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1 **Siderophore-mediated interactions determine the disease suppressiveness of**

2 **microbial consortia**

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21

ABSTRACT

Interactions between plant pathogens and root-associated microbes play an important role in determining disease outcomes. While several studies have suggested that steering these interactions may improve plant health, such approaches have remained challenging in practice. Because of low iron availability in most soils, competition for iron via secreted siderophore molecules might influence microbial interaction outcomes. Here we tested if bacterial interactions mediated by iron-scavenging siderophores can be used to predict the disease suppressiveness of microbial consortia against soil-borne *Ralstonia solanacearum* bacterial pathogen in tomato rhizosphere. Iron availability significantly affected the interactions within inoculated consortia and between the consortia and the pathogen. We observed contrasting effects of siderophores and other non-siderophore metabolites on the pathogen growth, while the siderophore effects were relatively much stronger. Specifically, disease incidence was reduced *in vivo* when the inoculated consortia produced siderophores that the pathogen could not use for its own growth. Employing siderophore-mediated interactions to engineer functionally robust microbial inoculants show promise in protecting plants from soil-borne pathogens.

IMPORTANCE

Soil-borne pathogens cause high losses of crop yield globally. The development of environmentally friendly approaches is urgently needed but this is still often constrained by complex interactions between root-associated microbes and pathogens. Here we demonstrate that the interactions within microbial consortia mediated by iron-scavenging siderophores play an important role in reducing

45 pathogen infection and enhancing plant health. This study provides a promise and
46 novel research direction for dealing with a wide range of microbial infections, both
47 soil-borne and human-related, through iron exploitation, which is important for the
48 colonization and infection of hosts by many pathogens.

49

50 **INTRODUCTION**

51 Soil-borne pathogens and pests represent a serious threat to agricultural production
52 causing up to 30% yield losses globally (1). One environmentally sustainable way to
53 improve plant health is to take advantage of plant-associated microorganisms that
54 form the first line of defense against pathogens by preventing their growth and
55 subsequent infections (2-5). The functionality of these natural communities is often
56 compromised in agricultural soils due to the extinction of several beneficial strains due
57 to high intensity agricultural practices (6, 7). Restoring microbiome ability to efficiently
58 suppress soil-borne diseases could, therefore, play a central element for future food
59 security. While several attempts have been made to augment microbiome functioning
60 by introducing potentially beneficial strains or consortia into the soil (8-10), the
61 outcomes are still highly variable. One explanation for this may be our poor
62 understanding of the underlying suppressive and facilitative ecological interactions
63 within the rhizosphere, which could limit the success of microbe-mediated
64 manipulations (11-13). In this study, we focused on understanding the role of
65 competition for iron in engineering disease suppressive microbial inoculants against
66 the plant pathogenic *Ralstonia solanacearum* bacterium.

67 The diversity of bacterial communities has been acknowledged to play an

68 important role in the resistance to pathogen infections (13, 14) and this positive
69 relationship is often thought to arise as a result of interactions within the bacterial
70 communities (15-17). Specifically, metabolic interactions and resource competition
71 have been shown to be important in determining pathogen infections in the soil (18,
72 19), and how these interactions take place within the inoculated consortia and
73 between the consortia and the pathogen is often essential for predicting disease
74 outcomes (12). For example, facilitative metabolic interactions within inoculated
75 consortia can potentially promote pathogen growth if it can also use the exchanged
76 metabolites for its growth (12, 20-22). However, if the metabolic interactions are more
77 specific, they might only benefit the members of the inoculated consortia and have no
78 effect, or even negative effects, on the pathogen growth via resource competition.
79 Interference competition via direct antagonism has also been shown important (23).
80 For example, production of secondary metabolites can drive negative interactions
81 between the inoculated strains, but also indirectly inhibit the pathogen if it is
82 susceptible to these metabolites (19, 24-26). Alternatively, these secondary
83 metabolites could be directly targeted towards the pathogen without having any
84 negative effect on the inoculated consortia (27). Together these findings suggest that
85 steering the interactions in microbial communities to suppress soil-borne pathogens
86 requires a profound understanding of the underlying ecological interactions both
87 within the inoculated consortia and between the consortia and the pathogen.

88 One potential way to manipulate microbial interactions could be via the
89 availability of limiting resources, such as carbon, phosphorus and nitrogen that are
90 essential for bacterial growth (13, 28, 29). Here we chose to study the effects of iron
91 as another important limiting resource in mediating microbial competition in the soil.

92 Iron is important for bacterial growth and metabolism (e.g., reduction of oxygen for
93 ATP synthesis and reduction of riboside precursors of DNA) and the demand for iron
94 often exceeds the available iron concentrations in the soil rhizosphere except for highly
95 acidic soils that typically have high ferric solubility (30-32). To capture iron, bacteria
96 either secrete or utilize at least one type of high-affinity iron-chelating compounds
97 called siderophores to compete for iron with other bacterial taxa (31-33). These
98 siderophores can be highly specific and only recognized by the receptors of the
99 siderophore-producing strains creating competition for iron (23, 34). Alternatively,
100 siderophores can be produced as public goods and taken up by other bacteria (31, 35),
101 which could support potential siderophore-mediated facilitation between strains (34,
102 36, 37). Consequently, the strength and type of interactions within bacterial
103 communities might be determined by iron competition which could also affect
104 pathogen growth and disease outcomes. Here we tested if siderophore-mediated
105 interactions between inoculated consortia and the pathogen can be used as an
106 efficient strategy to design suppressive microbial inoculants.

107 To evaluate the validity of such strategy we conducted microcosms experiments
108 where we first designed microbial consortia and measured how they interacted with
109 each other and with the pathogen via siderophores and other secondary (non-
110 siderophore) metabolites (for more details, please see the methods). Furthermore, we
111 used greenhouse experiments to explore how siderophore-mediated interactions
112 within the consortia and between the consortia and the pathogen affected the
113 outcomes of bacterial wilt disease using tomato as a host plant species. In both
114 experiments, we found that siderophores played a key role in shaping the competitive
115 interactions within the consortia and between the consortia and the pathogen, and,

116 that the strength of these interactions could be used to predict pathogen invasion in
117 the tomato rhizosphere. Competition for iron could thus be used as a framework to
118 design suppressive microbial inoculants that provide an efficient and predictable
119 strategy to control *R. solanacearum* disease outbreaks.

120

121 **RESULTS**

122 **Siderophore production by each member of inoculated consortia**

123 Five closely related, but non-pathogenic, *Ralstonia* strains (*Ralstonia mannitolilytica*
124 QL-A2, *Ralstonia mannitolilytica* QL-A3, *Ralstonia pickettii* QL-A6,
125 *Ralstonia taiwanensis* QL-117, and *Ralstonia pickettii* QL-140) were used to construct
126 inoculated consortia. These species were chosen as they often co-occur with the
127 pathogenic *R. solanacearum* strains in China and have been well characterized in our
128 previous studies (13, 28, 29). Siderophore production was indirectly measured using
129 the Chrome Azurol S (CAS) assay, which can reliably provide a relative comparison
130 between different strains and communities (for more details, please see the methods).
131 The mean siderophore production of each inoculated strain grown in monoculture was
132 significantly higher in iron-limited than in iron-rich growth conditions (Fig. S1; $P <$
133 0.001). Compared to the background signal of no siderophore production (7.67
134 µmol/L), all inoculated strains showed lower values under iron-rich conditions (Fig.
135 S1). This suggests that hardly any siderophores were produced when iron was readily
136 available. Siderophore production by the different consortia members span from 3.06
137 µmol/L in iron-rich conditions to 77.95 µmol/L in iron-limited conditions (Fig. S1).
138 Specifically, the strains QL-A6 and QL-140, produced surprisingly high amounts of

139 siderophores in iron-limited conditions (77.95 and 37.81 μmol/L, respectively; Fig. S1).

140

141 **Siderophore and non-siderophore metabolite-mediated effects of inoculated**

142 **consortia members on the pathogen growth**

143 To disentangle the effects of siderophores (S) and non-siderophore metabolites (M) on

144 the pathogen growth, we first determined the combined effects of total metabolites

145 (SM) produced by each consortia member under iron-rich and iron-limited conditions

146 (Fig. 1A and B). While the total metabolites of all five consortia members facilitated

147 pathogen growth under iron-rich conditions (Fig. 1A), their effects on pathogen growth

148 varied from inhibitive to facilitative under iron-limited conditions. More specifically,

149 four out of five consortia members (*Ralstonia mannitolilytica* QL-A2, *Ralstonia*

150 *mannitolilytica* QL-A3, *Ralstonia taiwanensis* QL-117 and *Ralstonia pickettii* QL-140)

151 inhibited, while *Ralstonia pickettii* QL-A6 strain promoted the pathogen growth (Fig.

152 1B and Fig. S2).

153 To determine the effects of non-siderophore metabolites (M), iron chelation was

154 implemented to the previous assays to inactivate all siderophores present in the

155 supernatant. Interestingly, this siderophore inactivation turned all inhibitory

156 interactions into facilitative (Fig. 1C and Fig. S2) and these effects showed similar

157 magnitude compared to the metabolite control treatment (MC), where supernatant

158 was derived from iron-rich growth conditions and thus contained only very little

159 siderophores (Fig. 1A and C and Fig. S2, all $P > 0.05$). To estimate the effects of

160 siderophores only (S), that is siderophore-mediated effects, we subtracted non-

161 siderophore metabolite (M) effects from the combined effects (SM). Our results

162 showed that siderophores-mediated effects by each consortia member mainly
163 inhibited the pathogen growth under iron-limited conditions (Fig. 1D and Fig. S2),
164 which was very similar to the combined effects of the total metabolites (SM) in the
165 same growth conditions (Fig. 1B and D and Fig. S2, Three out of five $P > 0.05$).
166 Altogether these results suggest that siderophore-mediated effects played a key role
167 in triggering pathogen inhibition and although these effects depended on the identity
168 of the consortia members, the magnitude of siderophore-mediated effects were
169 always relatively stronger than non-siderophore metabolite-mediated interactions.

170

171 **Siderophore and non-siderophore metabolite-mediated growth effects between**
172 **inoculated consortia members**

173 We applied the same strategy described previously to disentangle siderophore-
174 mediated effects from non-siderophore metabolite-mediated effects on the growth of
175 each member of the consortia in both iron-rich and iron-limited conditions (Fig. 1A
176 and B). While the total metabolite-mediated effects under iron-rich conditions mostly
177 showed facilitative effects on each strain growth (Fig. 1A, only 4 out of 20 pairwise
178 were inhibitive), they inhibited each other's growth under iron-limited conditions (Fig.
179 1B, only 7 out of 20 pairwise were facilitative). As observed with *R. solanecearum*, all
180 but one consortia member (*Ralstonia mannitolilytica* QL-A2, *Ralstonia mannitolilytica*
181 QL-A3, *Ralstonia taiwanensis* QL-117 and *Ralstonia pickettii* QL-140) mediated
182 inhibitive effects in iron-limited conditions, while *R. pickettii* QL-A6 strain mediated
183 facilitative effects between all consortia members (Fig. 1B and Fig. S3, $P < 0.01$). More
184 specifically, *R. pickettii* QL-140 inhibited all other consortia members, *R.*

185 *mannitolilytica* QL-A2 inhibited all the other consortia members except for *R.*
186 *taiwanensis* QL-117, *R. mannitolilytica* QL-A3 inhibited all other consortia members
187 except for *R. mannitolilytica* QL-A2 and *R. taiwanensis* QL-117, *R. taiwanensis* QL-117
188 inhibited all other consortia members except for *R. pickettii* QL-140 (Fig. 1B and Fig.
189 S3). While the non-siderophore metabolite-mediated facilitative interactions were
190 more common (12 vs. 7, Fig. 1B and C and Fig. S3), interactions between consortia
191 members were similar to the metabolite control treatment (Fig. 1A and C and Fig. S3,
192 only 6 out of 20 pairwise interactions showed slight differences). We also found that
193 siderophore-mediated interactions between consortia members were very similar to
194 the interactions exerted by total metabolites (Fig. 1B and D and Fig. S3, only 5 out of
195 20 pairwise interactions showed slight differences). Altogether, these results suggest
196 that within-consortia interactions were less sensitive to siderophore production
197 compared to consortia-pathogen interactions and that siderophores produced by *R.*
198 *pickettii* QL-A6 may potentially act as public goods.

199

200 **Siderophore and non-siderophore metabolite-mediated effects in pathogen-**
201 **consortia communities**

202 At the whole consortia level, we found that both the direct siderophore effect on
203 pathogen growth ($R^2 = 0.42$, $F_{1,102} = 76.1$, $P < 0.01$, Fig. 2A) and siderophore-mediated
204 interactions within consortia ($R^2 = 0.16$, $F_{1,102} = 20.1$, $P < 0.01$, Fig. 2B) positively
205 correlated with the overall siderophore production. Siderophore-mediated
206 interactions within consortia also positively correlated with siderophore-mediated
207 suppression of the pathogen ($R^2 = 0.17$, $F_{1,102} = 21.7$, P < 0.01, Fig. 2C). However, there

208 was a non-significant relationship between non-siderophore metabolite-mediated
209 interactions within inoculated consortia and between the non-siderophore metabolite
210 effects on the growth of the pathogen (Fig. S4; $P > 0.05$). This suggests that non-
211 siderophore metabolite effects may be different for the whole consortia compared to
212 single inoculant strains as previously observed (Fig. 1C). We further explored the
213 relative importance of community richness and strain identity effects on these
214 relationships. Siderophore production of the inoculant strains increased with
215 increasing consortia richness under iron-limited conditions ($R^2 = 0.06$, $F_{1,122} = 9$, $P <$
216 0.01, Table S1; no relationship observed in iron-rich control conditions). However,
217 strain richness only had a weak linear relationship with the siderophore-mediated
218 pathogen suppression ($R^2 = 0.05$, $F_{1,122} = 7$, $P < 0.01$, Table S2) and non-significant
219 relationship with the siderophore-mediated interactions within inoculated consortia
220 (not retained in the model, Table S3). Instead, we found strong strain identity effects
221 on siderophore production by the consortia. Specifically, the presence of strain *R.*
222 *pickettii* QL-A6 had a significant positive effect ($P<0.001$), strain *R. mannitolilytica* QL-
223 A2 ($P=0.016$) and *R. mannitolilytica* QL-A3 ($P=0.011$) had significant negative effects
224 and strains *R. taiwanensis* QL-117 and *R. pickettii* QL-140 had non-significant neutral
225 effects on siderophore production. In case of pathogen suppression, we found that
226 strains *R. pickettii* QL-A6 and *R. taiwanensis* QL-117 had positive effects and *R.*
227 *mannitolilytica* QL-A2, *R. mannitolilytica* QL-A3 and *R. pickettii* QL-140 negative effects
228 (Table S2, all $P<0.05$). These results suggest that siderophore-mediated effects were
229 always relatively more important than non-siderophore metabolite-mediated effects,
230 and that strain identity effects were stronger compared to consortia richness effects
231 in determining the strength of interactions within the consortia and between the

232 pathogen and consortia.

233

234 **Using siderophore and non-siderophore metabolite-mediated interactions to**
235 **predict disease incidence in tomato rhizosphere**

236 The proportion of wilted plants increased with time during the greenhouse
237 experiment and the disease spread fitted well with a logistic regression (Fig. 3A). While
238 the presence of inoculated consortia significantly decreased the disease incidence,
239 this effect depended on its composition (Fig. 3A; 58% reduction, $F_{1,125}$ = 82, P<0.01).
240 While siderophore production, siderophore effects on pathogen growth and
241 siderophore-mediated interactions within inoculated consortia all significantly
242 explained the disease spread, the relative importance of each factor varied along with
243 the different stages of infection (Fig. 3B-D and Table 1; the effect of consortia strain
244 richness was non-significant: *P*>0.05, Table S4). During the early stage of infection
245 before the disease onset (lag phase), siderophore production by inoculated consortia
246 was the main factor predicting the consortia suppressiveness (R^2 = 0.30, P<0.01, Fig.
247 3B and Table 1). In contrast, during the intermediate disease stage, the siderophore-
248 mediated interactions within inoculated consortia were the most important factor
249 influencing the maximum rate of disease onset (R^2 = 0.27, Fig. 3C and Table 1). During
250 the last disease stage, only the siderophore effect on the pathogen growth significantly
251 explained the proportion of wilted plants (R^2 = 0.08, Fig. 3D and Table 1). Moreover,
252 the effect of siderophore production became non-significant during the intermediate
253 and last disease stages, while siderophore-mediated interactions within inoculated
254 consortia became non-significant during the last disease stage. The non-siderophore

255 metabolite-mediated effects on the pathogen growth and non-siderophore
256 metabolite-mediated interactions within inoculated consortia had non-significant
257 effects on disease incidence (Fig. S5; $P > 0.05$). Furthermore, we found that the strain
258 identity had a strong effect on the lag phase before the disease onset (Table S4,
259 R^2=0.31 and $P<0.01$). Specifically, the presence of strains *R. pickettii* QL-A6 and *R.*
260 *taiwanensis* QL-117 reduced the lag time, strains *R. mannitolilytica* QL-A3 and *R.*
261 *pickettii* QL-140 increased the lag time and the strain *R. mannitolilytica* QL-A2 had non-
262 significant effect (Table S4). Together, these results suggest that siderophore-mediated
263 effects on disease incidence were stronger compared to non-siderophore metabolite-
264 mediated effects, while these effects varied depending on the stage of infection and
265 the presence of certain species in the inoculated consortia.
266

267 **DISCUSSION**

268 Synthetic microbial inoculants have been proposed as a way to suppress pathogens
269 and enhance plant health (38, 39). However the outcomes of such manipulations still
270 vary considerably due to poor establishment of the inoculants, which could be caused
271 by a mismatch in their ability to grow and survive in local abiotic and biotic
272 environmental conditions in the rhizosphere (28, 29). As iron is often an important
273 limiting resource in soil, we hypothesized that bacterial interactions mediated by iron-
274 scavenging siderophores could be used to predict the disease suppressiveness and
275 success of microbial inoculants. We found that siderophores produced by the
276 inoculated consortia played a more important role in the pathogen growth compared
277 to other secondary, non-siderophore metabolites indicative of their importance for

278 the pathogen suppression. Furthermore, while siderophore-mediated interactions
279 and inoculant strain identity effects played an important role in explaining pathogen
280 invasion in the plant rhizosphere, the effects of consortia richness had no effect. As a
281 result, depending on the consortia composition, siderophore-mediated effects could
282 either facilitate or suppress the pathogen growth likely depending on the specificity of
283 siderophores. Our findings hence suggest that siderophore-mediated interactions
284 within inoculated consortia and between the consortia and the pathogen are
285 important in predicting the effect of microbial inoculants on pathogen suppression.

286 Previous studies have showed that both siderophores and antibiotics are
287 important for suppressing the growth of pathogens (40, 41). Our result show that
288 siderophores play a much more important role in mediating effects on *Ralstonia*
289 *solanecearum* growth compared to non-siderophore metabolites and no direct
290 inhibition typical for antibiosis was observed. One potential explanation for these
291 results may be that all the strains included in the consortia belonged to the genus
292 *Ralstonia*, and due to this high relatedness, produced and were resistant to similar
293 antimicrobials (13). Moreover, as iron is essential for microbial metabolism, numerous
294 secondary metabolites such as antibiotics may not be expressed at high level under
295 limiting iron conditions (42) increasing the relative importance of siderophores in such
296 conditions. Furthermore, we have previously found that resource competition is the
297 main factor in mediating the effects between the same consortia members and the
298 pathogen for disease suppression (13, 29, 43). This study thus suggests that
299 interactions between these bacteria could be driven by a combination of iron and
300 carbon competition.

301 Recent studies have shown that siderophores can mediate both antagonistic and

302 facilitative effects in microbial communities depending on whether other microbes
303 have the matching receptors to uptake heterologous siderophores (44-48). In this
304 study, we used a relative comparison of siderophore effects using a well-established
305 CAS assays that indirectly measures the siderophore effects without quantifying the
306 absolute number or type of siderophores. Our results suggest that inoculated strains
307 can either inhibit or promote the pathogen and the resulting disease outbreaks
308 potentially depending on the compatibility of siderophores produced by each strain.
309 Other studies have reported that incompatible siderophores could potentially
310 constrain pathogen infection by reducing available iron in the environment (49-52).
311 Although bacteria belonging to the same genus may share the siderophore as public
312 goods, *e.g. Pseudomonas* (53), strain-specific siderophores may be produced to avoid
313 intra-specific competition and exploitation by social cheats (54, 55). As shown here,
314 even though the consortia members and the pathogen all belonged to the genus
315 *Ralstonia*, all but one strain, *R. pickettii* QL-A6, produced siderophores that had
316 antagonistic effects on the growth of the pathogen. These results thus support the
317 idea that siderophores produced by closely related bacterial strains that potentially
318 share the same ecological niche are likely to be private goods and accessed only by
319 themselves. While the direct purification of siderophores and deciphering of their
320 structural diversity remains challenging (56), in the future it will be important to
321 characterize siderophore-mediated effects observed in this study at the molecular and
322 genome level (51). In addition to simplified lab studies, siderophore production should
323 be quantified *in vivo* in complex rhizosphere bacterial communities and the survival of
324 inoculated strains determined using strains-specific markers (57) to better understand
325 the effects of siderophore-mediated competition in field conditions.

326 As previously demonstrated, interactions within inoculated consortia can reliably
327 predict the likelihood of microbial invasions (12). However, the exact mechanisms
328 driving these interactions between the inoculated consortia and the pathogen often
329 remain unclear. While siderophore-mediated bacterial interactions have been shown
330 to be important in natural environments, and within eukaryotic hosts under iron-
331 limited conditions (46, 58), our findings provide an ecological explanation of how
332 siderophore-mediated bacterial interactions could predict plant disease dynamics in
333 the agricultural context. Specifically, even though most of the pairwise interactions
334 between the consortia members and the pathogen were suppressive, the especially
335 strong facilitative effect by the strain QL-A6 on the pathogen could overshadow the
336 suppressive effects of otherwise suppressive consortia. This result emphasizes the
337 importance of strain identity when choosing strains for microbial inoculants. It is also
338 worth noting that the explanatory power of siderophore-mediated interactions in the
339 rhizosphere was moderate. Considering the complexity of natural systems, and lack of
340 control of several confounding and highly variable factors, this finding demonstrate
341 that siderophore-mediated interactions are likely to be very important for governing
342 microbial interactions in the soil. The predictive power of these analyses could
343 potentially be improved via incorporation of multiple interactions such as different
344 forms of resource competition, siderophore production *in vivo* and inoculant survival
345 success to achieve a more thorough and robust predictive framework for pathogen
346 invasions in complex rhizosphere environments (13, 24).

347 In conclusion, our results suggest that iron-scavenging siderophores can both
348 promote or constrain pathogen invasion depending on the consortia composition,
349 which ultimately determines the net strength and direction of siderophore-mediated

interactions. Hence, direct effects of siderophores on the pathogen growth and the siderophore-mediated interactions within inoculated consortia are both important for predicting pathogen suppression. We hope that these results can be broadly applied to control a wide range of microbial infections, both soil-borne and human-related, as iron exploitation is an important factor for pathogen colonization and infection with many hosts (59-61). In the context of functional microbial inoculant design, we suggest that including strains that trigger strong siderophore-mediated competitive interactions into inoculant consortia is important for reaching high biocontrol efficacy. For example, consortia whose siderophores can be used for the growth of non-pathogenic species, but which are inaccessible to the pathogen, might allow potentially stable coexistence of non-pathogenic competitors and strong continuous suppression of the pathogen.

MATERIALS AND METHODS

Bacterial strains and construction of inoculated consortia

We used *Ralstonia solanacearum* strain QL-Rs1115 (GenBank accession GU390462) tagged with the pYC12-mCherry plasmid(13) as a model pathogen in our study. The inoculated consortia comprised of five closely related *Ralstonia* strains (*Ralstonia mannitolilytica* QL-A2, *Ralstonia mannitolilytica* QL-A3, *Ralstonia pickettii* QL-A6, *Ralstonia taiwanensis* QL-117, and *Ralstonia pickettii* QL-140) that have previously shown to inhibit pathogen growth solely via resource competition without detectable toxin production (13). For the experiments, one colony of each strain, recovered from -80 °C 20 % glycerol stocks, was selected and grown in nutrient broth (NB, glucose 10.0

373 g L^{-1}, tryptone 5.0 g L^{-1}, beef extract 3.0 g L^{-1}, yeast extract 0.5 g L^{-1}, pH 7.0) with 170
374 r.p.m. agitation at 30 °C for 12 h. Bacteria were then washed three times by
375 centrifugation (5000 rpm, 5 min), resuspended in 0.85 % NaCl, and adjusted to a
376 density of 10^7 cells mL^{-1}. Inoculated consortia were constructed by using a full factorial
377 design including all possible strain combinations at one to five strain richness levels (in
378 total of 31 communities) following a substitutive design where all consortia had the
379 same final total bacterial densities (10^7 cells mL^{-1}) and equal ratio of all included strains
380 (12).

381

382 **Determining the siderophore production of the inoculated consortia and consortia**
383 **members**

384 The Chrome Azurol S (CAS) assays (62) were used to measure siderophore production
385 in iron-rich and iron-limited conditions. Even though the CAS assays does not measure
386 the type and absolute amount of produced siderophores, it is well-established method
387 for relative comparisons between different strains and communities (63). To establish
388 a control baseline for no siderophore production in the CAS assay, we used two
389 siderophore-negative mutants (*Pseudomonas aeruginosa* and *Burkholderia*
390 *cenocepacia* strains). These siderophore-negative strains showed a mean siderophore
391 production of 6.67 and 7.67 µM under iron-limited and iron-rich conditions,
392 respectively (63). These values were thus considered as the background signal of no
393 siderophore production in CAS assays in both conditions. Siderophore production of
394 inoculated consortia and defined siderophore-negative mutants of *Pseudomonas*
395 *aeruginosa* and *Burkholderia cenocepacia* strains were tested in MKB medium (K_2HPO_4

396 2.5 g L^{-1}, $MgSO_4 \cdot 7H_2O$ 2.5 g L^{-1}, glycerinum 15 mL L^{-1}, casamino acids 5.0 g L^{-1}, pH 7.2)
397 under both iron-limited and iron-rich conditions. The iron-rich condition was achieved
398 by adding iron (III) solution (1mM $FeCl_3 \cdot 6H_2O$, 10 mM HCl) into MKB medium (Final
399 concentration equaling 50 μM). Each inoculated strain or consortia was grown in both
400 iron-limited and iron-rich MKB medium using 96-well microplate assays. The wells
401 contained a total of 200 μL of liquid consisting of 185 μL of MKB media and 15 μL of
402 inoculum of each constructed consortia (10^7 cells mL^{-1}) and were incubated at 30 °C
403 with 170 r.p.m. orbital agitation for 48 h, which allowed all consortia reach stationary
404 phase. The cell-free supernatant was obtained by centrifugation (10, 000 rpm, 5 min)
405 and filtration (0.22-micron filter) and siderophore production was measured using a
406 universal CAS chemical assay. Briefly, one hundred microliters of each cell-free
407 supernatant, or fresh media as a control, were added to 100 μL CAS assay solution in
408 96-well microplate. After 2 h of static cultivation at room temperature, the optical
409 density (OD_{630}) of cell-free supernatants and uninoculated media controls were
410 measured using a spectrophotometer (SpectraMax M5, Sunnyvale, CA, USA).
411 Siderophore production was estimated using a reference curve based on the
412 relationship between OD_{630} values and known desferoxamine siderophore
413 (EmergenBio) concentrations increasing from 0 to 100 μg ml^{-1}.

414

415 **Testing siderophore effects on pathogen growth and interactions between consortia**
416 **members**

417 **a) Measuring siderophore and non-siderophore metabolite effects on the pathogen**
418 **growth**

419 The *R. solanacearum* pathogen strain QL-Rs1115 was exposed to supernatants
420 produced by all strains and consortia to test the effects of siderophores and other
421 secondary metabolites to its growth (Fig. S6). To disentangle these effects, we used
422 three types of supernatant treatments: (i) strain and consortia were grown in iron-
423 limited MKB medium to trigger siderophore production. This supernatant contained
424 hence both siderophore and non-siderophore metabolites and is henceforth referred
425 as siderophore-metabolite supernatant (SM), (ii) to test the effect of non-siderophore
426 metabolites on the pathogen growth, we treated SM supernatant with 50 μM $FeCl_3$ to
427 chelate produced siderophores via iron-chelation reaction. This supernatant contained
428 thus only non-siderophore secondary metabolites and is henceforth referred as non-
429 siderophore metabolite supernatant (M). Finally, (iii) we also used a metabolite-
430 control supernatant (MC) where we grew individual strains and consortia in iron-rich
431 conditions to obtain supernatant with secondary metabolites but only very little
432 siderophores. This control was used as positive control for iron-chelation treatment.
433 In addition, sterilized water was used as a control instead of the supernatant (C).

434 To test the effect of siderophores and non-siderophore metabolites on pathogen
435 growth in iron-limited conditions, we grew the pathogen in iron-limited medium with
436 supernatant that was derived from iron-limited conditions (i; SM). To test the effect of
437 non-siderophore metabolites in iron-limited conditions, we grew the pathogen in iron-
438 rich medium with supernatant that was derived from iron-limited conditions and
439 'inactivated' with $FeCl_3$ (ii; M). To test the effect of non-siderophore metabolites only
440 in iron-rich conditions, we grew the pathogen in iron-rich medium with supernatant
441 that was derived from iron-rich conditions (iii; MC). All measurements were conducted
442 in 96-well microplates with 180 μL of 10% MKB medium, 20 μL of cell-free consortia

443 supernatants and 2 µL inoculant of overnight pathogen culture (adjusted to OD_{600} =
444 0.5 after 12 h growth at 30°C with shaking). Pathogen-supernatant cultures were
445 incubated with shaking (rotary shaker set at 170 rpm min^{-1}) at 30 °C and change in
446 pathogen density measured as optical density with spectrophotometer after 24h
447 growth ($OD_{600;}$ SpectraMax M5 Plate reader). The effect of each supernatant on the
448 pathogen growth (SNG) was calculated as the relative growth effect (RG) compared to
449 the water-control (CG) treatment as follows: RG = (SNG / CG) - 1) *100, where SNG
450 refers to SM, M and MC supernatants described above. RG values below or above zero
451 thus indicated growth inhibition and facilitation, respectively, and were expressed as
452 percentage fold-changes. To quantify the effects of siderophores only (S), we used an
453 index based on the two supernatant treatments above by subtracting the metabolite
454 effect from the non-siderophore metabolite effects (SM-M = S).
455

456 **b) Determining siderophore and non-siderophore metabolite-mediated pairwise**
457 **interactions between inoculated consortia members**

458 To quantify the strength and direction of each pairwise interaction within consortia,
459 we tested how the supernatant of each member affected the growth of other four
460 consortia members. Briefly, we used the same experimental setup to obtain S, M and
461 SM supernatant as described previously to disentangle siderophore and non-
462 siderophore metabolite effects from each other. For each of the five consortia
463 members, 2 µL of overnight cultures (adjusted to OD_{600} = 0.5 after 12 h growth at 30°C
464 with shaking) were separately added into microplate wells containing 180 µL of 10%
465 MKB medium and 20 µL of cell-free supernatant of the other strains (all pairwise

466 interactions measured individually). The effect of different supernatants on the growth
467 of consortia members was calculated as the relative growth effect compared to the
468 water-control treatment as described previously.

469

470 **c) Determining siderophore and non-siderophore metabolite-mediated growth**
471 **effects by the multi-strain consortia on individual consortia members**

472 To calculate the mean siderophore and non-siderophore metabolite-mediated growth
473 effect by multi-strain consortia on each consortia member, we grew all inoculated
474 strains individually in the presence of supernatant mix produced by all other strains
475 present in every given consortium. For example, in consortia A+B+C, we measured the
476 consortia supernatant effects individually against A, B and C using the same
477 methodology as described previously. These pairwise consortia-consortia member
478 interactions were used to calculate the mean intensity of siderophore-mediated
479 interactions within the consortia as an average of these pairwise interactions.

480

481 **Determining the effects of inoculated consortia on bacterial wilt disease incidence**
482 **in tomato rhizosphere**

483 To quantify the suppressiveness of all inoculated consortia on the pathogen *in vivo*, we
484 used a 49 days long greenhouse experiment with tomato to measure changes in
485 bacterial wilt disease progression in the presence of 31 inoculated consortia (including
486 a positive control containing only the pathogen and a negative control without any
487 bacteria; all treatments were replicated three times). Surface sterilized tomato seeds
488 (*Lycopersicon esculentum*, cultivar 'Jiangshu') were germinated on water-agar plates

489 for three days before sowing into seedling plates containing seedling substrate
490 (Huainong, Huaian soil and Fertilizer Institute, Huaian, China). Tomato plants were
491 transplanted to seedling trays containing the natural soil from a rice field in Wuxi
492 (Jiangsu Province, China) at the three-leaf stage (11 days after sowing) and inoculated
493 with bacterial consortia using drenching method (final concentration of 10^8 cells per g
494 of soil). The pathogen was inoculated to the rhizosphere one week later using the
495 drenching method (final concentration of 10^6 CFU per g soil) and tomato plants were
496 grown for 38 days in a greenhouse with natural temperature variation ranging from 25
497 to 35°C and watered regularly. Seedling plates were arranged in a randomized order
498 and rearranged randomly every 2 days. The number of wilted plants per seedling plate
499 was used to determine disease severity as a disease index on a daily basis 17 days post
500 transplantation after first symptoms became visible.

501

502 **Statistical analyses**

503 General linear mixed models were used to examine the siderophore and non-
504 siderophore metabolite effects on the pathogen and consortia member growth in
505 mono, pairwise and consortia co-cultures. In these analyses, we conducted separate
506 models to explore consortia community richness, composition and strain identity
507 effects (presence and absence of certain strain) on strain interactions. Differences in
508 disease incidence was explained by three quantitative factors that were measured *in*
509 *vitro*: consortia siderophore production, consortia siderophore-mediated effect on the
510 pathogen growth and siderophore-mediated interactions between inoculated
511 consortia members. All variables were fitted as continuous variables and one separate

512 model was used for each variable. All analyses were performed using R 3.3.1 program
513 (www.r-project.org).
514

515 **Data availability**

516 All data has been deposited to Dryad Digital Repository with the following digital
517 identifier: https://doi.org/10.5061/dryad.6djh9w0xw
518

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530

531 **Author contributions**

532 SG, ZW, and YX designed experiments. SG, ZS, KC and XM performed experiments. SG
533 and TY analyzed data. QS, AJ, V-PF, MC and TP contributed intellectual input and

534 helped to interpret data. ZW led the research program. SG, TY, V-PF, ZW, CM and TP
535 wrote the manuscript; all authors discussed the results and commented on the
536 manuscript.

537 **Competing interests**

538 The authors declare no competing interests.

539

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733 **FIG 1 | The interactions mediated by siderophores and non-siderophore metabolites within the**
734 **inoculated consortia and between the inoculated strains and pathogen.** Network diagram showing the
735 strength and directionality of all pairwise interactions mediated by different metabolites between
736 inoculated strains and the effect of inoculated strains on the growth of the pathogen in iron-rich **(A)** and
737 iron-limited **(B)** conditions. The thickness of lines represents the strength of the facilitative or inhibitive
738 relationships and red and blue colors highlight inhibitive and facilitative effects, respectively. Both non-
739 siderophore metabolite **(C)** and siderophore-mediated **(D)** interactions within the inoculated consortia
740 affected the growth of pathogen in iron-limited conditions. Non-siderophore metabolite effects cover
741 the effects of all other secondary metabolites, except for siderophores, produced in the iron-limited
742 supernatant. The abbreviations A2, A3, A6, 117, 140, RS represent the inoculated strains QL-A2, QL-A3,
743 QL-A6, QL-117 and QL-140, respectively.
744

745 **FIG 2 | Siderophore-mediated effects on pathogen growth and interactions between consortia**
746 **members.** The direct siderophore effect on the pathogen growth (**A**) and siderophore-mediated
747 interactions within inoculated consortia (**B**) correlate with siderophore production by the inoculated
748 consortia. Direct siderophore-mediated effects on the pathogen growth correlates with siderophore-
749 mediated interactions within inoculated consortia (**C**). In all panels, each point refers to a defined
750 combination of consortia members.
751

752 **FIG 3 | The suppressiveness of inoculated consortia for the disease incidence progression in tomato**
753 **plant rhizosphere. (A)** Progression of bacterial wilt plant disease in the absence (black line) and
754 presence of inoculated consortia (teal line). Disease spread was fitted with the data using a logistic
755 regression to obtain three variables describing the dynamics of disease: lag time before disease onset
756 (early stage), the maximum rate of disease onset (intermediate stage) and fraction of wilted plants (late
757 stage). **(B-D)** Siderophore production, the direct siderophore effect on the growth of pathogen and
758 siderophore-mediated interactions within inoculated consortia on the disease progression during
759 different stages of infection. In all panels, each point refers to a defined combination of consortia
760 members.
761

762 **TABLE 1 General linear mixed model (GLM) comparing contributions of siderophore production, the**
763 **direct siderophore effect and siderophore-mediated interactions on the dynamics of disease**
764 **progression *in planta*.** All response variables were treated as continuous variables. The table shows the
765 most parsimonious models selected based on the AIC information. The up and downwards arrows
766 denote positive and negative effects on response variables, respectively.

767

768 **FIG S1 Siderophore production of defined siderophore non-producers (deletion mutants – left to the**
769 **vertical line) and inoculated strains in iron-rich (purple, A) and -limited (yellow, B) conditions (right to**
770 **the vertical line) based on CAS assay**. The averaged siderophore production values of the two
771 siderophore-deficient mutants represented a cutoff value (purple and yellow dashed lines) to
772 distinguish background CAS activity from real siderophore production. Data shows the mean of four
773 independent experiments and bars indicate the standard deviation. Different lowercase letters above
774 bars represent significances between strains measured in iron-rich (A) and iron-limited (B) conditions
775 at $P < 0.05$ (Duncan's multiple range test). The differences in siderophore production by the same strain
776 in both iron-rich and iron-limited conditions were determined by paired two-sided Student's *t* test (*P
777 < 0.05, **$P < 0.01$, ***$P < 0.001$; NS represents non-significant difference).

778

779 **FIG S2 Total metabolite (siderophore and non-siderophore) effects of inoculated strains on the**
780 **pathogen growth.** The direct total metabolite effect on the pathogen growth were measured under
781 iron-rich (MC: purple) and iron–limited (SM: yellow) conditions. The non-siderophore metabolite effects
782 (M: cyan-blue) on the pathogen growth under iron-limited condition are expressed as the effect by iron-
783 limited supernatant replenished with excess of iron to chelate produced siderophores in iron-limited
784 conditions. To quantify the effects of siderophores only (S: black), we subtracted the non-siderophore
785 metabolite effect from the total metabolite effect (SM-M = S). Data shows the mean of four
786 independent experiments and error bars indicate the standard deviation. Lowercase letters above each
787 bar represent the significance of each strain's effect under different treatments at $P < 0.05$ (Duncan's
788 multiple range test).

789

790 **FIG S3 Total metabolite (siderophore and non-siderophore) effects of inoculated strains on each other**
791 **growth.** The direct total metabolite effect on the pathogen growth were measured under iron-rich (MC:
792 purple) and iron–limited (SM: yellow) conditions. The non-siderophore metabolite effects (M: cyan-
793 blue) on the pathogen growth under iron-limited condition are expressed as the effect by iron-limited
794 supernatant replenished with excess of iron to chelate produced siderophores in iron-limited
795 conditions. To quantify the effects of siderophores only (S: black), we subtracted the non-siderophore
796 metabolite effect from the total metabolite effect (SM-M = S). Panel **A-E** represent the effect of
797 metabolites produced by QL-A2, QL-A3, QL-A6, QL-117 and QL-140 on each other, respectively. Data
798 shows the mean of four independent experiments and error bars indicate the standard deviation.
799 Lowercase letters above each bar represent the significance of each strain's effect under different
800 treatments at $P < 0.05$ (Duncan's multiple range test).
801
802 **FIG S4 Relationship between non-siderophore metabolite-mediated interactions within inoculated**
803 **consortia and non-siderophore metabolite effects on pathogen growth.** There was no relationship
804 between non-siderophore metabolite-mediated interactions within inoculated consortia and the direct
805 non-siderophore metabolite effect on pathogen growth ($P>0.05$).
806
807 **FIG S5 Determining the suppressiveness of inoculated consortia in tomato rhizosphere. (A-C)** The
808 direct non-siderophore metabolite effect on the growth of pathogen on the disease development
809 during each stage of infection. **(D-F)** The effect of non-siderophore metabolite-mediated interaction
810 within inoculated consortia on the disease development during each stage of infection.
811
812 **FIG S6 Overview of the experimental design used to assess the effect of siderophores produced by**
813 **strains and consortia on the growth of *Ralstonia solanacearum*.** To explore how competition from iron
814 affected interactions between the pathogen and consortia, we grew the pathogen in triplicates in
815 presence of supernatant form each of our consortia. Do determine whether the supernatant-mediated
816 effects on pathogen growth were due to iron competition or to non-siderophore metabolites, we set
817 up three different types of supernatant treatments: (i) strain and consortia were grown in iron-limited
818 MKB medium to trigger siderophore production. This supernatant contained hence both siderophores

819 and non-siderophore metabolites and is henceforth referred as siderophore-metabolite supernatant
820 (SM), (ii) to test the effect of non-siderophore metabolites on the pathogen growth, we treated SM
821 supernatant with 50 μM $FeCl_3$ to chelate produced siderophores via iron-chelation reaction. This
822 supernatant contained thus non-siderophore metabolites and is henceforth referred as non-
823 siderophore metabolite supernatant (M) and (iii) We also used a metabolite-control supernatant (MC)
824 which we grew strain and consortia in iron-rich conditions to obtain supernatant with secondary
825 metabolites but only very little siderophores. This control was used as positive control for iron-chelation
826 treatment. In addition, sterilized water was used as a control instead of the supernatant (C). We then
827 measured the effect of each supernatant on pathogen growth and calculated the net effect of
828 siderophores by subtracting the growth effect of the iron-replenished supernatant (SM) from the
829 growth effect of the iron-limited supernatant (M). The effect of each supernatant on pathogen growth
830 (SNG) was calculated as a relative growth effects (RG) compared to the water- control (CG) treatment
831 as follows: RG=((SNG/CG)-1)*100, where SNG refers to SM, M and MC. Values smaller and greater than
832 zero indicate growth inhibition and facilitation, expressed as percentage fold-change.
833
834 **TABLE S1 General linear mixed model (GLM) analyzing the effects of the strain richness (*Model 1-*
835 ***diversity effects*) and strain identity (*Model 2-identity effects*) on the siderophore production by**
836 **inoculated consortia under iron-limited and iron-rich conditions. In the Identity effect model, the**
837 **effect of each strain was analyzed as the presence vs. absence of each strain within the consortia (as**
838 **a binary predictor).** All response variables were treated as continuous variables and the table shows
839 the most parsimonious models selected based on the AIC information. The significant effects ($P < 0.05$)
840 are highlighted in bold and the up and downwards arrows denote positive and negative effects on
841 response variables, respectively.
842
843 **TABLE S2 General linear mixed model (GLM) analyzing the effects of the strain richness (*Model***
844 ***1-diversity effects*) and strain identity (*Model 2-identity effects*) on the direct siderophore and**
845 **non-siderophore metabolite effects on the growth of pathogen. In the Identity effect model, the**
846 **effect of each strain was analyzed as the presence vs. absence of each strain within the consortia**

847 **(as a binary predictor).** All response variables were treated as continuous variables and the table
848 shows the most parsimonious models selected based on the AIC information. The significant
849 effects (P < 0.05) are highlighted in bold and the up and downwards arrows denote positive and
850 negative effects on response variables, respectively.

851

852 **TABLE S3 General linear mixed model (GLM) analyzing the effects of the strain richness (*Model*
853 ***1-diversity effects*) and strain identity (*Model 2-identity effects*) on the siderophore and non-**
854 **siderophore metabolite-mediated interactions within inoculated consortia. In the Identity effect**
855 **model, the effect of each strain was analyzed as the presence vs. absence of each strain within**
856 **the consortia (as a binary predictor).** All response variables were treated as continuous variables
857 and the table shows the most parsimonious models selected based on the AIC information. The
858 significant effects (P < 0.05) are highlighted in bold and the up and downwards arrows denote
859 positive and negative effects on response variables, respectively.

860

861 **TABLE S4 General linear mixed model (GLM) analyzing the effects of the strain richness (*Model*
862 ***1-diversity effects*) and strain identity (*Model 2-identity effects*) on the dynamics of disease. In**
863 **the Identity effect model, the effect of each strain was analyzed as the presence vs. absence of**
864 **each strain within the consortia (as a binary predictor).** All response variables were treated as
865 continuous variables and the table shows the most parsimonious models selected based on the
866 AIC information. The significant effects (P < 0.05) are highlighted in bold and the up and downwards
867 arrows denote positive and negative effects on response variables, respectively.

868

869