* root exudates, promoted *Ps*WCS417r growth (**Fig. 2C**), suggesting an overall positive
150 correlation between amino-acid content in the root exudates and the growth of beneficial microbes
151 in the rhizosphere.
152
153 **Loss of *lht1* promotes root colonization by *Ps* WCS417r**
154 Having uncovered that *lht1* root exudates promote more *Ps* WCS417r growth, the next question
155 to address was whether this enhanced *in vitro* growth would translate to better root colonization.
156 To this end, Arabidopsis seedlings were grown for two weeks in MS-PhytoAgar plates incubated
157 vertically and then transferred to sterile plates with autoclaved-3MM paper as the substrate (**Fig.**
158 **S2A & B**). One day later, roots were flood-inoculated with *Ps* WCS417r. After inoculation, the
159 plates were incubated horizontally, and root colonization proceeded for 72h. At that time, roots
160 were weighed and processed to assess colonization as colony-forming units (CFUs) per root
161 weight. In line with the *in vitro* growth assays, the roots of *lht1* supported more bacterial growth
162 than the roots of wild-type seedlings (**Fig. 3A**). These data support the hypothesis that the higher
163 concentrations of AAs present in the rhizosphere of *lht1* plants have a positive impact on the
164 growth of PGPB such as *Ps* WCS417r. Next, to investigate the mechanisms underlying the
165 enhanced bacterial colonization of *lht1* roots, two processes known to contribute to the
166 establishment of a successful root-bacteria interaction, chemotaxis (8, 34, 35) and biofilm
167 formation (36, 37), were tested. The chemotaxis index was assessed using a modified capillary
168 assay previously described (34, 38) (**Fig. S3**). Unplanted MS media (MS medium that has not
169 been conditioned by roots) was used to assess the background swarming motility of *Ps* WCS417r.
170 The data showed a significant preference for *lht1* root exudates in both non-competitive (**Fig. 3B**)

Figure 2

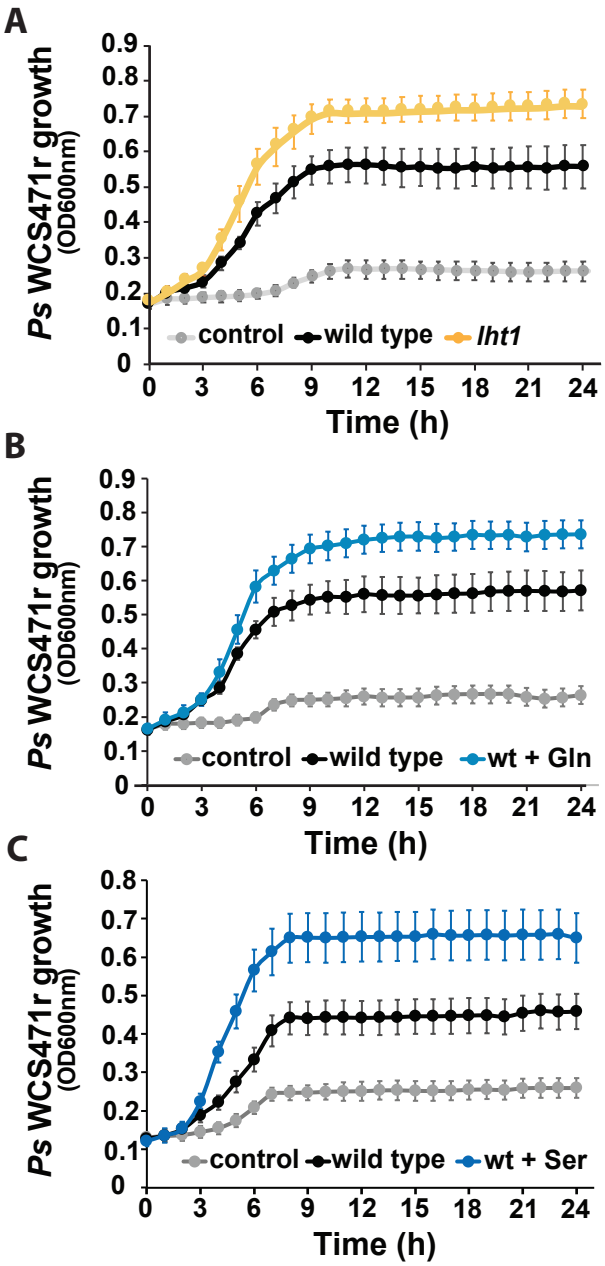

Figure 2. lht1-derived root exudates enhance Ps WCS417r growth. Across all panels in this figure, data represent 3 independent biological replicates. Exudates were obtained as described in Fig. 1A and Methods. In each biological replicate, at least 6 wells per condition were seeded with exudates or control media and longitudinally scored for growth every hour using OD600nm as the readout. Plates were incubated and scored in a temperature-controlled plate reader with shaking. Control corresponds to MS medium without exudate. Each dot in the curves represents the median growth of 6 wells at a given time point. Error bars are SEM. Statistical analysis was done using the CGGC (Comparison of Groups of Growth Curves) permutation test to compare pairs of samples (e.g., wild type vs. lht1) over the course of growth (24 hours). All differences between conditions were statistically significant. Further experimental and analysis details are described in the Methods section. (A) Ps WCS417r grows better in lht1-derived than WT exudates. (B) Ps WCS417r grows better in Gln-supplemented WT exudates. WT exudates were supplemented with 1 mM Gln (final concentration) or an equal volume of water as control. (C) Ps WCS417r grows better in Ser-supplemented WT exudates. WT exudates were supplemented with 1 mM Ser (final concentration) or an equal volume of water as control.

171 and competitive chemotaxis assays (**Fig. 3C-E**). To define whether the chemotaxis-enhancing
172 capacity of *lht1* exudates was driven, at least in part, by the AAs that promote bacterial growth,
173 wild-type exudates supplemented with Gln were also tested. Indeed, wild-type exudates
174 supplemented with Gln showed higher chemotaxis capacity and hence higher *Ps* WCS417r titers
175 than non-supplemented wild-type exudates (**Fig. 3F**). The capacity of *Ps* WCS417r to form
176 biofilms in wild-type and *lht1* root exudates, as well as in unplanted media as a control, was
177 assessed using the crystal violet staining method (37). We hypothesized that the increased
178 accumulation of AAs observed in the root exudates obtained from *lht1* plants could facilitate
179 biofilm formation and, in this way, the growth of *Ps* WCS417r in the rhizosphere. Consistent with
180 this hypothesis, *Ps* WCS417r showed an enhanced capacity to form biofilm in the *lht1* root
181 exudates compared to wild-type root exudates (**Fig. 3G**). Therefore, altogether, the AA transporter
182 LHT1 defines the AA content of the root exudates, the chemotaxis, the biofilm formation, and the
183 growth capacity of *Ps* WCS417r.
184
185 **Rhizospheric amino acids stimulate robust root colonization but compromise plant growth**
186 **in a microbial-dependent manner**
187 *Ps* WCS417r colonizes the root surface and promotes the growth of wild-type plants with a wild-
188 type rhizospheric composition. Since the AA-enriched exudates of *lht1* support more bacterial
189 growth than wild-type exudates, we hypothesized that wild-type plants exposed to *lht1* exudates
190 and *Ps* WCS417r (*lht1*+Ps) would grow larger than plants exposed to wild-type exudates and *Ps*
191 WCS417r (WT+Ps). Unexpectedly, this analysis showed the opposite outcome: *lht1* exudates and
192 *Ps* WCS417r inhibited the growth of wild-type seedlings (**Fig. 4A**). Importantly, as expected, when
193 wild-type seedlings were treated with *Ps* WCS417r only, they grew larger than untreated
194 seedlings. Similarly, *lht1* exudates alone did not inhibit the growth of wild-type seedlings (**Fig. 4A**).
195 Therefore, the growth inhibition depends on both *lht1* root exudates and *Ps* WCS417r. To narrow
196 down the mediators of this plant growth inhibitory effect, Arabidopsis growth was assessed after
197 exposure to Gln-supplemented wild-type root exudates and *Ps* WCS417r (WT-Gln+Ps). Like
198 *lht1*+Ps, WT-Gln+Ps also inhibited the growth of wild-type Arabidopsis seedlings (**Fig. 4B**). Ruling
199 out a direct toxic effect of Gln, seedling growth was not inhibited by 1 mM or 10 mM Gln (**Fig. 4B**),
200 implying a microbe-mediated inhibition of plant growth that is dependent on *Ps* WCS417r Gln
201 metabolism. Importantly, a high *Ps* WCS417r inoculation titer (OD_{600nm} =1.6) did not compromise
202 plant growth, suggesting that the high bacterial colonization in *lht1*+Ps and WT-Gln+Ps does not
203 contribute to plant growth inhibition (**Fig 4B**). To test the impact of other AAs that accumulate in
204 *lht1* root exudates, plant growth in wild-type exudates supplemented with 1 mM or 10 mM serine

Figure 3

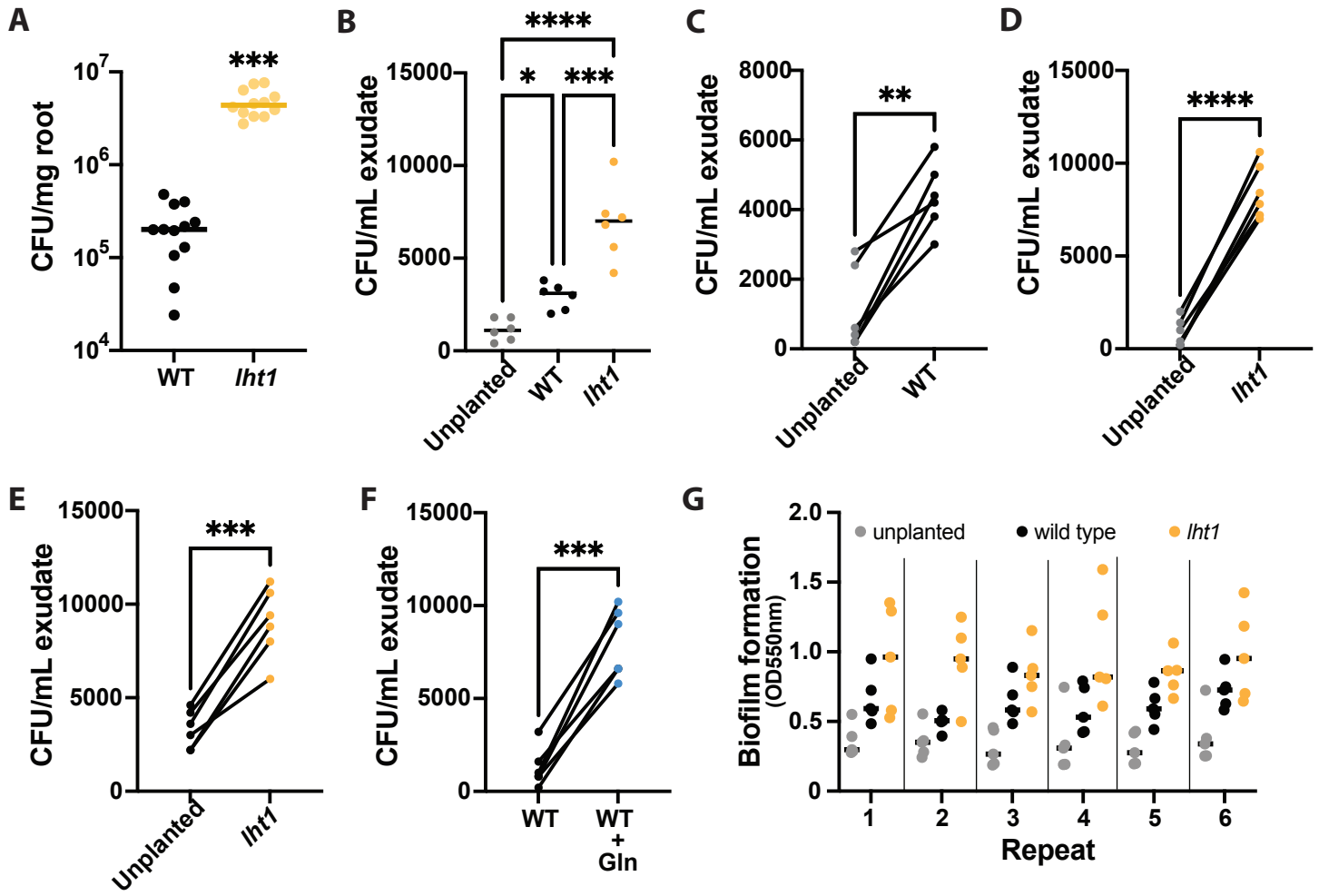

Figure 3. Ps WCS417r more effectively colonizes lht1 than wild-type roots. Across all panels in this figure, data are from at least 5-6 independent biological replicates. In each biological replicate, at least 5 seedlings/plants or their products (e.g., exudates) were analyzed. Each dot represents the median of 5 seedlings, 5 independently collected exudates, or 3 chemotaxis syringes per condition. In panels (A) the median of all biological replicates is shown as a bar. A two-sided Student's t-test was performed for statistical comparison of two means or a Welch's t-test for two means with unequal variances. For comparison of more than two means, a one-way ANOVA followed by Tukey's posthoc test, or a Kruskal-Wallis test for unequal variances followed by Dunn's posthoc test was performed. P values are represented as *≤0.05, **≤0.01, ***≤0.005, and ****≤0.001. Further experimental and analysis details are described in the Methods section. (A) Loss of LHT1 promotes root colonization by Ps WCS417r. The 1x MS-wetted 3MM paper in which 6 seedlings were growing was inoculated with Ps WCS417r at OD600nm= 0.00002. Seventy-two hours later, the roots were harvested, weighed, and ground; serial dilution were plated to count colony forming units (CFU) on LB agar with 50 μg/mL of rifampicin. Colonization is presented as CFU per mg of root fresh weight. (B) AAs-rich lht1 exudates attract Ps WCS417r more effectively than WT exudates. For this non-competitive chemotaxis assay, 200μl of filter-sterilized exudates of each condition were loaded in 1 mL syringes without the needles. The tip of each syringe was then dipped in an independent Petri dish containing chemotaxis buffer inoculated with Ps WCS417r at a final OD600nm= 0.002. After 1h, bacterial cells inside the syringe were harvested and diluted to measure CFU. Data are depicted as total CFU per mL of exudate. (C-D) Gln-supplemented WT exudates attract Ps WCS417r more effectively than non-supplemented WT exudates. For these competitive chemotaxis assays, 200 μL of filter-sterilized exudates of each condition were loaded in 1 mL syringes. Then, the tip of two syringes of different conditions was dipped in the same Petri dish containing chemotaxis buffer inoculated with Ps WCS417r at a final OD600nm= 0.002. After 30 min, bacterial cells inside each of the two syringes were harvested and diluted to measure CFU. Data are presented as total CFU per mL of each exudate. Competitive chemotaxis results are presented as follows: (C) Control 0.5x MS medium (without sucrose) versus WT exudate, (D) Control 0.5x MS medium (without sucrose) versus lht1 exudate, (E) WT versus lht1 exudate, and (F) WT exudate versus WT exudate supplemented with 1 mM Glutamine. (G) lht1 exudates better support Ps WCS417r capacity to produce biofilm. Six aliquots of 0.5x MS medium (without sucrose), wild-type root exudates, or lht1 root exudates were loaded on independent wells of a 96-well round-bottom plate and then inoculated with 2 μl freshly harvested Ps WCS417r grown overnight in LB medium After 48h of static growth, biofilms were stained with crystal violet, and absorbance at 550 nm was measured in a microplate reader as described in Methods. Each depicted dot represents the absorbance in each of 6 wells per condition. Biofilm formation was independently tested 6 times.

205 (Ser) was assessed. Interestingly, an excess of Ser significantly inhibited plant growth at both
206 concentrations tested and in the absence of *Ps* WCS417r (**Fig. 4C**). These data demonstrate that
207 Ser does not contribute to the plant growth inhibitory effect of *lht1* exudates mediated by *Ps*
208 WCS417r.
209 Across plant species, the response to abiotic and biotic stressors usually includes the inhibition
210 of plant growth (39–41). To identify the underlying mechanisms associated with plant growth
211 inhibition when *Ps* WCS417r colonizes an AA-rich rhizosphere, the expression of Arabidopsis
212 genes induced in response to various stressors that produce plant growth inhibition, was tested.
213 First, the elicitation of plant immunity is typically accompanied by the inhibition of plant growth
214 (42–44). To test if plant immunity was elicited by the combination of *lht1* exudates and *Ps*
215 WCS417r, the expression levels of *PR1* and *WRKY29* were assessed by RT-qPCR. The
216 expression of these genes is induced in response to a broad range of pathogenic cues (45, 46).
217 Arguing against a generalized activation of the plant defense in response to larger-than-normal
218 root colonization that could negatively impact plant growth, the data showed similar levels of *PR1*
219 or *WRKY29* expression in wild-type roots colonized with *Ps* WCS417r regardless of the origin of
220 the plant exudates (**Fig. 4D**). The levels and potency of the plant growth hormone indole-3-acetic
221 acid (IAA) are partially controlled by the activity of enzymes that conjugate amino acids to IAA,
222 producing IAA-derivatives less capable of promoting plant growth (47). Some members of the
223 GH3 family of IAA-amido synthetases whose encoding genes transcriptionally respond to stress
224 are necessary and sufficient to promote stress-induced plant growth inhibition, presumably by
225 lowering the effective concentration of active IAA (47). *GH3.3*, in particular, has been used as a
226 marker of stress-mediated growth inhibition (48). The expression of *GH3.3* in wild-type plants
227 exposed to *lht1* exudates and *Ps* WCS417r was significantly higher than that of wild-type plants
228 exposed to WT exudates and *Ps* WCS417r (**Fig. 4E**). Lastly, as excessive ammonia is known to
229 inhibit plant growth (11, 12, 49), we tested the hypothesis that *Ps* WCS417r may convert the
230 excess Gln present in *lht1* exudates or Gln-supplemented WT exudates into ammonia. Thus, the
231 capacity of *Ps* WCS417r to produce ammonia when grown in wild-type or *lht1* exudates was
232 assessed. In line with the ammonia-toxicity hypothesis, the levels of ammonia produced by *Ps*
233 WCS417r were higher in the *lht1* exudates than in the WT exudates (**Fig. 4F**). Notwithstanding
234 the lack of direct evidence, the increased levels of AAs in the *lht1* root exudates could enhance
235 *Ps* WCS417r-mediated production of ammonia, which could, in turn, contribute to inhibiting plant
236 growth. Overall, the data presented in this section suggest that LHT1-mediated regulation of the
237 root exudate's amino-acidic composition is critical to maintaining the concentration and
238 representation of AAs that is optimal for the beneficial effect of root colonization by PGPB.

Figure 4

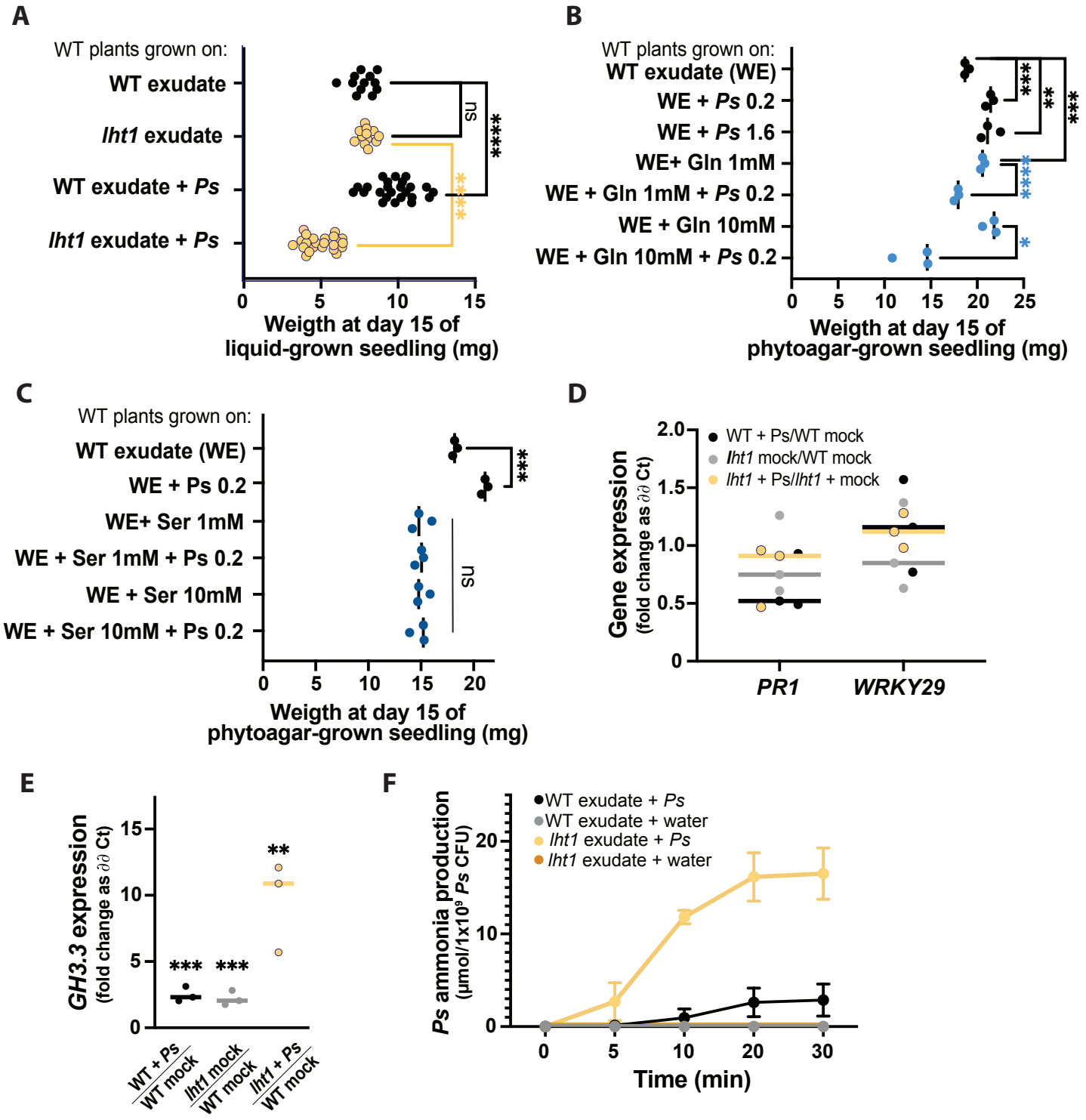

Figure 4. Excess AAs in lht1 exudates combined with Ps WCS417r inhibit wild-type Arabidopsis growth. Across all panels, each dot represents an independent biological replicate. The median of all biological replicates is depicted as a bar. A two-sided Student's t-test was performed for statistical comparison of two means or a Welch's t-test for two means with unequal variances. For comparison of more than two means, a one-way ANOVA followed by Tukey's posthoc test, or a Kruskal-Wallis test for unequal variances followed by Dunn's posthoc test was performed. P-values are represented as *≤0.05, **≤0.01, ***≤0.005, and ****≤0.001. Further experimental and analysis details are described in the Methods section. (A) lht1 exudates inhibit the growth of wild-type Arabidopsis when inoculated with Ps WCS417r. Fifteen-day -old wild-type seedlings growing on 12-well plates with floating mesh on WT or lht1 exudate with or without Ps WCS417r OD600nm= 0.2. Each dot represents the median weight of ≥3 seedlings per condition. The experiment was repeated 6 times; the median of all biological replicates is depicted as a bar. (B) Gln-supplemented WT exudates combined with Ps WCS417r inhibit the growth of wild-type Arabidopsis. Assay was carried out as in (A) except that two different Ps WCS417r inoculum titters were used (OD600nm=0.2 or 1.6) and that WT exudates were supplemented with Gln 1mM or 10 mM and inoculated or not with Ps WCS417r (OD600nm=0.2). (C) Ser-supplemented WT exudates directly inhibit the growth of wild-type Arabidopsis. Assay and controls as in (B), except that WT exudates were supplemented with 1 mM or 10 mM Ser (instead of Gln). (D) Pathogen-responsive genes are not induced in plants concomitantly exposed to Ps WCS417r and lht1 exudates. Wild-type seedlings were exposed for 24h to WT exudate (depicted as WT mock), WT exudate + Ps WCS417r (final OD600nm= 0.2), lht1 exudate (depicted as lht1 mock), or lht1 exudate + Ps WCS417r (final OD600nm= 0.2) as described herein (A) and in Methods. Gene expression in the roots was assessed using RT-qPCR. Fold change was calculated using ∂∂Ct. The specific comparisons are depicted in the X-axis. Each dot corresponds to an independent biological replicate (calculated based on 2 experimental replicates), and the median of the 3 biological replicates is depicted as a bar. (E) GH3.3, a mediator of the stress-triggered growth inhibition response, is induced in plants concomitantly exposed to Ps WCS417r and lht1 exudates. Expression of the gene encoding the auxin-conjugating enzyme GH3.3 is measured in the conditions described in (D). (F) Ps WCS417r produces more ammonia from lht1 than from wild-type exudates. Ammonia excreted by Ps WCS417r while incubated on WT or lht1 exudates was assessed at 0, 5, 10, 20, and 30 min. Exudates obtained in three independent experiments were assessed for ammonia independently but simultaneously. After the indicated times, the incubation-media supernatants were harvested, filter sterilized, and their ammonia content was assessed using an ammonia-assay kit (AbCam; Cat # ab102509) following manufacturer's guidelines. Each dot represents the median ammonia concentration (µmol/mL), and error bars correspond to the SEM.

239

240 **DISCUSSION**

241

242 **LHT1 activity is essential to maintain a balanced composition of amino acids in the root**
243 **exudates.**

244

245 Plants release a fraction of their metabolites into the rhizosphere as root exudates. Since some
246 of the exudate-dependent microbes present in the rhizosphere promote plant growth, the
247 metabolites present in root exudates represent an investment rather than an expenditure for the
248 plant. It has been proposed that, like seeds or growing organs (e. g. new leaves), belowground
249 microorganisms are additional sinks of plant photosynthates (50). Therefore, as with other plant
250 sinks, it is reasonable to hypothesize that plants would regulate the transport of metabolites to
251 and from the rhizosphere. Furthermore, it had been hypothesized that active recycling of low
252 molecular weight compounds from the rhizosphere is particularly important for plants to control
253 the growth of rhizosphere microbes and to ward off pathogen invasion of root tissues (51). Yet,
254 whether plants exert control over the accumulation of plant-derived metabolites in the rhizosphere
255 through AA uptake was unclear. The present study was based on two related hypotheses: (i)
256 plants would regulate the levels of AAs in the rhizosphere, including the AAs released by the
257 plants themselves, and (ii) some of the AA importers that contribute to the uptake of AAs from the
258 growth medium and the soil, may also contribute to retrieve plant-derived AAs back into the plant.
259 In support of these hypotheses, the data showed that a loss-of-function mutant of the AA importer
260 LHT1 leads to an increased accumulation of AAs in the root exudates of *Arabidopsis* (**Fig. 1A,**
261 **C**). Furthermore, our search for specific exuded metabolites, able to change the structure of the
262 rhizosphere, identified Gln, among other AAs, enriched in the root-exudates of the *lht1* (**Fig. 1B**)
263 whose sole supplementation is sufficient to boost the growth of the *Ps* WCS417r (**Fig. 2A, B**).
264 Unexpectedly though, the increased bacterial growth in exudates and increased root colonization
265 (**Fig. 3A**) did not translate into increased plant growth (**Fig 4A**). Together, the data suggest that
266 wild-type plants maintain a balanced concentration of AAs in the exudates that is optimal to
267 promote PGPB growth and, in turn, to maximize plant growth, and that LHT1 contributes to
268 maintaining this optimal concentration of AAs in the exudates. In line with an optimized plant-
269 microbe interaction that is, at least in part, modulated by LHT1, one hour after inoculation of
270 Arabidopsis roots with *Ps* WCS417r, *LHT1* expression increases in root tissues (52), suggesting
271 a mechanism by which microbes and plants communicate with each other to maintain levels of

272 AAs in the rhizosphere that sustain optimal PGPB growth and metabolism, and enhanced plant
273 growth.
274
275 **The re-uptake of root-exuded amino acids is necessary to modulate bacterial metabolism**
276 **and promote plant growth.**
277 The root-associated microbiome is mainly located in the rhizosphere, the narrow (1-3 mm) region
278 of soil surrounding the root that is rich in plant-made metabolites exuded by the roots (53). Several
279 PGPB species that live in the rhizosphere have been shown to either produce plant hormones or
280 regulate the levels of plant hormones that have a positive effect on plant growth (54, 55). The
281 mechanisms by which *Ps* WCS417r promotes plant growth, however, are not fully understood.
282 To explain the detrimental effects of Gln-supplemented *Ps* WCS417r on plant growth, several
283 hypotheses can be formulated, some of which would be worth testing in future studies. One
284 hypothesis would be that excessive levels of AAs could have a direct detrimental effect on the
285 physiology of the plant itself. This may be proven correct for some AAs, including Ser, which was
286 sufficient to inhibit Arabidopsis root and shoot growth (**Fig. 4C**). Gln has been reported to inhibit
287 root growth of the Ler (Lansberg erecta) ecotype but not the Sha (Shakdara) ecotype of
288 Arabidopsis (56), suggesting that the root growth of the Col-0 ecotype used in our experiments
289 could also be inhibited by an excess of Gln. However, in the experimental setup used in our study,
290 the detrimental effect of Gln was only evident in the presence of *Ps*WCS417r (**Fig. 4B**). For
291 instance, even relatively high concentrations (20 mM) of Gln alone did not inhibit plant growth
292 (**Fig. 4B)**. In contrast, the combination of *Ps*WCS417r with 1 mM Gln causes subtle growth
293 inhibition, and 10 mM robustly inhibits plant growth (**Fig. 4B**). This observation suggests that the
294 microbe-mediated bioconversion of Gln is required to inhibit plant growth. Bioconversion by soil-
295 dwelling microbes has been extensively studied for chemicals of anthropogenic origin (*e.g.*,
296 pesticides) (57, 58) but much less is known about PGPB bioconversion of plant-derived
297 compounds. Nevertheless, it is well-documented that microbes convert Gln into ammonium, and
298 that high ammonium levels inhibit the growth of roots and shoots in Arabidopsis (59), especially
299 in conditions of low potassium levels (11, 12, 49). Furthermore, the genome of *P. simiae* encodes
300 at least five enzymes capable of converting glutamine into glutamate and ammonia
301 (www.Biocyc.org). This ammonia-producing reaction, together with the inhibition of the ammonia-
302 consuming reaction carried out by glutamine-synthase, would be favored in the Gln-rich
303 environment of the *lht1* rhizosphere. In agreement with the literature, *Ps* WCS417r produced high
304 ammonia levels when *lht1* exudates were used as growth medium (**Fig. 4F**). However, in the
305 conditions used for assessing plant growth in this study, the high availability of potassium in the

306 MS medium may alleviate ammonia toxicity. Considering that the high ammonia level present in
307 the MS medium (20 mM) does not inhibit plant growth, the local concentrations of ammonia
308 produced by *Ps* WCS417r Gln metabolism would need to be at least higher than 20 mM to be
309 able to inhibit plant growth. Thus, in future studies, it would be relevant to test whether *Ps*
310 WCS417r strains with reduced capacity to release ammonium from glutamine (*e.g.*, a *glsA*
311 mutant; locus tag PS417_15505) would maintain the capacity to grow robustly in exudates
312 supplemented with Gln and perhaps promote plant growth to a larger extent compared to the wild-
313 type strain. Other byproducts of PGPB metabolism may also affect plant growth; thus, in future
314 studies, it would be informative to perform metabolic profiling of root exudates post-PGPB growth
315 to identify metabolites that are only present at high levels in the supernatant of *Ps* WCS417r
316 grown in *lht1* exudates. A complementary hypothesis would be that *Ps* WCS417r-generated
317 byproducts of Gln metabolism activate a stress response in the plant that includes growth
318 inhibition. Reduced plant growth in response to abiotic and biotic stress is well documented. The
319 metabolism of plant growth hormones such as IAA mediates the growth inhibitory response to
320 stress (47). The GH3 family of proteins are important player in the plant inhibitory metabolism of
321 IAA upon stress. In line with the notion that the Gln metabolism by *Ps* WCS417r described here
322 may promote the formation of IAA-derivatives that suppress plant growth, Gln-supplemented *Ps*
323 WCS417r, but not Gln or bacteria alone, promoted the induction of the Arabidopsis gene encoding
324 the IAA-amido synthetase GH3.3. This result provides a clue for future dissection of the
325 mechanism by which Gln metabolism in *Ps* WCS417r inhibits plant growth.
326
327 **CONCLUSIONS**
328 Data presented in this study establishes that Arabidopsis uses AAs reuptake to control the organic
329 composition of the rhizosphere as a relevant strategy to foster enduring beneficial interactions
330 with their root microbiota. Modulation of the concentration of AAs in the exudates is, at least in
331 part, executed by LHT1. In the absence of LHT1-mediated re-uptake, the excessive levels of AAs
332 in the rhizosphere cause microbe-mediated inhibition of plant growth. Future studies will define
333 how Gln metabolism in *Ps* WCS417r leads to plant growth inhibition. Among other possibilities,
334 the alkalinization of the rhizosphere and the impact on auxin levels as potential sources of plant
335 growth inhibition need to be further investigated.
336
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339 University of Virginia School of Medicine for LC-MS quantification of AA in root exudates. This

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Author Contributions

IDKA and CHD conceived and designed the experiments. IDKA and CHD performed experiments with help from BTK. IDKA analyzed the data with input from CHD. IDKA and CHD wrote the manuscript. All authors read and approved the final version for publication.

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354 **METHODS**

355 **Plant materials**

356 For all experiments, Arabidopsis wild-type plants were Col-0 and mutants were of Col-0
357 background. All T-DNA lines belong to the SALK Collection (60) and were obtained from the
358 Arabidopsis Biological Resource Center, Ohio State University. Homozygous mutant lines were
359 selected by PCR genotyping as previously described (Zhang et al 2022a). Seeds were always
360 surface-sterilized using 10% bleach three times for two minutes, followed by three washes with
361 sterile water, and resuspended in 0.1% PhytoAgar (PlantMedia; Cat#40100072-2). and stratified
362 in the dark at 4 ºC for at least two days.

363

364 **Growth of seedlings in vertical plates**

365 Stratified seeds were germinated on square plates (100 mm x 100 mm square plates; Fisher
366 Scientific; Cat#FB0875711A) containing sterile full-strength (1x) Murashige and Skoog (MS)
367 basal medium with vitamins (PhytoTech # M519), supplemented with 0.5% sucrose (Sigma;
368 S7903), 0.5 g/L MES (Sigma; M8250), and 0.7% PhytoAgar (PlantMedia #40100072-1). The pH
369 was corrected to 5.7 with KOH. Plates were sealed with parafilm and incubated vertically in a
370 reach-in plant growth incubator (Conviron Adaptis 1000, Canada) at 25 ± 0.2 ºC, 75% RH, 16h
371 Light/8h Dark, and 100 µmoles/m^2/s light intensity for the times indicated in the relevant figure
372 legends. Uniformly growing seedlings were then selected and transferred to new plates containing
373 autoclaved-3MM paper cut to fit 100 mm x 100 mm square plates and wetted to saturation with
374 0.5x liquid MS medium pH 5.7 without sucrose. These plates were incubated horizontally under
375 the same conditions as above for one day to allow roots to attach to the 3MM paper.

376

377 **Root exudate collection assays**

378 Root exudates were collected using a modification of a previously published method(31).
379 Arabidopsis seeds were sown on an autoclaved polytetrafluoroethylene (PTFE) mesh (McMaster-
380 Carr; Cat#1100t41) floating on the surface of 1 mL 1x MS liquid medium containing 0.5% sucrose
381 in 12-well tissue culture plates (USA Scientific; Cat#CC7682-7512). Plates were incubated in
382 reach-in plant growth incubators (Conviron Adaptis 1000, Canada) at 25 ± 0.2 ºC, 75% RH, 16h
383 Light/8h Dark, and 100 µmoles/m^2/s light intensity. Sterilized and stratified seeds were sown on
384 the floating mesh. Twelve days after sowing, the medium was replaced with 0.5x MS liquid
385 medium without sucrose, and plants were allowed to grow for three additional days. Importantly,
386 in these conditions, roots grow submerged in medium while shoots remain on the air space of the

387 well. Root exudates were then collected and filter-sterilized through 0.22 µm filter for further
388 processing.
389
390 **Colorimetric/Fluorometric Quantitation of Amino Acids**
391 Total amino acid content of root exudates was assessed using the L-Amino Acid Quantitation
392 Colorimetric/Fluorometric Kit (BioVision #K639-100) following the manufacturer's instructions with
393 modifications. Briefly, a 12.5 µL reaction mix containing 11.5 µL L-amino acid assay buffer, 0.5
394 µL L-amino acid probe, and 0.5 µL L-amino acid enzyme mix was added to each well containing
395 12.5 µL of the test samples, L-amino acid standards to generate a standard curve, or unplanted
396 media to estimate and later subtract the background signal. The reactions were incubated in the
397 microtiter plate reader (SpectraMax® i3x, Molecular Devices) for 30 minutes at 37 ºC. The
398 fluorescent signal (Ex/Em = 535/587 nm) was recorded every 5 min. AA quantification was
399 performed on 5-6 independent samples per condition.
400
401 **Liquid Chromatography—Mass Spectrometry Analysis of Amino Acids**
402 Root exudates were vacuum-dried and reconstituted in 100 µL of 0.1% formic acid. Samples were
403 analyzed at the University of Virginia-Biomolecular Analysis Facility Core following a standard
404 protocol (Nemkov et al., 2019). Serial dilutions of standards of every AA were ran for every
405 analysis. Five µL of samples were injected into a UHPLC system (Ultimate 3000, Thermo, San
406 Jose, CA, USA) and separated through a 3-min isocratic elution (5% acetonitrile, 95% water, 0.1%
407 formic acid) on a 1.7 µm C18 column (Kinetex XB-C18, Phenomenex, Torrance, CA, USA) at 250
408 µL/min and 25°C. High-resolution mass spectrometry analysis was performed using a triple
409 quadrupole orbitrap mass spectrometer (Q-Exactive HF-X, Thermo Scientific). Glycine data was
410 omitted from final analysis as unplanted MS medium contained background levels of glycine (2
411 mg/L). Data analysis was performed with the proprietary Thermo XCalibur software
412 (https://www.thermofisher.com/order/catalog/product/OPTON-30965#/OPTON-30965), and
413 targeted peak detection was achieved using ICIS peak integration algorithm. Thermo Quantitative
414 analysis software (Quan Browser) was then used to generate calibration curves, followed by
415 determining the concentration of the amino acids in the root exudate and unplanted samples. LC-
416 MS analysis was performed on 6 independent samples per condition.
417
418 **Bacterial growth on root exudates**
419 *Pseudomonas simiae* WCS417r was maintained on LB plates supplemented with 50 µg mL^{-1}
420 rifampicin. A single colony was randomly picked from a plate and grown overnight in 100 mL of

421 LB at 28 ºC and 230 rpm till the cultures reached OD_{600} = 0.4 – 0.8. The bacteria were then
422 harvested by centrifugation, washed three times with sterile water, and the OD_{600nm} was adjusted
423 to the required inoculation titer with sterile water. Root exudates (100 µL) of each condition were
424 aliquoted on six or more wells of a 96-well microtiter plate and inoculated with *Ps* WCS417r.
425 Plates were incubated under constant agitation at 28 ºC, and the culture density was monitored
426 over time by reading OD_{600nm} in a microtiter plate reader (SpectraMax® i3x, Molecular Devices).
427
428 **Root colonization**
429 Seedings were grown on vertical plates as described above. Two weeks after sowing, six (6)
430 uniformly growing seedlings were transferred to each of 5 plates containing 3MM paper wetted
431 with 5 mL half-strength MS without sucrose. Plates were horizontally incubated for one day to
432 allow seedlings to attach to the paper surface. The next day, the edge of the paper opposite to
433 the seedling's-shoot side was flooded with 0.5x MS alone (contamination control) or inoculated
434 with freshly harvested *Ps* WCS417r diluted to OD_{600nm}= 0.0002. Bacteria were allowed to colonize
435 the roots for 72h. The capacity of roots to support bacterial growth was analyzed in 6 to 30
436 individual seedlings per condition. To assess colony forming units (CFU) per mg of root, the root
437 mass was recorded right after harvesting. Immediately after weighing, the root tissue was ground
438 with metal beads in a TissueLyser (QIAGEN, Inc). Aliquots of the ground roots were base-ten
439 serial diluted in sterile water and plated on OmniTray plates (Nunc™) containing LB agar medium
440 supplemented with 50 µg/mL of rifampicin. Plates were incubated at 28 ºC overnight, and colonies
441 were counted under a microscope. Only replicates with no colonies in the "contamination control"
442 were included in the analysis.
443
444 **Competitive chemotaxis assay**
445 Competitive chemotaxis assay was designed by modifying capillary assays previously described
446 (34, 38), using syringes (**Fig. S3**). Briefly, *Ps*WCS417r cultures grown and harvested as described
447 above were resuspended at OD_{600}=0.002 in chemotaxis buffer (10 mM potassium phosphate pH
448 7.2, 1 mM $MgCl_2$, and 0.1 mM EDTA). Bacterial suspensions (40 mL) were pipetted into empty
449 sterile Petri dishes. Then, 200 µL of the test samples (*e.g.*, unplanted MS, wild-type-root exudates,
450 or *lht1*-root exudates) were loaded into each of two 1 mL sterile syringes (without needles) and
451 the tips of the syringes were dipped below the liquid surface in the Petri dish containing the *Ps*
452 WCS417r in chemotaxis buffer suspension. The syringes loaded with exudates were allowed to
453 attract bacteria from the suspension for 30 min. The syringes were removed from the bacterial

454 suspension, and colony-forming units in the exudates contained in the syringes were assessed
455 via dilution and plating as described above (see root colonization assay for details).
456
457 **Biofilm formation assays**
458 Unplanted medium, wild-type root exudates, and *lht1* root exudates were tested for their capacity
459 to promote biofilm formation upon *Ps* WCS417r inoculation as follows. Each well of a 96-well
460 round-bottom plate (Catalog # CLS2797, Sigma) was first loaded with 200 µl of root-exudate
461 sample and then inoculated with 2 µL of freshly harvested *Ps* WCS417r (see details above)
462 resuspended at OD_{600}=0.02. Biofilms were allowed to grow statically for 48h at 28 ^{O}C.
463 Subsequently, the medium and non-adherent bacterial cells were carefully removed by pipetting
464 and wells were washed with sterile water without disturbing the biofilms attached to the bottom of
465 the plate. The plates were air-dried for 5-10 minutes. Next, the wells were stained with 125 µL of
466 a 0.1% (w/v) solution of crystal violet in water for 15 minutes. The stain was gently rinsed with
467 water, without disturbing the stained biofilm. One hundred and fifty (150) µL of a 6:3:1 sterile
468 water:methanol:acetic acid solution was added to each well to solubilize the crystal violet for 15
469 minutes. To quantify biofilm biomass, 125 µL of the 150 µL solution of stained biofilm were
470 transferred into a well of a polyvinyl chloride 96-well flat-bottom plate (Catalog # CLS2595,
471 Sigma), for absorbance assessment at 550 nm in a microplate reader (SpectraMax® i3x
472 (Molecular Devices). Biofilm formation was tested on 3 to 6 independent samples per condition.
473
474 **Plant growth on exudates ± *Ps* WCS417r**
475 The capacity of *Ps* WCS417r to promote the growth of Arabidopsis seedlings when combined
476 with WT exudates, *lht1* exudates, or WT exudates supplemented with Gln or Ser was assessed
477 as follows. Seedings were grown on 1x MS PhytoAgar with 0.5% sucrose. Plates were incubated
478 in reach-in plant growth incubators (Conviron Adaptis 1000, Canada) at 25 ± 0.2 ^{o}C, 75% RH,
479 16h Light/8h Dark, and 100 µmoles/m^2/s light intensity. After two weeks, six (6) uniformly growing
480 seedlings were transferred to each of 3 to 7 plates containing 3MM paper wetted with 5 mL half-
481 strength MS without sucrose. Plates were horizontally incubated for one day to allow seedlings to
482 stabilize. The next day, the seedlings in each plate were root flooded (liquid added to the edge of
483 the paper opposite to the seedlings-shoot side) with half-strength MS alone (contamination
484 control) or with freshly harvested *Ps* WCS417r diluted to OD_{600nm}= 0.2 in the following carriers:
485 Plate 1) WT exudate, and Plate 2) *lht-1* exudate. In independent experiments we tested half-
486 strength MS alone as contamination control or *Ps* WCS417r diluted to OD_{600nm}= 0.2 in the
487 following carriers: Plate 1) WT exudate, Plate 2) WT exudate supplemented with 1mM Gln, Plate

488 3) WT exudate supplemented with 10mM Gln, Plate 4) WT exudate supplemented with 1mM Ser,
489 and Plate 5) WT exudate supplemented with 10mM Ser. We also included an “overgrowth control”
490 in which we root-flooded the seedlings with *Ps* WCS417r diluted in WT exudate at final OD_{600nm}=
491 1.6 (instead of 0.2); this control was included to test whether overgrowth of *Ps* WCS417r would
492 be sufficient to inhibit plant growth.
493
494 ***Ps* WCS417r excreted-ammonium assessment**
495 The capacity of *Ps*WCS417r to produce ammonia through metabolizing exudates of interest was
496 measured using a modified version of the Berthelot colorimetric method (**Fig. S4**). Briefly, 24 wells
497 of a 96-well plate were filled with 90µl of WT or *lht1* exudate, or 0.5x MS media as control. Twelve
498 wells of each condition and of each independent source of exudate (*e.g.*, 12 wells of WT exudate
499 obtained in biological replicate 1, 12 wells of WT exudate obtained from biological replicate 2,
500 etc.) were inoculated with freshly harvested and three times washed *Ps*WCS417r to reach a final
501 OD_{600nm} = 0.5, while the remaining 12 wells of each condition and biological replicate were left
502 bacteria-free to measure the basal levels of ammonia in each exudate (negative controls). Plates
503 were incubated at 28ºC with constant shaking. After 5, 10, 20, and 30 minutes, the content of 3
504 wells per condition was transferred to 1.5 mL tubes and centrifuged for 15 min at 15,000g. The 3
505 supernatants from each treatment and control condition were pooled and filtered sterilized. Two
506 microliter aliquots of the supernatants were transferred to wells of a 96-well plate prefilled with
507 98µl of assay buffer of the ammonia assay kit from AbCam (AbCam Cat # ab102509), and
508 ammonia levels were quantified according to the manufacturer's guidelines. Each supernatant
509 was tested in triplicate, and, as described above, three independent exudates per condition were
510 tested in parallel.
511
512 **Plant tissue gene expression analysis**
513 Root/leaf tissues were harvested one at a time into previously weighed tubes, and the weight of
514 the tissues was measured. Immediately after weighing a sample, it was flash-frozen in liquid
515 nitrogen and kept at -80ºC until processing. RNA was isolated using TRIzol® Reagent (Fisher
516 Scientific; Cat#15596018), DNAse-I treated (Promega) and quantified in a NanoDrop-ND1000
517 spectrophotometer (Thermo Fisher Scientific Inc). Two (2) µg of total RNA were used for cDNA
518 synthesis with MMLV (Promega) and random hexamers (Invitrogen). qPCR was performed
519 using real-time SYBR-Green quantification. There were two technical replicates per condition
520 per biological replicate. The reactions were performed an ABI 7500 Fast Real-Time PCR

521 system (Applied Biosystems). Data were analyzed using the ΔΔCt method (61). Gene
522 expression was measured on ≥3 independent samples per condition.
523
524 **Statistical analysis**
525 Data analyses and graphs were generated using Excel and GraphPad. A two-sided Student's *t*-
526 test was performed for statistical comparison of two means, or a Welch's *t*-test for two means with
527 unequal variances, when relevant. For comparison of more than two means, a one-way ANOVA
528 followed by Tukey's posthoc test, or a Kruskal-Wallis test for unequal variances followed by
529 Dunn's posthoc test was performed, as indicated in the relevant figure legends.
530 For statistical analysis of bacterial growth curves, the CGGC (Comparison of Groups of Growth
531 Curves) permutation test (62) was used to compare pairs of samples (i.e., unplanted vs. wild type;
532 unplanted vs. *lht1*; wild type vs. *lht1*) over the course of growth (24 hours). The test statistic (mean
533 *t*) is the two-sample *t*-statistic to compare the OD_{600} values between the two groups at each hour,
534 averaged over the course of growth (24 hours). A P-value was obtained for the test statistic by
535 simulation. Samples were randomly allocated to each of the two groups and the mean *t* was
536 recalculated for 10,000 data sets generated through this permutation. The P-value is the
537 proportion of permutations where the mean *t* is greater in absolute value than the mean *t* for the
538 original data set.
539

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Figure S1

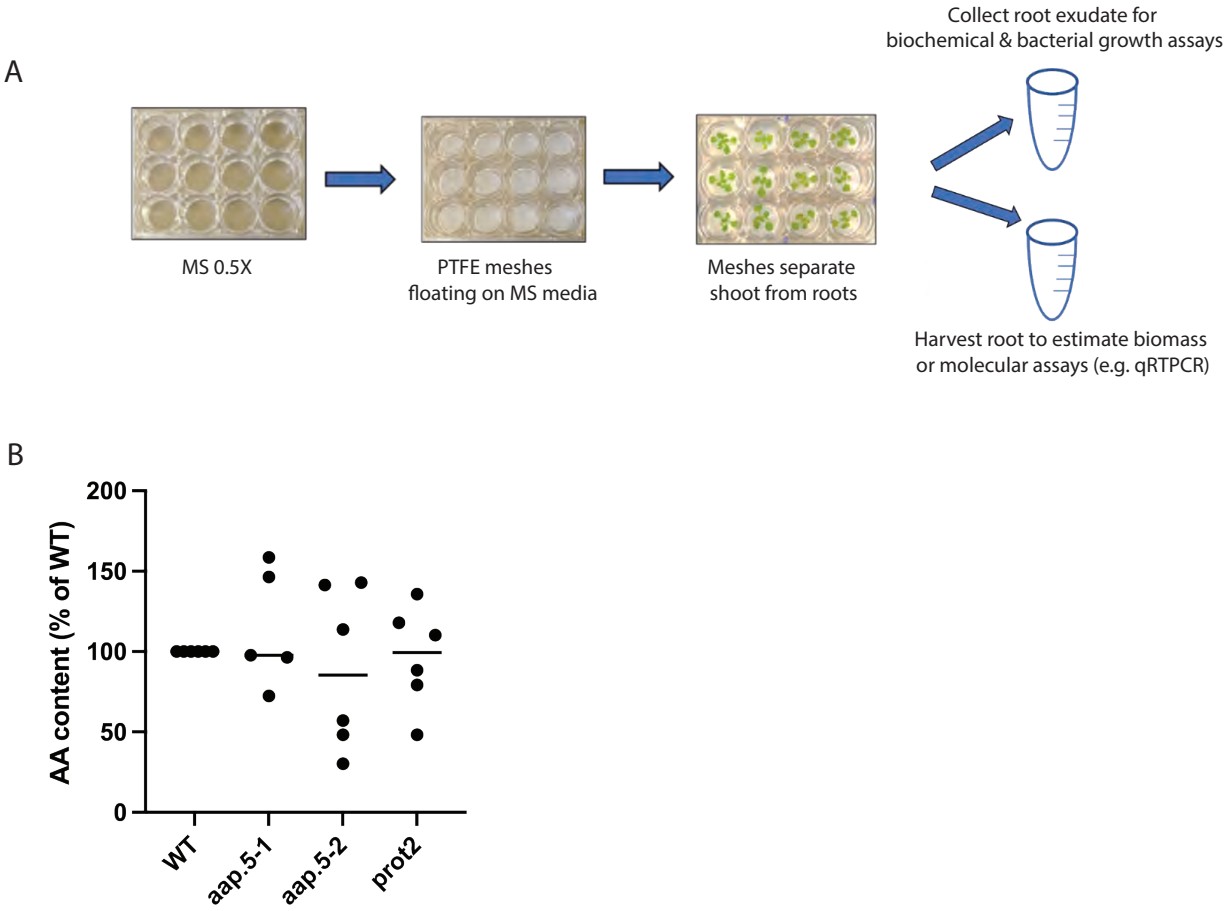
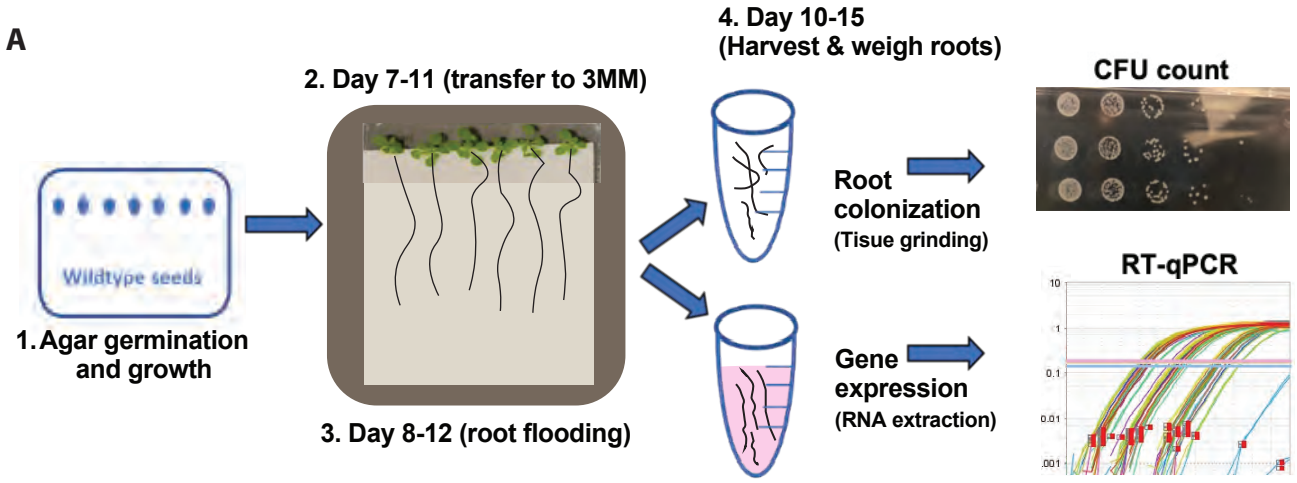


Figure S1. (A) Workflow to obtain root exudates. Twelve-well plates were loaded with 1 mL of 1x MS buffer supplemented with 0.5% sucrose and 0.5 g/L MES. Seeds were sown on sterile polytetrafluoroethylene (PTFE) meshes and placed floating on top of the medium. After 12 days, the medium was replaced with 0.5x MS liquid medium without sucrose, and plants were allowed to grow for three additional days. Importantly, in this condition, roots grow submerged in the medium while shoots remain in the air space of the well. Root exudates were then collected and filter-sterilized for further processing or testing. **(B)** Total AA content in *aap5* and *prot2* root exudates is similar to that of WT plants. Seeds of sequence-verified homozygous mutants were sown, and exudates were obtained as described in (A). Colorimetric quantification of AAs was carried out as described in Methods. Each dot corresponds to a biological replicate and is the median AA content per mg of roots of 5 seedlings per genotype relative to the wild type (as a percentage). Experiments were repeated 6 independent times. One-way ANOVA followed by Tukey's posthoc test shows no statistically significant differences between WT and the mutant genotypes.

Figure S2



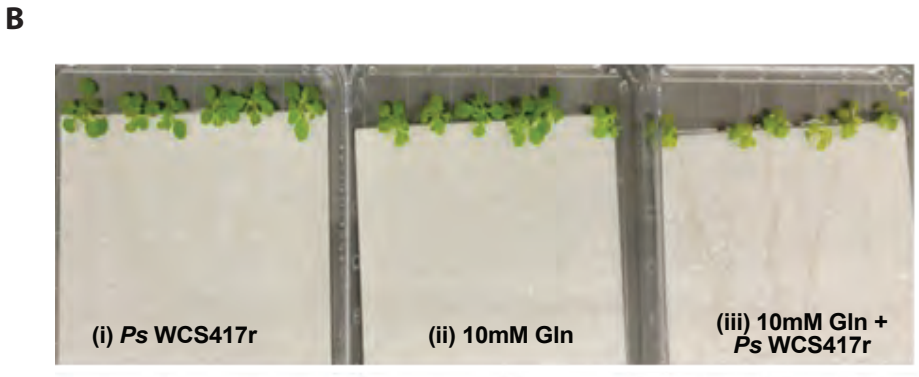


Figure S3. Workflow of competitive chemotaxis assay. (1) A Petri dish is filled with 40 mL of *Ps* WCS417r freshly resuspended chemotaxis buffer at a final OD600nm =0.002 Filter-sterilized root exudates (200 µL) or fresh MS medium (negative control) were loaded into 1 mL sterile syringes (without the needles). The tips of the two syringes to be tested in the competitive assay were then immersed just under the surface of the bacterial suspension contained in the Petri dish. **(2)** After 30 min, the content of each syringe was transferred to independent 1.5 mL tubes. **(3)** Serial dilutions were plated on LB-agar plates to count colony-forming units (CFU).

Figure S3

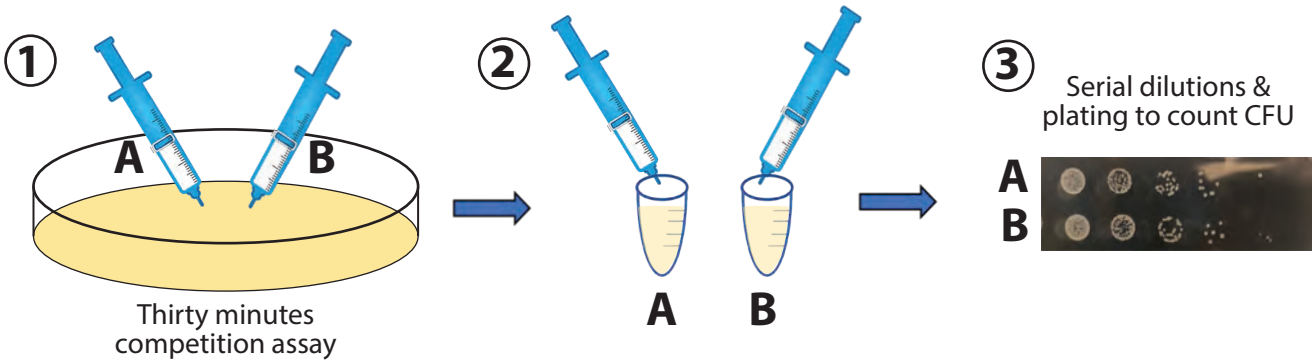


Figure S2. (A) Workflow to assess colonization and gene expression in roots. **(1)** Stratified seeds are germinated in 1x MS agar with 0.5% sucrose. **(2)** At days 7 to 11, uniformly growing seedlings were transferred to square plates containing autoclaved 3MM paper wetted with 0.5x MS without sucrose (composed image to reveal roots position and size). Plates were sealed and incubated horizontally for 1 day to allow roots to attach to the 3MM paper. **(3)** At days 8 and 12, the bottom of the 3MM paper is flooded with a suspension of *Ps* WCS417r (final OD600nm= 0.2) or sterile 0.5x MS as a no-growth (contamination) control. The bacteria are resuspended in exudates obtained from WT or lht1, and WT exudates were supplemented with AAs as indicated. 4. After 24-72h of exposure to exudates *Ps* WCS471r AAs, roots are harvested, weighed, and processed to obtain RNA or to count bacterial colony-forming units (CFU). (B) Representative pictures of seedlings in square plates on 3MM paper substrate. At day 13 roots were flood-treated with: (i) Ps WCS417r OD600nm=0.2 (control), (ii) 10 mM Gln, or (iii) *Ps* WCS417r and Gln at final OD600nm=0.2. The images show symptoms 3 days after treatment.

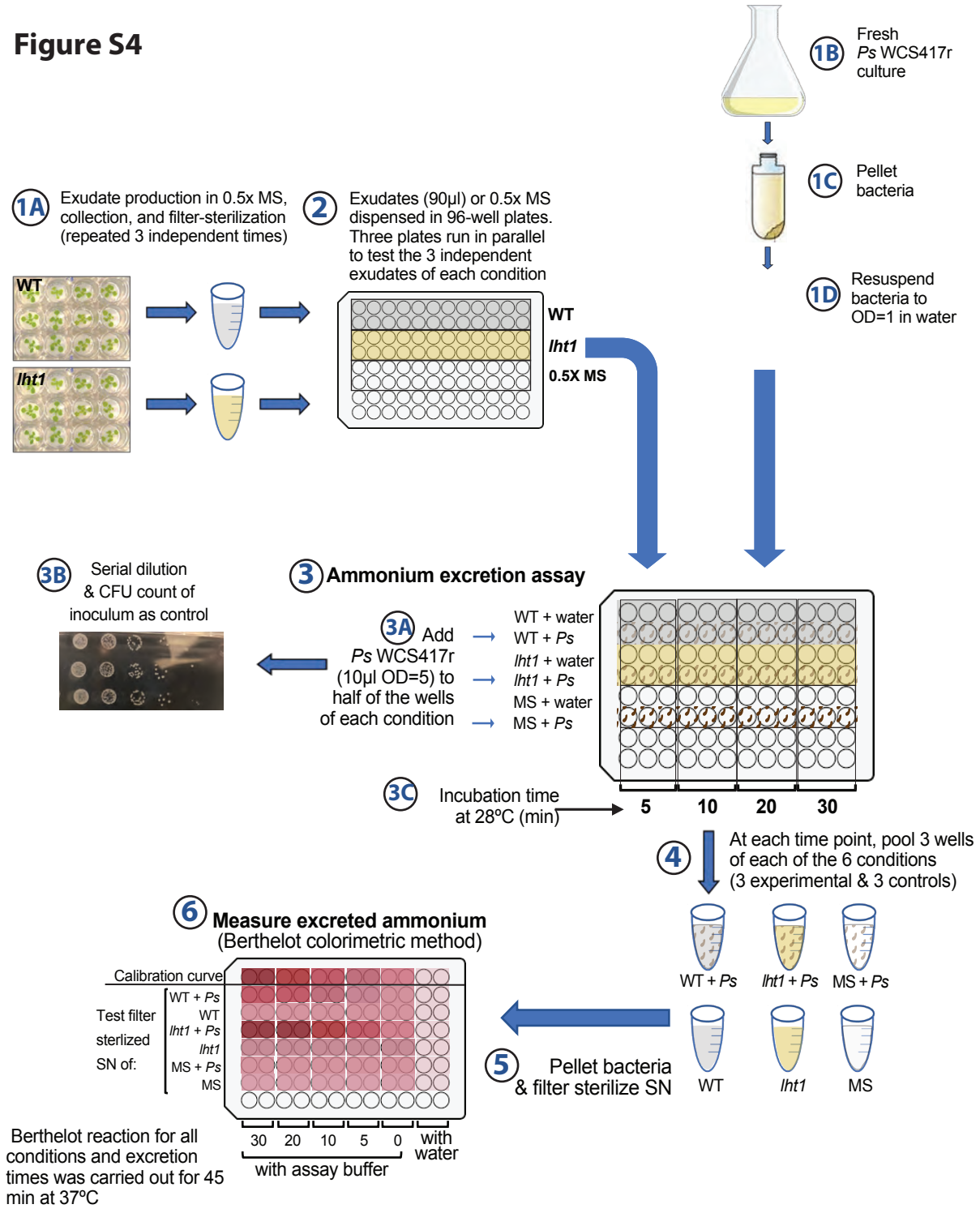


Figure S4. **Workflow of *Ps* WCS417r excreted-ammonium assay.** WT and *lht1* exudates were collected **(1A)** as described in Methods. Aliquots of 90 µL of each exudate were transferred to 24 wells of a 96-well plate **(2)**. While 12 wells of each exudate were inoculated with freshly harvested **(1A)** and three times washed **(1B)** *Ps* WCS417r to reach a final OD600nm = 0.02 **(1C)**, the remaining 12 wells of each condition were left bacteria-free to be used as negative controls. Plates were incubated at 28 ºC with constant shaking **(3)**. At 5, 10, 20, and 30 minutes, 3 wells of each experimental and 3 wells of each control condition were harvested **(3A)**. In parallel, 10 µL aliquots were used to assess CFUs (3B). After centrifugation for 5 min at 10,000g, the 3 supernatants from each treatment and control condition were pooled **(4)** and filtered-sterilized **(5)**. Aliquots of the supernatants were used to quantify ammonia **(6)** using the ammonia assay kit from AbCam (Cat # ab102509), according to the manufacturer's guidelines. The experiment was repeated 3 times with independent batches of fresh exudates.