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# Siderophore-mediated interactions determine the disease suppressiveness of microbial consortia

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**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

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

## INTRODUCTION

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

The diversity of bacterial communities has been acknowledged to play an

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

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

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

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

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

## RESULTS

### Siderophore production by each member of inoculated consortia

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

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

**Siderophore and non-siderophore metabolite-mediated effects of inoculated consortia members on the pathogen growth**

To disentangle the effects of siderophores (S) and non-siderophore metabolites (M) on the pathogen growth, we first determined the combined effects of total metabolites (SM) produced by each consortia member under iron-rich and iron-limited conditions (Fig. 1A and B). While the total metabolites of all five consortia members facilitated pathogen growth under iron-rich conditions (Fig. 1A), their effects on pathogen growth varied from inhibitive to facilitative under iron-limited conditions. More specifically, four out of five consortia members (*Ralstonia mannitolilytica* QL-A2, *Ralstonia mannitolilytica* QL-A3, *Ralstonia taiwanensis* QL-117 and *Ralstonia pickettii* QL-140) inhibited, while *Ralstonia pickettii* QL-A6 strain promoted the pathogen growth (Fig. 1B and Fig. S2).

To determine the effects of non-siderophore metabolites (M), iron chelation was implemented to the previous assays to inactivate all siderophores present in the supernatant. Interestingly, this siderophore inactivation turned all inhibitory interactions into facilitative (Fig. 1C and Fig. S2) and these effects showed similar magnitude compared to the metabolite control treatment (MC), where supernatant was derived from iron-rich growth conditions and thus contained only very little siderophores (Fig. 1A and C and Fig. S2, all $P > 0.05$). To estimate the effects of siderophores only (S), that is siderophore-mediated effects, we subtracted non-siderophore metabolite (M) effects from the combined effects (SM). Our results

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

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

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

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

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

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

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

pathogen and consortia.

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

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

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

## DISCUSSION

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

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

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

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.

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

In conclusion, our results suggest that iron-scavenging siderophores can both promote or constrain pathogen invasion depending on the consortia composition, 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

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 r.p.m. agitation at 30 °C for 12 h. Bacteria were then washed three times by centrifugation (5000 rpm, 5 min), resuspended in 0.85 % NaCl, and adjusted to a density of $10^7$ cells $mL^{-1}$. Inoculated consortia were constructed by using a full factorial design including all possible strain combinations at one to five strain richness levels (in total of 31 communities) following a substitutive design where all consortia had the same final total bacterial densities ($10^7$ cells $mL^{-1}$) and equal ratio of all included strains (12).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

**Statistical analyses**

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

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

## Data availability

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


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## Author contributions

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

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

## Competing interests

The authors declare no competing interests.

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

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

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

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

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

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

**FIG S3 Total metabolite (siderophore and non-siderophore) effects of inoculated strains on each other growth.** The direct total metabolite effect on the pathogen growth were measured under iron-rich (MC: purple) and iron–limited (SM: yellow) conditions. The non-siderophore metabolite effects (M: cyan-blue) on the pathogen growth under iron-limited condition are expressed as the effect by iron-limited supernatant replenished with excess of iron to chelate produced siderophores in iron-limited conditions. To quantify the effects of siderophores only (S: black), we subtracted the non-siderophore metabolite effect from the total metabolite effect (SM-M = S). Panel **A-E** represent the effect of metabolites produced by QL-A2, QL-A3, QL-A6, QL-117 and QL-140 on each other, respectively. Data shows the mean of four independent experiments and error bars indicate the standard deviation. Lowercase letters above each bar represent the significance of each strain's effect under different treatments at $P < 0.05$ (Duncan's multiple range test).

**FIG S4 Relationship between non-siderophore metabolite-mediated interactions within inoculated consortia and non-siderophore metabolite effects on pathogen growth.** There was no relationship between non-siderophore metabolite-mediated interactions within inoculated consortia and the direct non-siderophore metabolite effect on pathogen growth ($P>0.05$).

**FIG S5 Determining the suppressiveness of inoculated consortia in tomato rhizosphere. (A-C)** The direct non-siderophore metabolite effect on the growth of pathogen on the disease development during each stage of infection. **(D-F)** The effect of non-siderophore metabolite-mediated interaction within inoculated consortia on the disease development during each stage of infection.

**FIG S6 Overview of the experimental design used to assess the effect of siderophores produced by strains and consortia on the growth of *Ralstonia solanacearum*.** To explore how competition from iron affected interactions between the pathogen and consortia, we grew the pathogen in triplicates in presence of supernatant form each of our consortia. Do determine whether the supernatant-mediated effects on pathogen growth were due to iron competition or to non-siderophore metabolites, we set up three different types of supernatant treatments: (i) strain and consortia were grown in iron-limited MKB medium to trigger siderophore production. This supernatant contained hence both siderophores

and non-siderophore metabolites and is henceforth referred as siderophore-metabolite supernatant (SM), (ii) to test the effect of non-siderophore metabolites on the pathogen growth, we treated SM supernatant with 50 µM $FeCl_3$ to chelate produced siderophores via iron-chelation reaction. This supernatant contained thus non-siderophore metabolites and is henceforth referred as non-siderophore metabolite supernatant (M) and (iii) We also used a metabolite-control supernatant (MC) which we grew strain and consortia in iron-rich conditions to obtain supernatant with secondary metabolites but only very little siderophores. This control was used as positive control for iron-chelation treatment. In addition, sterilized water was used as a control instead of the supernatant (C). We then measured the effect of each supernatant on pathogen growth and calculated the net effect of siderophores by subtracting the growth effect of the iron-replenished supernatant (SM) from the growth effect of the iron-limited supernatant (M). The effect of each supernatant on pathogen growth (SNG) was calculated as a relative growth effects (RG) compared to the water- control (CG) treatment as follows: RG=((SNG/CG)-1)*100, where SNG refers to SM, M and MC. Values smaller and greater than zero indicate growth inhibition and facilitation, expressed as percentage fold-change.

**TABLE S1 General linear mixed model (GLM) analyzing the effects of the strain richness (*Model 1-diversity effects*) and strain identity (*Model 2-identity effects*) on the siderophore production by inoculated consortia under iron-limited and iron-rich conditions. In the Identity effect model, the effect of each strain was analyzed as the presence vs. absence of each strain within the consortia (as a binary predictor).** All response variables were treated as continuous variables and the table shows the most parsimonious models selected based on the AIC information. The significant effects ($P < 0.05$) are highlighted in bold and the up and downwards arrows denote positive and negative effects on response variables, respectively.

**TABLE S2 General linear mixed model (GLM) analyzing the effects of the strain richness (*Model 1-diversity effects*) and strain identity (*Model 2-identity effects*) on the direct siderophore and non-siderophore metabolite effects on the growth of pathogen. In the Identity effect model, the effect of each strain was analyzed as the presence vs. absence of each strain within the consortia**

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

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

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