An ERF transcription factor StPTI5, a novel regulator of endophyte community maintenance in potato

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ABSTRACT

Potato is exceptionally sensitive to a wide range of stresses. Its successful interaction with beneficial symbionts would allow for environmentally friendly plant protection. We investigated the processes

involved in colonisation of potato with beneficial *Bacillus subtilis*. RNAseq showed that at the time of biofilm establishment, immune responses in interacting roots were attenuated, and complex transcriptional network was triggered, including ERFs, MYBs, NACs and WRKYs. Interestingly, the response is intensified if plants are inoculated by two antagonistic B. subtilis strains. We further focused on the role of strongly induced ERF transcription factor PTI5. While PTI5 is not involved in the establishment of biofilm on roots, we show that bacterial abundance increases in PTI5-silenced plants. Remarkably, root colonization by the arbuscular mycorrhizal fungus *Rhizophagus irregularis* was also higher in the PTI5-silenced plants, making PTI5 an interesting breeding target. Such mechanistic understanding of plant–microbe interaction paves the way for sustainable crop management.

INTRODUCTION

Plant-microbe and microbe–microbe interactions in the rhizosphere determine plant health and productivity.[1] Beneficial microorganisms are common in rhizosphere soils. Some can endophytically colonize plants, frequently improving plant nutrition and plant growth, and protect plants from disease and abiotic stresses through a wide variety of mechanisms.[2,3] They can be efficient and environmentally friendly alternatives to chemical pesticides and fertilizers, due to their multiple benefits for the plants and their long shelf life, which is comparable with that of agrochemicals.[4] However, the current limitation for a wider adoption of microbial inoculants in agriculture is reproducibility of the results under field conditions.[5] Additional knowledge, such as a deeper understanding of the mechanisms involved in plant colonization and maintenance of the plant microbiome is required to improve the use of microbes as plant protectants.[6]

The plant immune system is central in shaping the plant response to beneficial and pathogenic microorganisms, regulating the extension and successful colonization of plant tissues by mutualistic organisms and preventing the invasion of the parasitic ones. Recognition of pathogens leads to the activation of interconnected plant hormonal signaling pathways mediating downstream

55 transcriptional reprogramming resulting in effective defense responses.[7,8] When plant interacts with
56 beneficial microbes, microbe triggered immunity (MTI) is activated similarly as in interaction with
57 pathogens.[9] Beneficial microbes developed several strategies to overcome this immune response and
58 colonize the plant. One option to avoid effective recognition is by modifying the microbe-associated-
59 pattern, recognized by immunity, for example flagellin. Alternatively, the microbes develop tolerance
60 to toxic compounds produced, e.g. reactive oxidative species (ROS) or they actively suppress MTI.[10]
61 They can also modify metabolism of plant to allow for easier penetration, for example suppress
62 synthesis of suberin.[11]

63 Successful colonization by beneficial bacteria can induce resistance to pathogenic bacteria and
64 herbivores. Mechanisms of induced resistance (IR) are not well understood, but it has been shown to
65 involve jasmonic acid (JA), salicylic acid (SA) and ethylene signaling pathways.[11, 12, 13] Several individual
66 transcription factors have been shown also to be important for the establishment of the IR state. As
67 described for bacteria, root colonization by beneficial fungi can also boost plant immunity. For
68 example, root colonization by arbuscular mycorrhizal fungi is tightly regulated and the modulation of
69 immune responses during the symbiosis establishment can also leads to induced resistance in systemic
70 tissues.[15] Therefore, understanding the fine-tuned regulation of plant immune responses during
71 colonization by beneficials is essential for optimizing bioinoculant-based strategies in agriculture,
72 allowing their establishment and promoting their benefits in plant health.

73 In the case of bacteria, social behavior has been proven to be crucial for the success in colonizing plant
74 tissues. Bacteria often exist in multicellular groups of cells called biofilms,[16] where they engage in
75 competition for resources but also in cooperative (synergistic) interactions that enhance community
76 productivity.[17] These social engagements are most often mediated through secreted molecules that
77 are believed to have a profound impact on bacterial diversity, spatial organization, and ecological
78 functions.[16,18] *B. subtilis* species are capable of different forms of social interactions besides biofilm
79 formation, such as quorum sensing (QS), group motility (swarming), and kin discrimination.[19] Non-kin

80 *B. subtilis* strains form a striking boundary between approaching swarms,[20,21] exclude a non-kin
81 competitor from a common swarm,[22] and do not mix on *Arabidopsis thaliana* roots, while kin strains
82 merge on solid medium and cohabit root surfaces.[20] *B. subtilis* strains also produce a range of
83 antibiotics, surfactants, secondary metabolites, and bioactive volatiles expected to modulate social
84 interactions between bacteria and with the plant.[20,21,23,24]

85 *B. subtilis* QS system comprises the signalling peptide ComX, which positively regulates the synthesis
86 of surfactin (encoded by Srf operon), one of the most potent surfactants and a lipopeptide
87 antibiotic.[19,25] Surfactin acts as a signalling molecule during biofilm formation, promotor of horizontal
88 gene transfer and as an inducer of physiological changes in plants during colonization with
89 bacteria.[25,26,27,28,29] Surfactin is also freely shared between kin *B. subtilis* strains, but not between non-
90 kin.[22] Despite evidence for *in situ* QS dependent expression of surfactin, the link between QS and
91 surfactin on plant roots and how QS affects plant colonisation and plant immune response is less
92 understood.

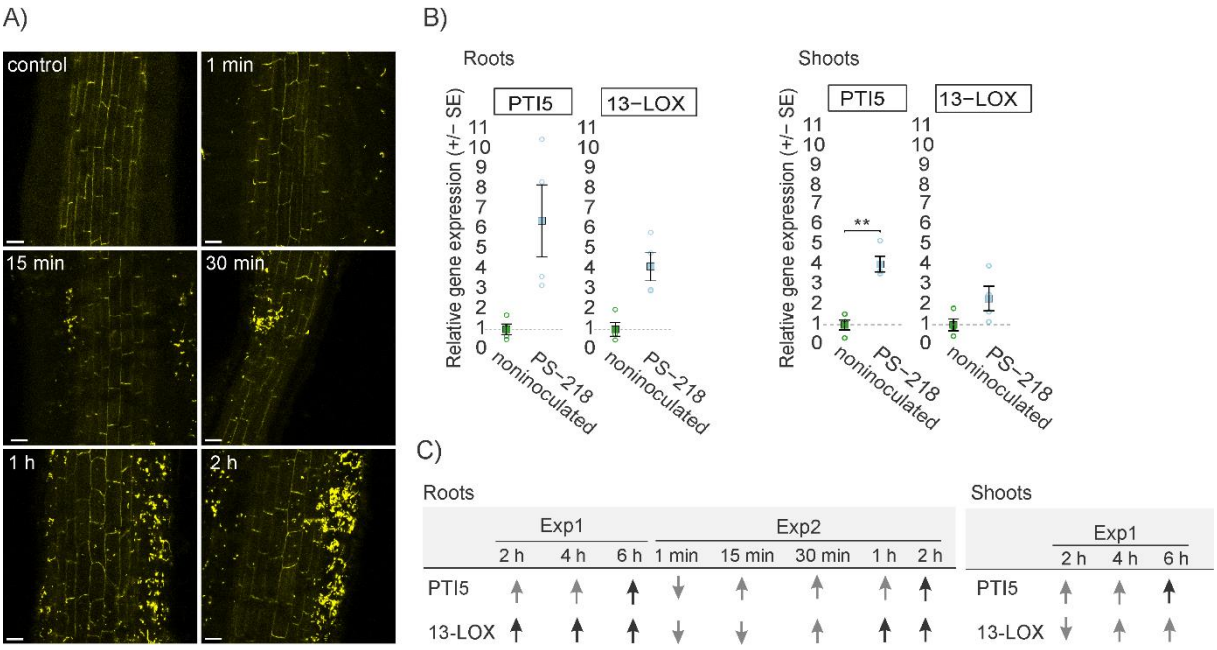
93 As potato, the third most important crop in the world, is exceptionally sensitive to a wide range of
94 environmental stresses,[31] studying its interaction with beneficial microbes is crucial to ensure an
95 efficient environmentally friendly system of plant protection based on modulation of its microbiome.
96 The aim of this study was to investigate mechanisms involved in the interaction between potato and
97 *B. subtilis*. Different *B. subtilis* strains and mutants were used to investigate the principles of bacterial
98 social interactions and their interaction with the plant. We identified transcription factor PTI5 as a
99 potential regulatory hub for *B. subtilis* endophytic colonization and further investigated its role in the
100 plant–bacteria interaction using transgenic plants with silenced PTI5. We here show that *B. subtilis*
101 abundance is increased in systemic tissue of plants with silenced PTI5, suggesting that PTI5 maintains
102 optimal *B. subtilis* abundance in colonized plants. Interestingly, a similar function was confirmed also
103 for the establishment of the mutualistic symbiosis between potato and arbuscular mycorrhizal fungi.

104

RESULTS

Stable biofilm formation on potato roots coincides with first transcriptional responses in plant roots

We here studied the response of potato to colonization by two different *B. subtilis* strains (PS-216 and PS-218). Both formed biofilm on potato roots (Figure S1). As our aim was to capture both initial events in biofilm establishment as well as long-term effects of potato–*B. subtilis* interaction, we first determined timing of biofilm establishment on potato roots and transcriptional responses in the plant. We selected eight genes known to be involved in potato immune response as potential indicators of potato MTI to *B. subtilis* colonization[32,33] and followed their expression in potato roots and shoot tissue. The genes showed diverse expression profiles, which were for some genes tissue specific (Figure S2, Table S1). Two genes, an ERF transcription factor PTI5, which requires an active ethylene pathway for the activation and mediates crosstalk of ethylene and SA pathways[33] and a gene involved in JA synthesis, 13LOX,[32] were reproducibly upregulated in roots and shoots 24 hours post-bacterial inoculation (Figure 1, Figure S2, Table S1). The results were similar for both tested *B. subtilis* strains (Exp1 and Exp2 in Figure S2, Table S1).



121 **Figure 1: Dynamics of biofilm formation and plant responses of potato to *B. subtilis*.** A) Biofilm formation (YFP
122 fluorescence) was followed on potato roots after incubation with *B. subtilis* PS-218 YFP for a specified time. Scale
123 is 50 µm. B) Potato response to bacteria colonization is robustly followed by expression of PTI5 and 13-LOX.
124 Relative expression of PTI5 and 13-LOX in roots and shoots of *B. subtilis* PS-218 YFP inoculated and non-
125 inoculated plants, after overnight incubation. Relative gene expression was scaled to the average gene
126 expression of non-inoculated group. Welch's t-test was used to determine differences between treatments.
127 Individual measurements (circles), mean (squares) and standard error are shown. Asterisks (*) denote a
128 statistically significant difference (p-value < 0.05). Data from all experiments are given in Table S1. C) Dynamics
129 of PTI5 and 13-LOX expression (up- or downregulation) in roots and shoots of potato plants after inoculation with
130 high *B. subtilis* strain PS-218 culture density (10^7 CFU/mL) and incubation for a specified time. Data of all
131 experiments are available in Table S1. Up- or downregulation was determined by comparing expression in
132 inoculated plants with expression in non-inoculated plants. ↑: upregulation, ↓: downregulation, **↑**: statistically
133 significant upregulation determined by Welch's t-test, **↓**: statistically significant downregulation determined by
134 Welch's t-test. Exp: experiment.

135

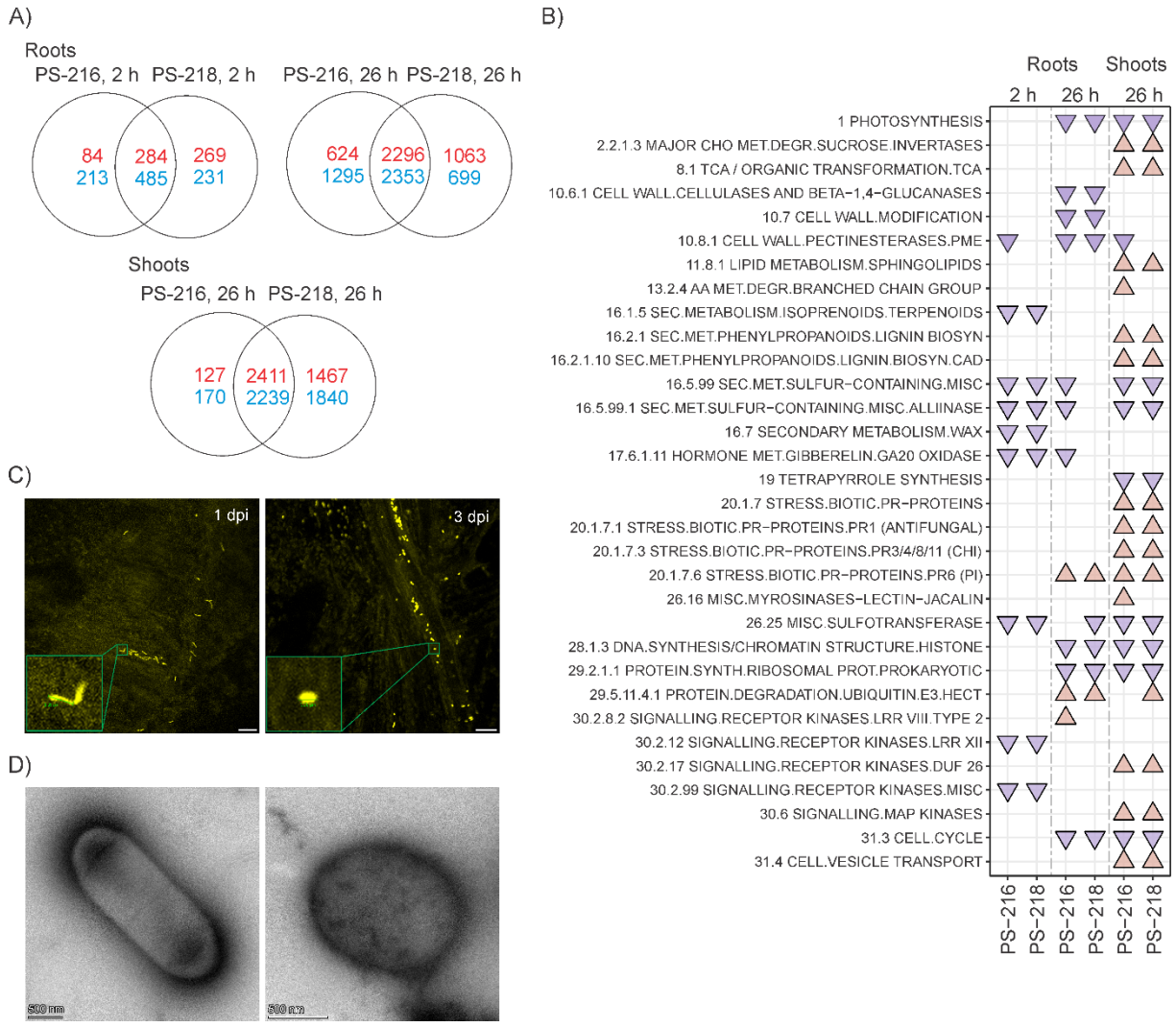
136 To study the dynamics of biofilm formation, we set up an experiment with higher density bacteria
137 inoculum (10^7 CFU/mL). In such set-up, biofilm reproducibly began forming after the first 15 minutes
138 of incubation, and it was consistently present over the entire root surface after a 2-hour incubation
139 period (Figure 1). Interestingly, the induction of PTI5 and 13-LOX genes was also detected when the
140 biofilm was formed uniformly on the root (Figure 1, Figure S2, Table S1).

141

142 **Complex regulatory network is triggered when potato is colonized with *B. subtilis***

143 To investigate plant response to *B. subtilis* colonization in more detail, we next performed RNA-seq
144 analysis of colonized roots and systemic shoot tissue. Our special interest was in the starting point of
145 colonization, as we intended to capture initial *B. subtilis* recognition related responses in the plant.
146 Therefore, roots were analyzed at the time of biofilm formation (2 hours post-inoculation (hpi)) and

147 24 hours later (26 hpi). The response of the plant was stronger at later time points (Figure 2A, Figure
148 S3). Moreover, shoots were analyzed at this later time to explore potential activation of MTI and/or
149 IR. For both root and shoot tissue, we identified a large overlap in differentially expressed genes for
150 plants inoculated with two different strains (Figure 2A, Figure S3).



151

152 **Figure 2: Potato adjusts to colonization with *B. subtilis* by repression of immune signaling and cell wall**
153 **synthesis.** A) Number of genes, regulated by *B. subtilis* in plant roots and shoots after 2-h and 26-h incubation
154 with PS-216 YFP and PS-218 mKate2. Blue: downregulated genes, red: upregulated genes. B) Visualization of
155 results in the context of biological pathways. For each comparison between *B. subtilis*-inoculated and non-
156 inoculated plants, gene sets enriched for up- or downregulated genes (q-value < 0.1) are shown and designated
157 as red triangles or blue triangles for shoots and roots at different time points. MapMan ontology BINs were used
158 to generate gene sets. Sample group comparisons of inoculated to non-inoculated (in columns) are designated
159 with bacterial strain names used for inoculation. PS-216: PS-216 YFP, PS-218: PS-218 mKate2. See Table S2 for all
160 results. C) *B. subtilis* morphology is changed after internalization in plants. Left: *B. subtilis* morphology 1 dpi (day

161 post-inoculation). Right: *B. subtilis* morphology 3 dpi. See Figure S4 for more data. Scale is 25 µm. D) Electron
162 micrograph of free-living (left) and *in planta* living *B. subtilis* PS-216 (right). Scale is 500 nm.

163

164 Many processes related to primary line of defense were downregulated in roots at the time of biofilm
165 formation, including diverse immune receptors, genes involved in cell wall modifications and in
166 synthesis of terpenoids and waxes (Figure 2B). Interestingly, among the genes that were upregulated
167 in roots already at the time of biofilm formation were in majority genes coding for transcription factors,
168 hormonal synthesis, signalling components and components of protein degradation. Several ERF
169 transcription factors were upregulated besides the already identified PTI5, as well as many MYBs,
170 WRKYs, bZIP and NAC transcription factors. We also identified several lipoxygenases upregulated (JA
171 synthesis), but also genes involved in ethylene and gibberellin synthesis as well as in Ca^{2+} signalling
172 (Table S3). The response of most of these genes was intensified in later time point in both roots and
173 shoots (Table S3).

174 Remarkably, more induced gene sets were detected in shoots. The processes activated in shoots
175 resemble IR as studied so far in other plants. Receptor kinases were upregulated as were MAP kinases
176 and executors of plant defense, such as beta-glucanases, alpha amylase inhibitors, PR1 proteins and
177 PR5 proteins (Figure 2B, Table S2, Table S3). Among the genes associated with transcriptional
178 regulation, NPR1 was also specifically upregulated in shoots only.

179 In addition, we also followed the extent of bacterial internalization within the plant. We detected
180 bacteria in stem already 1 day post-inoculation (dpi) (Figure S4). Interestingly, while plant responds to
181 *B. subtilis* colonization, also *B. subtilis* adapts once internalized as its morphology starts to change to
182 more rounded form as early as 2 dpi (Figure 2C, Figure S4). Also using electron microscopy, we can see
183 that most free-living bacteria are multiplying, while no multiplication was detected in *in planta* living
184 bacteria (Figure 2D, Figure S5).

185 **Biofilm formation and plant responses are attenuated in potato roots inoculated with *B. subtilis***
186 **quorum sensing and surfactin-deficient mutants**

187 To better understand the mechanisms that trigger plant responses, we inspected the role of the *B.*
188 *subtilis* ComX peptide mediated QS and surfactin production in potato–*B. subtilis* interaction. We used
189 two PS-216 mutant strains, one with deletion of the *comQXP* gene cluster, coding for the genes
190 involved in QS, which also regulate surfactin production (PS-216 Δc*omQXP*),[25] and the other strain with
191 a mutation in the *srfA* operon, which is unable to produce surfactin (PS-216 Δ*srfA mutant*). Both
192 mutants are reported to still form biofilms in liquid media.[34] The biofilm formed by both mutants on
193 potato root was barely detectable at 26 hours after inoculation, while strong biofilm formation was
194 observed for wild-type (WT) bacteria (Figure 3A, Figure S6, Table S4). The reduced ability to form
195 biofilm was also reflected in the potato response to *B. subtilis*. The induction of JA synthesis and
196 ethylene signalling markers observed in response to the WT was attenuated in the plant response to
197 the mutants. Similarly, the colonization associated downregulation of apoplastic ROS generator
198 RBOHD and photosynthesis marker CAB occurred to a lesser extent in response to the mutants (Figure
199 3B, Table S5).

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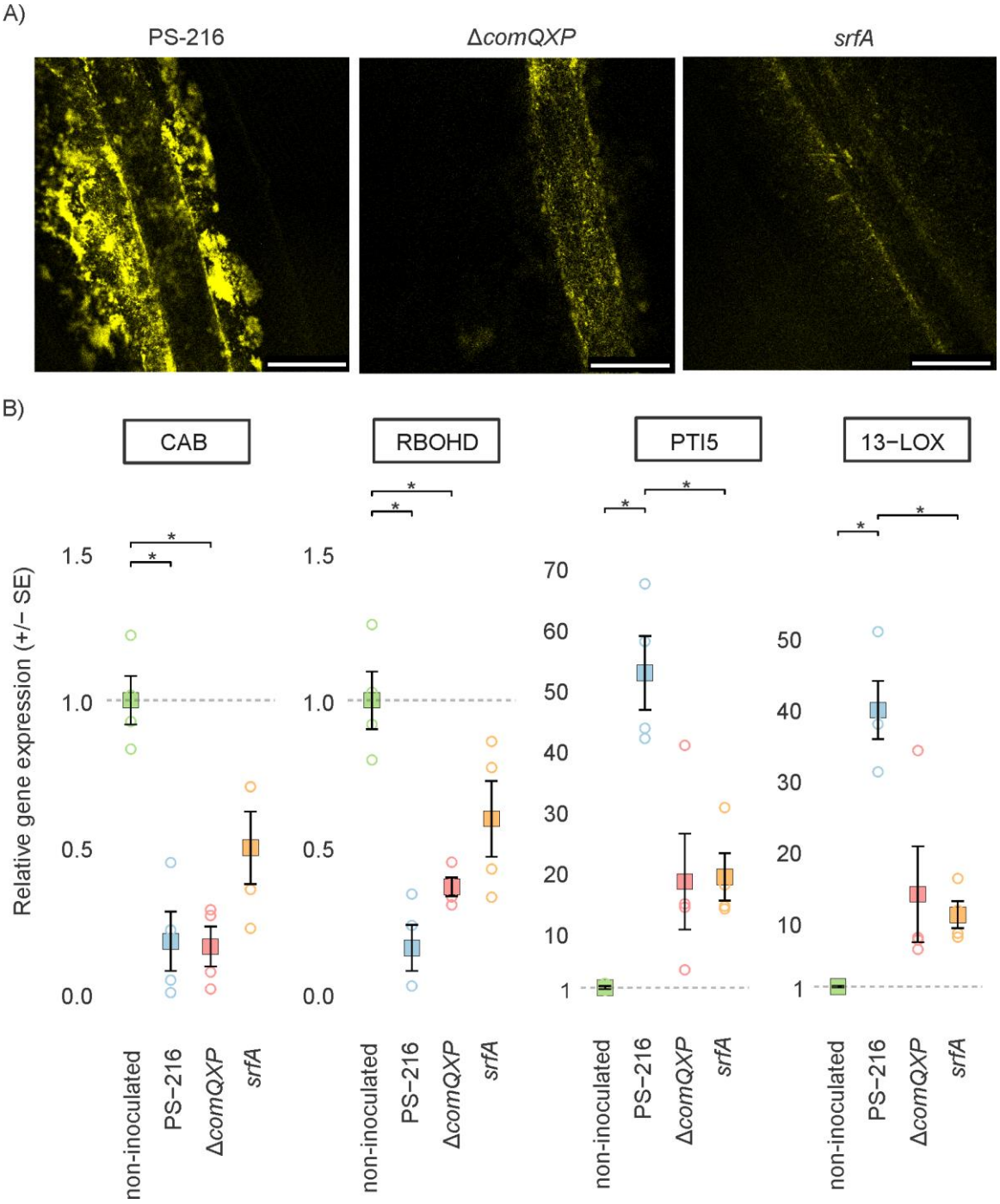

Figure 3: Induction of plant responses is dependent on efficient *B. subtilis* biofilm formation on roots. A) Biofilm formation on potato roots after 26-h incubation with YFP-labelled *B. subtilis* PS-216 (left), quorum sensing-negative *B. subtilis* YFP-labelled mutant PS-216 Δ*comQXP* (middle) and PS-216 srfA mutant (right) culture. Scale is 500 µm. B) Expression of selected genes (RBOHD, 13-LOX, CAB, PTI5) in non-inoculated potato roots and after 26-h incubation with *B. subtilis* PS-216 YFP, PS-216 Δ*comQXP* YFP and PS-216 *srfA* YFP. Relative gene expression was scaled to the average gene expression of non-inoculated group. Welch's t-test was used to determine differences between groups. Individual measurements (circles), mean (squares) and standard error are shown.

209 Asterisks (*) denote statistically significant difference (p-value < 0.05). See Table S5 for the results of all genes
210 for both roots and shoots.

211

212 To confirm that biofilm establishment is required for successful activation of plant responses, and that
213 *B. subtilis* secondary metabolites are not sufficient, we treated plants with the conditioned media in
214 which bacteria were growing. Results showed that the conditioned media alone did not trigger any
215 response in treated plants, albeit containing surfactin (Figure S7, Table S6). Therefore, we conclude
216 that *B. subtilis* biofilm formation on the roots is required for induction of potato response.

217

218 **Non-kin intraspecies interaction of *B. subtilis* strains on root intensifies potato responses**

219 To understand plant responses to colonizing bacterial communities, which is relevant for application
220 in practice, we set up an experiment inoculating plants with two genetically different, categorized
221 previously as non-kin, and two isogenic (only differently fluorescently labelled) *B. subtilis* strains. When
222 roots were inoculated with PS-216 and PS-218 strains separately, both equally colonized the roots
223 (Figure 4A, first two columns). Similarly, when differentially labelled isogenic strains were combined,
224 the resulting biofilm on the root contained similar abundances of each strain, with cells of each strain
225 spatially well mixed (Figure 4A, third column). On the other hand, when non-kin strains PS-216 and PS-
226 218 were combined, we observed biofilm with segregation of the two strains (Figure 4A, last column).
227 Moreover, one of the strains dominated on the roots, with only a small amount of biofilm formed by
228 the other strain (Figure 4A, last column).

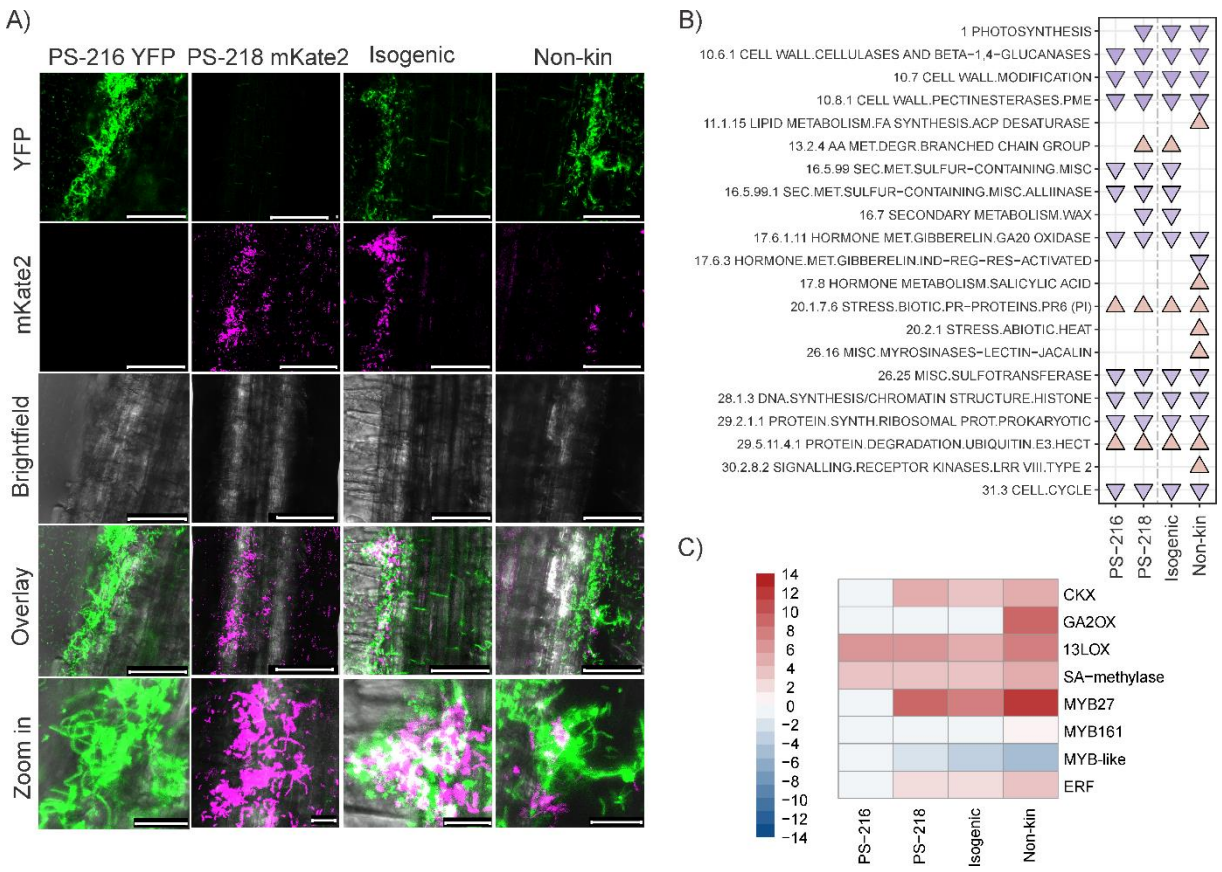

Figure 4: Social interactions of *B. subtillis* strains on potato roots intensify potato immune response. A) Colonization of potato roots by isogenic and non-kin *B. subtilis* strains after 4-h incubation. From top to bottom: YFP fluorescence, mKate2 fluorescence, brightfield, overlay of images, zoom in of overlay images. From left to right: PS-216 YFP, PS-218 mKate2, PS-218 YFP and PS-218 mKate2 (isogenic – self interaction), PS-216 YFP and PS-218 mKate2 (non-kin interaction). White color (overlay) shows colocalization of YFP and mKate2. The scale is 25 µm for **zoom in** images and 100 µm for all others. B) Visualization of responses in the context of biological pathways. For each comparison between *B. subtilis*-inoculated and non-inoculated plants, gene sets enriched for up- or downregulated genes (q-value < 0.1) are shown and designated as red triangles or blue triangles, respectively after 2-h incubation. MapMan ontology BINs were used to generate gene sets. Sample group comparisons to non-inoculated ones (in columns) are designated with bacterial strain names used for inoculation. PS-216: PS-216-YFP, PS-218: PS-218-mKate2, isogenic interaction: YFP-labelled PS-218 and mKate2-labelled PS-218, non-kin interaction: YFP-labelled PS-216 and mKate2-labelled PS-218. See Table S2 for all results. C) Examples of genes with intensified response when plant is interaction with two non-kin strains. Log2 values are shown. Blue: downregulated genes, red: upregulated genes, according to scale on the left.

We next performed RNA-seq analysis of roots at the time of uniform establishment of *B. subtilis* biofilm (2 hpi). In roots, the intensity of the transcriptional response to the non-kin strain mixture was higher

247 than that to the mixture of two isogenic, differentially labeled strains (Figure 4, Figure S3). Regarding
248 biological pathways, some receptor kinases (BINs 26.16 and 30.2.8.2) were specifically upregulated in
249 the non-kin interaction, as well as some enzymes from fatty acid biosynthesis (BIN 11.1.15) and heat
250 shock proteins (BIN 20.2.1) (Figure 4B, Table S2). In addition, 422 genes had more than 2-fold stronger
251 response in the interaction with non-kin strains compared to monoculture or the isogenic mixture.
252 Among the more induced ones, are two MYB transcription factors, salicylate
253 carboxymethyltransferase, gibberellin 2-oxidase 2, peroxidase and beta-galactosidase genes (Table
254 S3).

255

256 ***B. subtilis* abundance is increased in plants with silenced PTI5**

257 We next overlayed data with plant stress signaling prior knowledge network, built from experimental
258 data on protein–protein interactions, protein–DNA interactions and metabolic pathways (skm.nib.si).[35]
259 The central signaling module that was regulated in the studied interaction was ethylene signaling,
260 linked to regulated JA synthesis, SA signaling and ROS signaling (Figure 5A; Figure S8; Table S7). Thus,
261 we focused on ERF transcription factors, as the role of ethylene signaling was so far not in depth
262 addressed in interaction of plants with beneficial microbes. Among ERFs, PTI5 was found to be more
263 than 50-fold induced when plants were exposed to *B. subtilis* monocultures or two-strain mixtures,
264 already early after colonization (after 2-hour incubation). This could indicate its role as regulatory hub
265 in plant colonization and/or biofilm formation. To test this role, we produced transgenic potato with
266 reduced expression of PTI5. First, we followed biofilm formation in transgenic plants with silenced
267 PTI5. Results showed that PTI5 is not involved in biofilm formation on roots (Figure 5B, Table S8), as
268 no difference in biofilm formation was detected between non-transgenic (NT) and PTI5-silenced plants
269 (Figure 5B, Figure S9, Table S8).

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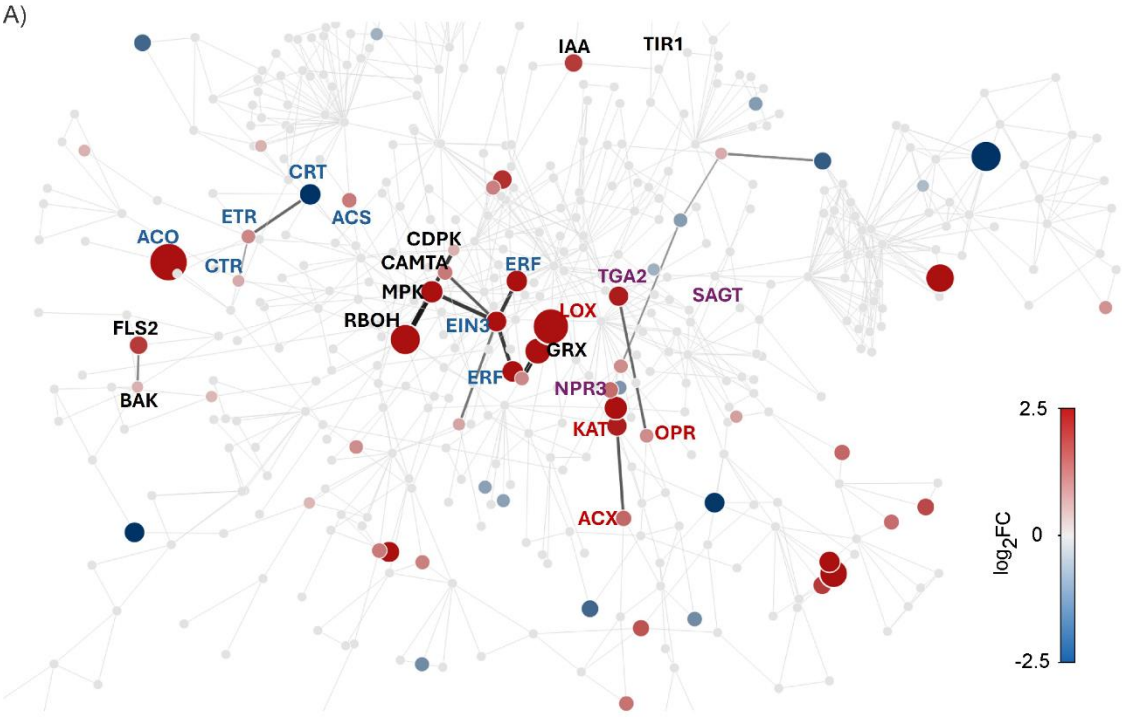
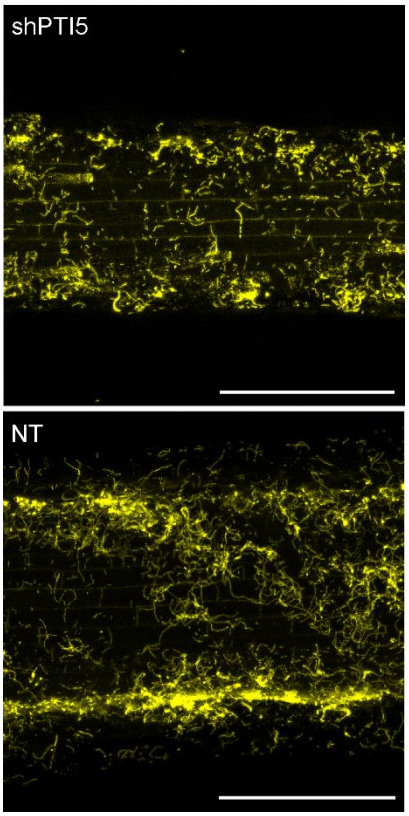
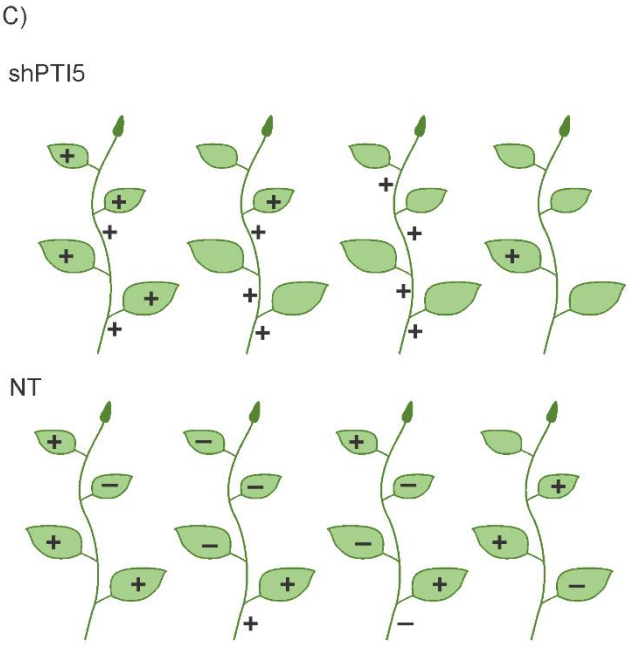






	No of samples		% of	p-value
	all	positive	positive	
shPTI5	15	15	100	0,0035
NT	17	9	52.9	

Figure 5: ERF transcription factor PTI5 is required for balanced colonization of potato plants. A) Plant immune signalling modules that were regulated following incubation of roots with non-kin mixture of *B. subtilis* strains. Genes and proteins are represented as nodes and interaction between them (protein–protein interaction, protein–DNA interaction) as edges of the network. Differentially expressed genes from ethylene (blue), JA (red),

276 SA (violoet) and other (black) signalling modules are shown. Black edge connected two differentially expressed
277 genes. All non-differentially expressed genes and edges are shown in grey. The size of the node indicates the
278 difference in expression between PS-216 and PS-218 inoculated and non-inoculated roots (log2FC). See Table S7
279 for the name and description of regulated genes, and Figure S8 for information on central signalling modules
280 that were regulated in potato roots in isogenic interaction (PS-218 YFP + PS-218 mKate2). B) *B. subtilis* PS-218
281 YFP root colonization (yellow fluorescence) of transgenic potato with silenced PTI5 (shPTI5) and non-transgenic
282 potato plant (NT) after 2-h incubation. Scale is 250 µm. C) Bacillus abundance in shPTI5 and NT potato plants.
283 The scheme shows leaves of four shPTI5 and four NT plants in which the presence of *B. subtilis* was (+) or was
284 not (-) detected by qPCR in plants from soil which were incubated for 2 h in PS-218 YFP before being planted and
285 grown in soil for one week. Leaves with no mark were not analyzed. The table presents a statistically significant
286 difference in the percentage of positive samples (leaves, nodes and internodes) between NT and shPTI5 plants
287 determined by Fisher's exact test. See Table S9 for details. The results of line L6 are shown and were confirmed
288 on another transgenic line shPTI5 L2 (Figure S9, Table S10).

289

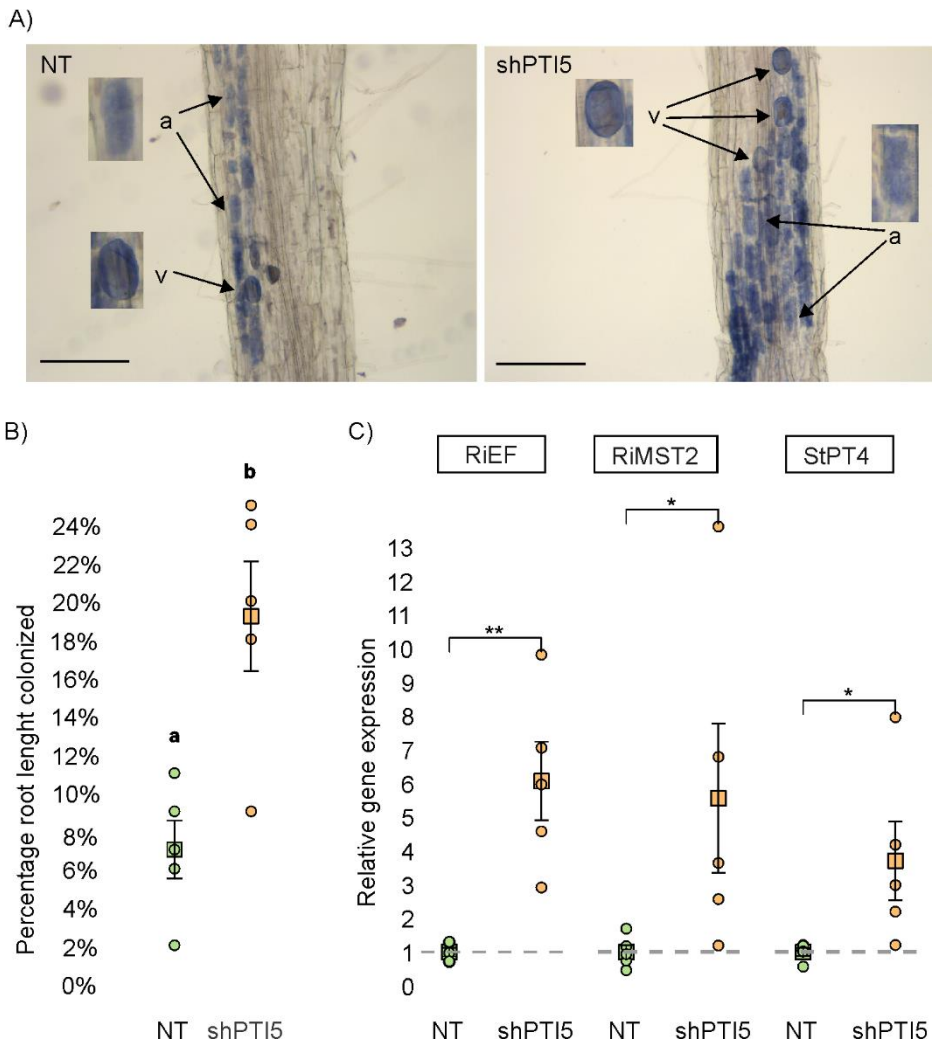
290 PTI5 was also upregulated in potato shoots. To test if it has a role in maintenance of *B. subtilis*
291 endophytic community we followed *B. subtilis* abundance in shoots of NT and PTI5-silenced plants. *B.*
292 *subtilis* abundance in systemic tissue was higher in PTI5-silenced plants, revealing that the balance in
293 the abundance of endophytic *B. subtilis* community was perturbed (Figure 5C, Table S9, Table S10).

294

295 **PTI5 is also regulating the extent of potato root colonization by arbuscular mycorrhizal fungi**

296 Considering the role of PTI5 in regulating endophytic colonization of *B. subtilis*, we aimed to explore if
297 this role was specific for this interaction or could be extended to other beneficial symbiosis. We
298 decided to explore the potential role of PTI5 in regulating the extension of root colonization by
299 arbuscular mycorrhizal fungi. This symbiosis is known to be under plant control to keep the interaction
300 under mutualistic levels and avoid excessive colonization.[36,37] With this aim, we explored the
301 colonization levels and symbiosis functionality in NT and PTI5-silenced lines. As shown in Figure 6, *R.*
302 *irregularis* successfully colonized potato roots, with intercellular hyphae, arbuscules and vesicles
303 present in the cortex. Remarkably, the extension of colonization, including the abundance of
304 arbuscules and vesicles was higher in the PTI5-silenced lines (Figure 6A). Indeed, the percentage of

305 root length colonized by the fungus was more than 2-fold higher in the silenced lines (Figure 6B), in
306 agreement with the higher amount of fungal DNA in those plants. To check if the functionality of the
307 mycorrhizal symbiosis was altered in those lines, we checked the expression levels of plant and fungal
308 transporters involved in the nutrient exchange between the symbionts within the arbuscules: the
309 potato phosphate transporter StPT4, and the fungal monosacharide transporter RiMST2, both
310 transcriptionally upregulated in arbusculated cells and defined as markers for symbiotic functionality
311 (Figure 6C). Both markers were upregulated in the PTI5-silenced lines, confirming the functionality of
312 the symbiosis.



313

314 **Figure 6: Mycorrhizal colonization of potato roots in non-transgenic potato plant (NT) and PTI5-silenced**
315 **genotype (shPTI5).** A) Representative images of mycorrhizal colonization by *R. irregularis* in the different
316 genotypes. Fungal structures within the root cortex of potato plants are stained in blue (a: arbuscules, v: vesicles).
317 Scale is 200 µm. B) Percentage of root length colonized by the arbuscular mycorrhizal fungi *R. irregularis*. C)

318 Expression analysis of *R. irregularis* constitutive gene (RiEF) and symbiosis marker genes, the *R. irregularis*
319 monosaccharide transporter (RiMST2) and the potato phosphate transporter (StPT4), as well as regression
320 analysis using Dunn's pairwise comparisons were used to determine the differences between the genotypes.
321 Different letters show statistical differences. Permutation test was used to determine the differences between
322 the genotypes. Individual measurements (circles), mean (squares) and standard error are shown. Asterisks
323 denote a statistically significant difference (*: p-value < 0.05, **: p-value < 0.01). The results of analysis of line L6
324 are shown and were confirmed on another transgenic line shPTI5 L2 (Figure S10).

325

326 **DISCUSSION**

327 Microbial inoculants have been proven as efficient and environmentally friendly alternatives to
328 chemical pesticides and fertilizers.[38,39] Current limitation in adopting such agricultural practices is their
329 variable efficiency in the field due to lack of understanding of mechanisms involved in the regulation
330 of plant colonization and maintenance of the plant microbiome.[6,40] Therefore, studying the
331 mechanisms regulating the interactions between crop plants and microbes is crucial to ensure
332 robustness of such plant protection approach. As the third most important crop in the world,
333 exceptionally sensitive to a wide range of stresses, potato is one of the most suitable candidates for
334 plant growth-promoting rhizobia (PGPR) based products.[31] We here established a model system for
335 such studies, exploring selected *B. subtilis* soil isolates individually or in mixtures to establish the
336 molecular basis of their interactions with plants. Furthermore, we explored the potential extension of
337 our findings by testing other widespread mutualistic interaction, the arbuscular mycorrhizal symbiosis.

338 *B. subtilis* initially suppresses immune response in potato roots (e.g. downregulation of diverse
339 receptor kinases) thereby facilitating its own colonization, while it boosts induction of plant immunity
340 related genes in shoots at a later stage (Figure 2, Table S2), as it was shown also in other studies of
341 plants with beneficials as part of induced resistance phenomena.[13,41] Here we show that live bacteria
342 are required for triggering of the plant response, as the conditioned media in which bacteria were
343 growing did not trigger the response (Figure S7). Moreover, the intensity of the plant response was
344 dependent on the extent of biofilm formation on the potato root (Figure 3). Both, the mutant in

345 surfactin production and mutant in QS (also deficient in surfactin production) showed a reduced ability
346 to form biofilm on roots. This is interesting as the same strain was able to form floating biofilms
347 (pellicles),[42] suggesting that the ComX QS control of the biofilm development depends also on plant
348 factors. Besides residing on the plant roots, *B. subtilis* can also colonize the plant endophytically.
349 Internalised bacteria stop dividing and change the morphology (Figure 2, Figure S5). The round
350 bacterial form closely resembles L-form bacteria, which are characterized by modified or absent cell
351 walls.[42,43,44] We also tested the extent of bacteria internalisation with QS impaired strains, which was
352 interestingly not affected (Table S11).

353 To get insights into the complexity of plant–microbe interactions, we extended our studies to
354 application of two different *B. subtilis* strains to explore how bacterial diversity may influence the plant
355 response. We studied pairs of strains with different kin discrimination types of intraspecies interactions
356 — isogenic strains were predicted to be cooperative, and non-kin strains to be antagonistic.[22] We
357 observed that non-kin interaction of two *B. subtilis* strains invigorates the plant response, especially in
358 immune and abiotic signalling components (Figure 4, Table S3). These results imply that even minor
359 taxonomic changes in the phytobiome can lead to substantial differences in the plant responses.
360 Increasing evidences support that plants actively manage their microbiota for balancing growth with
361 immunity.[46] It has been reported that *B. subtilis* can promote plant growth via direct pathways such as
362 producing phytohormones (e.g., auxin, gibberellin, cytokinin, and abscisic acid).[46,47,48] Both strains used
363 in this study produce auxins (Table S12). Interestingly, we here show that *B. subtilis* additionally
364 regulates phytohormone metabolism *in planta*. Gibberellin 2-oxidase 2 and cytokinin oxidase, which
365 catalyze the irreversible degradation of cytokinin and gibberellin phytohormones, have been among
366 the most induced genes by inoculation of non-kin *B. subtilis* strains (Figure 4, Table S3). This could
367 suggest that equilibrium between growth and immunity is shifted towards immunity in plants
368 inoculated by non-kin *B. subtilis* strains, as a functional relation between lower activity of gibberellin
369 signalling and reduced plant disease severity has previously been confirmed in potato.[50] In accordance,
370 indole-3-acetic acid-amido synthetase GH3.8 was in our study induced in *B. subtilis* inoculated plants

371 at late time point in both shoots and roots (Table S3). GH3.8 functions as IAA-amino synthetase that
372 prevents the accumulation of free indole acetic acid (IAA), which increases plant disease resistance,
373 growth and development.[51]

374 Not much is however known on the mechanisms that allow for successful colonisation of plants with
375 beneficial microbes while avoiding colonization by harmful pathogens. MTI is known to be induced also
376 when plant is exposed to beneficials.[11] In interaction of Arabidopsis with beneficial *Pseudomonas* strain
377 it was found that suberin biosynthesis is down regulated to allow for better penetration of bacteria.[11]
378 Similarly, in our study cell wall metabolism was downregulated in root colonization (Figure 2, Figure
379 4). In addition, synthesis of toxic terpenoids was downregulated in roots at the biofilm forming stage
380 (Figure 2). Suppression of toxic ROS is crucial for successful colonisation of plants by microbes.[24] We
381 found ROS scavenger peroxidase and ROS generator RBOHD induced and repressed, respectively,
382 following inoculation by *B. subtilis* (Figure 3). Several studies also reported the increase of peroxidase
383 in different plant–*Bacillus* interactions, and in some cases this induction have been associated to
384 pathogen resistance.[51,52,53,54,55,56]

385 Plant colonisation is frequently linked to induction of SA, JA and ethylene signalling pathways and
386 transcriptional rewiring, of which MYB72, MYC2 and NPR1 transcription factors are the most studied
387 ones, and are proposed to be relevant for IR responses.[13,58] Here we found upregulation of the 13-LOX
388 pathway leading to JA synthesis, as previously described in other plant-PGPR interactions (Figure 1,
389 Figure 3).[58,59,60] Similarly, several studies reported activation of SA signalling pathway.[14] We found NPR1
390 upregulated in shoots (Table S2, Table S3). In corroboration with previous studies, we also identified
391 several MYB transcription factors induced in both roots and shoots (Table S3).

392 Modulation of ethylene signalling in plant microbiome interactions have been pinpointed as a central
393 element in their regulation and their impact on plant responses to stress.[62] For example, ethylene have
394 been proposed to regulate biofilm formation and root colonization by beneficial bacteria[63,64] and the
395 arbuscular mycorrhizal symbiosis,[65] and it is required for IR by beneficial bacteria and fungi.[66,67] We

396 also detected induction of the ethylene signalling pathway in *B. subtilis* inoculated roots, including ETR,
397 EIN3 and several ERF transcription factors (Figure 5, Figure S8, Table S3). Moreover, 1-
398 aminocyclopropane-1-carboxylate oxidase, which catalyzes the final step in ethylene biosynthesis, was
399 in our study induced in all tissue and time points, further suggesting involvement of ethylene signalling
400 pathway in potato response to *B. subtilis* (Figure 5, Figure S8, Table S3).
401 ERF transcription factors, the members of the AP2/ERF family, play prominent roles in plant immunity,
402 although the function of most of them remains unknown.[13,59,68,69] A different set of ERFs was regulated
403 in roots and shoots in our study. Among them, ERF transcription factor PTI5 was strongly upregulated
404 in all studied conditions. PTI5 was first identified in tomato as interacting partner of the *R* gene *Pto*
405 that confers resistance against the bacterial pathogen *Pseudomonas syringae* pv. tomato.[70,71] On the
406 other hand, we have previously shown that PTI5 negatively regulates potato defence response to
407 pathogens potato virus Y and *Ralstonia Solanacearum*, suggesting its role as susceptibility factor in
408 potato immunity.[33] Interestingly, PTI5 is transcriptionally regulated by EIN3 in the presence of SA only
409 and on the protein level regulated by autophagy degradation.[33] Results of this study suggest that PTI5
410 has a negative regulatory role also in the maintenance of endophytic community in potato, as both *B.*
411 *subtilis* abundance in systemic tissues and mycorrhizal colonization of roots were increased in PTI5-
412 silenced potato plants (Figure 5, Figure 6). Results thus suggest that high PTI5 levels increase
413 susceptibility of potato to pathogens and reduce the capability of plant to interact with beneficial
414 organisms, making it a breeding target for the future sustainable potato. Taking it altogether, we here
415 set a cornerstone in understanding how beneficial microbes and plant communicate to regulate
416 endophytic colonization and maintain mutualism and have identified a way for improved colonization
417 by beneficial through silencing of PTI5.
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419
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AUTHOR CONTRIBUTIONS

Concept and design: TL, MJP, IMM, KG. Acquisition, analysis or interpretation of data: TL, BK, KP, KS, TGK, MZ, MP, PS, AV, VL, TMP, JMP, MJP, IMM, KG. Drafting of manuscript: TL, KG. Critical revision of the manuscript for important intellectual content: MZ, MP, PS, MJP, IMM, KG.

METHODS

Plant material

Potato plants cv. Rywal (NT) and shPTI5-Rywal were grown in stem node tissue culture for two weeks after *in-vitro* micropropagation under controlled environmental conditions (22 ± 2°C in the light and 19 ± 2°C in the dark with 70–90 µmol/m2/s2 radiation (OSRAM L 58 W/77 FLUORA lamps, Germany) and a 16-h photoperiod) as previously explained.[72] For studying systemic *B. sublilis* spread, plants were planted in soil two weeks after node segmentation and grown under controlled environmental conditions for one week as previously described.[73] shPTI5-Rywal transgenic lines were prepared by introducing shPTI5 construct[33] into NT using *A. tumefaciens* LBA4404.[74] The transformed bacteria were used for the transformation of NT stem internodes from *in vitro* plantlets as described in Lukan et al.[73] using appropriate selection media.

444 ***B. subtilis* strains**

445 WT *B. subtilis* strains PS-216 and PS-218[75] tagged with red fluorescent protein (mKate2) and yellow
446 fluorescent protein (YFP) gene linked to a constitutive hyperspank promotor were used, namely PS-
447 218 *amyE::*$P_{hypercI03}$*-YFP* (Sp) (PS-218 YFP), PS-216 *amyE::*$P_{hypercI03}$*-YFP* (Sp) (PS-216 YFP), PS-218
448 *amyE::*$P_{hyperspank}$*-mKate2* (Chl) (PS-218 mKate2) and PS-216 *amyE::*$P_{hyperspank}$*-mKate2* (Chl) (PS-216
449 mKate2).[20,22] PS-216 mutants with impaired surfactin production PS-216 *amyE::PhypercI03-YFP* (Sp)
450 *srfA::Tn917 (mls)* (PS-216 *srfA* YFP)[22] and QS mutant PS-216 *ΔcomQXP::kan amyE::*$P_{hypercI03}$*-YFP* (Sp) (PS-
451 216 Δ*comQXP* YFP) (this work) were used. QS mutant PS-216 ΔcomQXP::kan amyE::PhypercI03-YFP
452 (Sp) was constructed by transformation of PS-216 amyE::PhypercI03-YFP (Sp)[22] with plasmid pED302,[76]
453 carrying a kanamycin cassette between *degQ* and *comA*, resulting in a deletion of the *comQXP* locus.

454

455 **IAA production detection**

456 The production of IAA was assessed following the protocol of Pramanik et al.[77] Fresh bacterial cultures
457 were prepared from single colonies in LB medium supplemented with 0.1 % tryptophan and incubated
458 in the dark at 30°C with shaking at 120 rotations per minute (rpm) for 48 hours. After incubation,
459 cultures were centrifuged at 10,000 rpm for 5 minutes, and the supernatant was transferred to a
460 microtiter plate in triplicates. Equal volumes of Salkowski reagent (prepared by adding 1 mL of 0.5 M
461 ferric chloride to 50 mL of 35 % perchloric acid, mixed well, and stored in a brown bottle) were added
462 to each well. The mixture was incubated in the dark at room temperature for 30 minutes. Blanks were
463 prepared similarly, using only LB medium with 0,1 % tryptophan. Absorbance was measured
464 spectrophotometrically at 536 nm, and IAA concentrations were calculated using a standard curve
465 prepared with concentrations ranging from 10–100 µg/mL of IAA.

466

467

468 **Potato inoculation with *B. subtilis***

469 On day one, *B. subtilis* from permanent culture was plated on a solid LB medium with appropriate (see
470 methods section *B. subtilis* strains) antibiotics (100 µg/mL spectinomycin or 5 µg/mL chloramphenicol)
471 and incubated overnight at 37°C. On day two, a single colony was resuspended in 3 mL of LB medium
472 with antibiotics and grown overnight at 200 rpm and 37°C. On day three at noon, 30 µL of the bacteria
473 culture was inoculated in 3 mL of LB medium without antibiotic and grown for 3 hours at 200 rpm at
474 37°C (OD_{600} between 0.4 and 0.6). Bacterial culture was then diluted in 3 mL of MS30 (5 g of Murashige
475 and Skoog Basal Medium with vitamins and 30 g of sucrose per 1 L of bidistilled water, pH 5.8) medium
476 to OD_{600} 0.02.

477 For studying marker genes expression after potato incubation in bacterial culture of low density, two
478 weeks old potato plants from tissue cultures were added to diluted bacterial culture. Note that diluted
479 bacterial culture was incubated 16 hours at 11 rpm before adding potato plants. Shoots were
480 separated from the culture by parafilm cover and incubated for 21 hours at 11 rpm in a growth
481 chamber prior sampling for quantitative PCR (qPCR) (Exp1 and Exp2 in Figure S2). When studying if the
482 conditioned media triggered any response in treated plants (Table S6), plants were added to a culture
483 (shoots were separated from the culture by parafilm cover) and discarded after 17-hour incubation.
484 New plants were added to conditioned medium and incubated for 26 hours.

485 For studying *B. subtilis* morphology after internalization in plants, two weeks old potato plants in tissue
486 cultures grown in the solid MS30 medium were inoculated with 300 µL of bacterial culture, which was
487 spread on the medium and after a specified time (Figure S4), stem and leaves were observed under
488 confocal microscope.

489 In all other experiments, on day four in the morning (after 16-hour incubation), bacterial cultures were
490 vortexed, except when studying the role of QS and surfactin (Table S5, Table S6). Cultures of different
491 strains were mixed in a ratio of 1:1 when interactions were studied. In 3 mL of bacterial cultures, two-
492 week old potato plants from tissue cultures were added (shoots were separated from the culture by

493 parafilm cover) and incubated for a specified time (15 min to 26 hours), depending on the experiment.
494 When studying *B. subtilis* abundance in shPTI5 transgenic plants with qPCR (Table S9, Table S10), plants
495 were planted in soil after a 2-hour incubation. In all experiments, shoots were not in contact with
496 bacterial culture.

497

498 **Mycorrhizal symbiosis establishment and quantification**

499 The mycorrhizal inoculum was maintained as monoxenic culture consisting in an *in vitro* culture of
500 transformed carrot roots colonized by the arbuscular mycorrhizal fungus *R. irregularis* (MUCL 57021)
501 (REKA Group. B.V., Bleiswijk, The Netherlands), produced and maintained growing in Gel-Gro (ICN
502 Biochemicals, Aurora, OH, USA) as previously described in Chabot et al.[78] Potato plantlets growing in
503 125 mL pots with a mixture of soil, sand and vermiculite (3:2:1, v:v:v) were inoculated by adding a 1
504 cm piece of the monoxenic culture, containing approximately 50 *R. irregularis* spores, fungal hyphae
505 and colonized carrot roots. In the non-inoculated treatment, a piece of medium containing only
506 uninfected carrot roots was applied to the non-mycorrhizal controls. Plants were grown in a growth
507 chamber (day/night cycle: 16 h, 24°C/8 h, 19°C; relative humidity: 50 %). The experiment was repeated
508 twice and eight independent plants per genotype were analyzed.

509 Mycorrhizal colonization of roots and symbiosis establishment was evaluated histochemically and
510 molecularly mycorrhizal structures within the roots were observed and quantified after clearing the
511 roots in 10 % KOH and staining the fungal structures with 5 % black ink in 2 % acetic acid solution as
512 described in García at al.[79] Percentage of mycorrhizal colonization of roots was determined following
513 the gridline intersection method[80] using a Nikon SMZ1000 stereomicroscope. To quantify the total
514 amount of fungus within the root and assess symbiotic functionality we analysed the expression levels
515 of marker genes by qPCR (see below).

516

517

518 **Confocal microscopy**

519 Stellaris 8 Confocal microscope with HC PL FLUOTAR 10x/0.30 DRY objective (Leica Microsystems) was
520 used to detect emission of YFP and mKate2 on the roots of plants in the experiment for investigating
521 timing of biofilm formation, *B. subtilis* morphology after internalization in plants, biofilm formation
522 pattern when studying bacteria–plant social interactions, biofilm presence on the roots of plants
523 sampled for RNA-seq, biofilm formation in transgenic plants with silenced PTI5 and prior all qPCR
524 experiments to confirm roots colonization. Confocal microscope Leica TCS LSI macroscope with Plan
525 APO 5 x objective (Leica Microsystems) was used to detect emission of YFP when investigating biofilm
526 formation on the roots of mutants that have attenuated surfactin production. The emission of YFP was
527 followed after excitation with 488 nm laser in the window 505–560 nm. The emission of mKate was
528 followed after excitation with 589 nm laser in the window between 605–650 nm. Regions of interest
529 (ROI) were scanned unidirectionally with scan speed of 400 Hz and frame average at least 2. The images
530 were processed to obtain maximum projections from Z-stacks for all channels and merged using Leica
531 LAS X software (Leica Microsystems). Mean intensity of fluorescence on confocal images was
532 determined in LAS X software (Leica). Statistically significant differences were determined by Student's
533 t-test.

534

535 **Transmission electron microscopy (TEM)**

536 Samples were prepared for examination with TEM using negative staining method. Pellets of
537 centrifuged liquid culture or leaf homogenate infiltrated with *B. subtilis* were resuspended in 0.1 M
538 phosphate buffer with 2 % polyvinylpyrrolidone. Six µL of resuspension was applied to copper grids
539 with or without glow discharge (400 mesh, formvar-carbon coated) for 5 minutes. Afterwards, the
540 remaining resuspension was dabbed with filter paper. This procedure was repeated two more times.
541 Afterwards, the grids were washed with bidistilled water and stained with one droplet of 1 % (w/v)
542 water solution of uranyl acetate. Two grids were prepared for each sample. The grids were observed

543 by transmission electron microscope TALOS L120 (ThermoFisher SCIENTIFIC, The Netherlands),
544 operating at 100 kV and representative micrographs were acquired (camera Ceta 16M) using Velox
545 software.
546

547 **qPCR analysis**

548 The shoots and elongation zones of roots of *B. subtilis*-inoculated and non-inoculated (see description
549 of inoculation chapter) potato plants were sampled in four or five biological replicates (plants). RNA
550 was isolated by RNeasy Plant Mini Kit (Qiagen) from shoots and RNeasy Plant Mini Kit (Qiagen) or
551 RNeasy Micro Kit (Qiagen) from roots according to the manufacturer's protocols. Concentration and
552 purity of DNase-treated (0.5 µL of DNase I per µg RNA; Qiagen) total RNA was evaluated using
553 NanoDrop ND1000 spectrophotometer (Nanodrop technologies) and agarose gel electrophoresis and
554 then reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher
555 Scientific) or High-Capacity RNA-to-cDNA Kit (Thermo Fisher Scientific). To determine *B. subtilis*
556 abundance in systemic shoot tissue, genomic DNA was isolated by MagMAX™ Plant DNA Isolation Kit
557 (Thermo Fisher Scientific) according to the manufacturer's protocol. Concentration and purity were
558 evaluated as stated above.

559 Samples were analyzed in the setup for qPCR as previously described.[32] The expression of 8 genes
560 (RBOHD, HSP70, PTI5, 13-LOX, PR1B, BGLU2, CPI8, CAB) involved in different steps of immune signaling
561 (Table S13 for a full list of genes and their description) was determined and normalized to the
562 expression of two validated reference genes, COX and elongation factor 1 (EF-1), as described
563 previously.[81] See Lukan et al.[32] for primer and probe information. Relative copy numbers of *comQ* from
564 *B. subtilis* were determined and normalized to relative copy numbers of NPR1 (a single copy gene,
565 https://spuddb.uga.edu/). The standard curve method was used for relative gene expression
566 quantification using quantGenius (http://quantgenius.nib.si).[82]

567 For molecular assessment of mycorrhizal colonization, the expression of the constitutive *R. irregularis*
568 elongation factor 1 (RiEF) gene and the markers of symbiotic functionality, the fungal RiMST2 and the
569 plant StPT4, both involved in the nutrient exchange between the symbionts in the arbuscules were
570 determined in roots by qPCR using gene-specific primers (Table S13). Relative quantification of specific
571 mRNA levels was performed using the comparative 2-Δ(ΔCt) method.[83] Expression values were
572 normalized using the normalizer gene EF-1α encoding the potato translation elongation factor-1α. [84]
573 Five independent biological replicates per treatment were analyzed.
574 Statistical analysis was conducted in R version 4.3.1.[85] Relative gene expression was scaled to the
575 average relative gene expression of the non-inoculated group.[86] Mean, standard deviation, standard
576 error of the mean and 95 % confidence intervals were calculated per gene, tissue type and genotype
577 and visualised using the ggplot2 package v3.3.6.[87] Pairwise Welch's t-test with holm correction for
578 multiple testing adjustment was employed to quantify the difference between the groups. Results
579 were visualised using rstatix v0.7.0[88] and ggpubr v0.4.0[89] R packages.
580
581 **RNA-seq analysis**

582 RNA was isolated from shoots and elongation zones of roots by RNeasy Plant Mini Kit (Qiagen) and
583 treated with RNA Clean & Concentrator-5 (DNase Included) (Zymo Research). In RNA-seq Exp 1, RNA-
584 seq was for roots performed after 2- and 26-hours incubations with *B. subtilis* culture and for shoots
585 after 26 hours of incubation, both for monocultures (PS-216 YFP, PS-218 YFP) and non-inoculated
586 samples (incubation in MS30 medium) as a control. In RNA-seq Exp 2, RNA-seq was for roots performed
587 after 2 hours of incubation with *B. subtilis* culture for monocultures (PS-216 YFP, PS-218 YFP, PS-218
588 mKate2), isogenic mixture (PS-218 mKate2 + PS-218 YFP), non-kin mixture (PS-218 mKate2 + PS-216
589 YFP) and non-inoculated samples (incubation in MS30 medium) as a control. Four biological replicates
590 (plants) were analyzed for each interaction (Table S14).

591 Raw paired-end Illumina reads were adapter-trimmed and reads below average PHRED basecall quality
592 score 20 were removed using Trim Galore (https://github.com/FelixKrueger/TrimGalore).[90]
593 Sequencing quality control was performed using FastQC v0.11.9.[91] Taxonomic classification of reads
594 was performed using Centrifuge v1.0.4[92] in conjunctions with the 2018 nt database. Reads were
595 mapped to *Solanum tuberosum* Phureja clone DM 1-3 genome with STAR v2.7.5c[93] using the merged
596 genome annotations GFF3.[94] Only uniquely mapped fragments were counted (--
597 outFilterMultimapNmax 1) and additional alignment parameters were set to restrict erroneous
598 mapping (--outFilterMismatchNoverReadLmax 0.02, --quantTranscriptomeBan Singleend, --
599 outFilterType BySJout, --alignSJoverhangMin 10, --alignSJDBoverhangMin 1, --alignIntronMin 20, --
600 alignIntronMax 10000, --alignMatesGapMax 10000). Centrifuge and mapping results showed
601 substantial contamination of sample BS472, therefore we excluded this sample from further analysis.

602 Gene Set Enrichment Analysis[95] was performed using MapMan[96] based gene sets (gmt file available at
603 https://github.com/nib-si/ptda)[97] and non-filtered normalized read counts, comparing different
604 Bacillus treatments to non-inoculated plants to identify significantly altered regulation of processes
605 and functionally related gene groups (FDR corrected q-value < 0.10). Results of all GSEA comparisons
606 were merged using R v4.3.1.[85] GSEA figure was generated using adjusted gseaFromStats function from
607 biokit v0.1.1 package.[98]

608 Differential gene expression analysis was performed in R using the *limma* package.[99] Low expressed
609 genes were filtered by retaining only genes with raw read counts above 100 in at least 11 samples for
610 RNA-seq Exp 2 and above 50 counts in at least 4 samples for RNA-seq Exp 1. Root and leaf samples
611 were analyzed separately. Normalized counts were transformed using voom function, followed by
612 linear model fit, and statistics calculation for defined contrasts using the eBayes function. Genes with
613 Benjamini-Hochberg FDR adjusted p-values < 0.05 and $|\log_2 FC| > 1$ were considered significantly
614 differentially expressed. Venn diagrams were plotted using a modified limma::vennDiagram function.

615 Raw and normalized read counts were deposited at GEO under accession number GSE232028. Results
616 of RNA-seq were validated by qPCR (Table S1).

617

618 **Network analysis**

619 Mechanistic Plant Stress Signalling (PSS) (https://skm.nib.si/)[35] reaction network was pre-processed
620 using DiNARs'[100] pre-processing app. Interactions among different molecular entities (such as genes,
621 proteins or transcripts) are defined on functional cluster level, mostly encompassed of *A. thaliana* gene
622 identifiers. Therefore, *Solanum tuberosum* gene identifiers[94] were first translated to Arabidopsis gene
623 identifiers using BLAST+ reciprocal best hit (RBH) search[101] on proteomes (file available at
624 https://github.com/NIB-SI/DiNAR/tree/master/TranslationTables). Top three potato identifiers per
625 Arabidopsis identifier were considered and further prioritised to the functinal cluster using DiNARs'
626 prioritisation script. Experimental data were superimposed onto the PSS network and visualised using
627 DiNAR. Separate condition specific networks, as defined by differential expression contrasts, were
628 exported as individual interactive .html files for further examination.

629

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Supplemental information

Figure S1: Biofilm on potato roots after incubation in *B. subtilis* culture, imaged at different time points.

Figure S2: Expression profiles of selected genes.

Figure S3: Number of genes, regulated by *B. subtilis* in plants incubated in *B. subtilis* culture of high density (107 CFU/m^{L}).

Figure S4: Observation of *B. subtilis* cells in potato tissue after internalization.

Figure S5: B. subtilis cells as observed in liquid culture (A) and potato leaf homogenate (B).

Figure S6: Biofilm formation on potato roots inoculated with *B. subtilis* mutants that have attenuated surfactin production.

Figure S7: *B. subtilis*-produced secondary metabolites in the medium do not contribute significantly to the PTI5 and 13-LOX upregulation.

Figure S8: Regulated central signaling modules in potato roots in isogenic and non-kin interactions.

961 Figure S9: *B. subtilis* root colonization of transgenic potato lines with silenced PTI5 (shPTI5 L2 and L6)
962 and non-transgenic (NT) potato plant.

963 Figure S10: Mycorrhizal colonization of potato roots in non-transgenic potato plant (NT) and PTI5
964 silenced genotype line 2 (shPTI5).

965 Table S1: Standardised qPCR gene expression data.

966 Table S2: Results of gene set enrichment analysis.

967 Table S3: Differential gene expression table for comparisons between plants inoculated with *B. subtilis*
968 and non-inoculated plants.

969 Table S4: The amount of biofilm on potato roots.

970 Table S5: Relative gene expression in potato inoculated with *B. subtilis* mutants that have attenuated
971 surfactin production.

972 Table S6: Expression of selected potato genes induced by conditioned medium in which *B. subtilis* was
973 growing.

974 Table S7: Plant stress signaling (PSS) prior knowledge network node information with corresponding
975 RNA-seq differential expression.

976 Table S8: The amount of biofilm formation on potato roots for non-transgenic (NT) and shPTI5 plants.

977 Table S9: *B. subtilis* abundance in shPTI5 L6 and non-transgenic (NT) potato plants determined by qPCR.

978 Table S10: *B. subtilis* abundance in shPTI5 L2 and non-transgenic (NT) potato plants determined by
979 qPCR.

980 Table S11: Abundance of *B. subtilis* PS-216 and QS impaired strain PS-216 *srfA* in Rywal non-transgenic
981 (NT) potato plants, determined by qPCR.

982 Table S12: The production of indole acetic acid (IAA) was assessed following the protocol of Pramanik
983 et al.[77]

984 Table S13: Selected genes and corresponding primers and probes sequences for expression analyses
985 with quantitative PCR.

986 Table S14: Sample information for RNA-seq experiments.

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