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Bacteria establish an aqueous living space as a crucial virulence mechanism

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Abstract

High humidity has a profound influence on the development of numerous phyllosphere diseases in crop fields and natural ecosystems, but the molecular basis of this humidity effect is not understood. Previous studies emphasize immune suppression as a key step in bacterial pathogenesis. Here we show that humidity-dependent, pathogen-driven establishment of an aqueous intercellular space (apoplast) is another crucial step in bacterial infection of the phyllosphere. Bacterial effectors, such as *Pseudomonas syringae* HopM1, induce establishment of the aqueous apoplast and are sufficient to transform non-pathogenic *P. syringae* strains into virulent pathogens in immune-deficient Arabidopsis under high humidity. Arabidopsis quadruple mutants simultaneously defective in a host target (MIN7) of HopM1 and in pattern-triggered immunity could not only recapitulate the basic features of bacterial infection, but also exhibit humidity-dependent dyshomeostasis of the endophytic commensal bacterial community in the phyllosphere. These results highlight a new conceptual framework for understanding diverse phyllosphere-bacterial interactions.

Introduction

The terrestrial phyllosphere (the above-ground parts of plants) represents one of the most important habitats on Earth for microbial colonization. Although the vast majority of phyllosphere microbes exhibit benign commensal associations and maintain only modest populations, adapted phyllosphere pathogens can multiply aggressively under favorable environmental conditions and cause devastating diseases. In crop fields, phyllosphere bacterial disease outbreaks typically occur after rainfalls and a period of high humidity[1-3], consistent with the famous "disease triangle" (host-pathogen-environment) dogma formulated more than 50 years ago[4]. The molecular basis of the

55 profound effect of high humidity on bacterial infection of the phyllosphere is not

56 understood.

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58 Many plant and animal pathogenic bacteria, including the model phyllosphere bacterial

59 pathogen *Pseudomonas syringae*, carry a type III secretion system (T3SS), which is

60 used to deliver disease-promoting "effector" proteins into the host cell as a primary

61 mechanism of pathogenesis[5,6]. Studies of how individual type III effectors promote

62 bacterial disease in plants and animals show that effector-mediated suppression of host

63 immunity is a common theme in both plant-bacterial [7-9] and animal-bacterial

64 interactions [10,11]. However, due to the apparent molecular complexities in bacterial

65 diseases, the fundamental question as to what minimal set of host processes that must

66 be subverted to allow basic bacterial pathogenesis to occur has not been answered in

67 any plant or animal pathosystem.

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69 **Immune-suppression and pathogenesis**

70 To test the hypothesis that host immunity may be the only process that needs to be

71 subverted for bacterial pathogenesis in the phyllosphere, we performed infection assays

72 in Arabidopsis polymutants severely defective in multiple immune pathways: (i)

73 *fls2/efr/cerk1* (*fec*), which is mutated in three major pattern recognition receptor (PRR)

74 genes relevant to *P. syringae* pv. *tomato* (*Pst*) DC3000 infection[12], (ii) *bak1-5/bkk1-*

75 *1/cerk1* (*bbc*; see Methods), which is compromised in immune signaling downstream of

76 multiple PRRs[13,14], and (iii) *dde2/ein2/pad4/sid2 (deps),* which is defective in all three

77 major defense hormone pathways (salicylic acid, jasmonate and ethylene)[15]. Two

78 nonpathogenic mutant derivatives of *Pst* DC3000 were used: the *hrcC⁻* mutant

79 (defective in type III secretion)[16] and the DC3000D28E mutant, in which the T3SS

80 remains intact, but 28 of 36 type III effectors are deleted[17]. As shown in Fig. 1a, *hrcC⁻*

81 and DC3000D28E mutants grew very poorly not only in wild-type Col-0, but also in

82 immune-compromised mutants when infiltrated into the apoplast, suggesting that host

83 immunity is unlikely to be the only process subverted by *Pst* DC3000 during infection.

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High humidity required for pathogenesis

During the active pathogenesis phase, phyllosphere bacterial pathogens such as *Pst* DC3000 live mainly in the air-filled apoplast, which is connected directly to open air through epidermal pores called stomata. The water status inside the apoplast could therefore be influenced by air humidity during pathogen infection. In crop fields, phyllosphere bacterial disease outbreaks typically occur after rainfalls and a period of high humidity[1-3,18], following the "disease triangle" dogma in plant pathology. In addition, one of the earliest and common symptoms of phyllosphere bacterial diseases is the appearance of "water soaking" in infected tissues, although whether water-soaking plays an active role in bacterial pathogenesis remains unclear. These key phenomena could be demonstrated in the laboratory. Whereas *Pst* DC3000 multiplied to a very high level under high humidity (~95%; mimicking high humidity after rains in crop fields), it multiplied to a much lower level under low humidity (< 60%) (Fig. 1b), as reflected also in a lower disease severity (Fig. 1c). The ability of *Pst* DC3000 to multiply increased as humidity rose; in contrast, the *hrcC⁻* mutant multiplied poorly under all tested humidity conditions (Fig. 1d). The most aggressive infection by *Pst* DC3000 was associated with the appearance, usually within one day after infection, of water soaking in the infected Arabidopsis leaves under high humidity (Fig. 1e). Water-soaked spots could also be observed in *Pst* DC3000-infected leaves of another host species, tomato (Fig. 1f). Real-time imaging (Supplementary Video 1) showed that the initial water-soaked spots mark the areas of later disease symptoms (necrosis and chlorosis), and revealed, interestingly, that water soaking was a transient process and it disappeared before the onset of late disease symptoms. Using a *Pst* DC3000 strain tagged with a luciferase reporter (DC3000-*lux*[19]), we found that water soaking areas and luciferase signals are detected nonuniformly across the leaf, but they overlap extensively (Fig. 1g, Extended Data Fig. 1), revealing that water-soaked areas are where bacteria multiply aggressively in the phyllosphere before the onset of late disease symptoms.

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P. syirngae water-soaking effectors

The DC3000D28E mutant never caused water soaking under any condition (e.g., high humidity/inoculum). We therefore transformed each of the 28 *Pst* DC3000 effector genes, individually, back to the DC3000D28E mutant to identify the effector(s) that cause water soaking. Most effectors did not (see Fig. 2a for *avrPto*, as an example); but *hopM1* and *avrE* (together with their respective type III secretion chaperone genes *shcM* and *avrF*) did (Fig. 2a). We found this result interesting because, although HopM1 and AvrE show no sequence similarity, they were previously shown to be functionally redundant in virulence and they are highly conserved in diverse *P. syringae* strains and/or other phytopathogenic bacteria[20,21]. Moreover, transgenic overexpression of 6xHis:HopM1[22] or 6xHis:AvrE[23] under control of dexamesathone (DEX)-inducible promoter (10 μM DEX used) also caused water soaking under high humidity (Fig. 2b). In contrast, transgenic expression of AvrPto, like D28E (*avrPto*), did not. These results show that HopM1 and AvrE, either delivered by bacteria or when overexpressed transgenically inside the plant cells, are each sufficient to cause water soaking.

Bacterial mutant analysis showed that HopM1 and AvrE are necessary for *Pst* DC3000 to cause water soaking during infection, as the *avrE⁻/hopM1⁻* double mutant[20] could not cause water soaking, even when the inoculum of the *avrE⁻/hopM1⁻* mutant was adjusted to reach a similar population with *Pst* DC3000 when water soaking was assessed (Fig. 2c). In contrast, *Pst* DC3000 and the *avrE⁻* and *hopM1⁻* single mutants[20] caused strong initial water soaking (Extended Data Fig. 2a) and later disease symptoms (Extended Data Fig. 2b) and multiplied aggressively in a high humidity-dependent manner, while the *avrE⁻/hopM1⁻* double mutant multiplied poorly regardless of the humidity setting (Fig. 2d). Transgenic expression of 6xHis:HopM1 in Arabidopsis (in these experiments 0.1 nM was used to induce low-level expression of HopM1 so that HopM1 alone does not cause extensive water soaking) restored the ability of the *avrE⁻/hopM1⁻* double mutant to cause water soaking and multiply highly under high humidity (Extended Data Fig. 2c, d). These results revealed that, unlike the other 34 effectors present in the

143   *avrE⁻/hopM1⁻* double mutant, the virulence functions of HopM1 and AvrE are uniquely

144   dependent on external high humidity.

145

146   Why would the virulence functions of HopM1 and AvrE be dependent on the external

147   humidity? We hypothesized that perhaps the primary function of HopM1 and AvrE is to

148   create an aqueous apoplast *per se* (i.e., bacteria "prefer" to living in an aqueous

149   environment in the apoplast), the maintenance of which requires high humidity as the

150   leaf apoplast is directly connected to open air through stomata. If so, it may be possible

151   to substitute the function of HopM1 and AvrE by simply providing water to the apoplast.

152   To directly test this hypothesis, we performed transient water supplementation

153   experiments in which Col-0 plants infiltrated with the *avrE⁻/hopM1⁻* mutant were kept

154   water-soaked, transiently, for the first 12 h to 16 h to mimic the kinetics of transient

155   water soaking normally occurring during *Pst* DC3000 infection (Supplementary Video 1).

156   Remarkably, transient apoplast water supplementation was sufficient to restore the

157   multiplication (100- to 1000-fold) of the *avrE⁻/hopM1⁻* mutant almost to the level of *Pst*

158   DC3000 (Fig. 2e), as well as appearance of severe disease symptoms (Fig. 2f). As

159   controls, *Pst* DC3000, the *hrcC⁻* mutant and CUCPB5452 (which contains *avrE* and

160   *hopM1* genes but has much reduced virulence due to deletion of other type III

161   effectors[24]) grew only slightly better (<10 fold) with transient water-supplementation

162   (Fig. 2e). These results demonstrate that the primary virulence function of HopM1 and

163   AvrE can be effectively substituted by supplying water, transiently, to the apoplast.

164

165   **HopM1's host target in water soaking**

166   To investigate the mechanism by which HopM1 creates aqueous apoplast, we focused

167   on the host targets of HopM1 in Arabidopsis. We have previously shown that HopM1 is

168   targeted to the trans-Golgi-network/early endosome (TGN/EE) in the host cell and

169   mediates proteasome-dependent degradation of several host proteins, including MIN7

170   (also known as BEN1), which is a TGN/EE-localized ADP ribosylation factor-guanine

171   nucleotide exchange factor involved in vesicle trafficking[22,25,26]. Although the *min7*

172   mutant plant partially allows increased bacterial multiplication[22,25], the exact role of

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MIN7 during pathogen infection remains enigmatic. A previous study showed that HopM1's virulence function is fundamentally different from that of canonical immune-suppressing effectors, such as AvrPto[17]. In light of our discovery of HopM1's primary role in creating water-soaking in this study, we tested the intriguing possibility that MIN7 may be a key player in modulating apoplast water soaking in response to bacterial infection. Excitingly, we found that the *min7* mutant plant allowed apoplast water soaking to occur in the absence of HopM1/AvrE (i.e., during infection by the *avrE⁻/hopM1⁻* mutant; Fig. 3a, Extended Data Fig. 3c), and allowed the *avrE⁻/hopM1⁻* mutant to multiply (Extended Data Fig. 3a, b). Thus, genetic removal of MIN7 is sufficient to mimic the virulence function of HopM1, albeit partially, in causing apoplast water soaking. The *min7* mutant plant is defective in endocytic recycling of plasma membrane (PM) proteins and has an abnormal PM[26], suggesting that HopM1 degrades MIN7 possibly to compromise host PM integrity as a mechanism to create an infection-promoting aqueous apoplast (Extended Data Fig. 4).

If apoplast water soaking is an essential step of pathogenesis, we hypothesized that plants may have evolved defense mechanisms to counter it. Indeed, we found that *Pst* DC3000 (*avrRpt2*)-triggered effector-triggered immunity (ETI)[27] completely blocked water-soaking, even when the inoculum of *Pst* DC3000 (*avrRpt2*) was raised to reach a population similar to *Pst* DC3000 when water soaking was assessed (Fig. 3b, c, Extended Data Fig. 5a-b). When transferred from high (~95%) to low (~50%) humidity, *Pst* DC3000 (*avrRpt2*)-infected leaves quickly wilted, indicating extensive ETI-associated programmed cell death. In contrast, *Pst* DC3000-infected, water-soaked leaves returned to pre-infection healthy appearance (Fig. 3b), indicating little host cell death during apoplast water soaking. Furthermore, *Pst* DC3000 (*avrRpt2*)-triggered ETI stabilized the MIN7 protein (Fig. 3d). These results therefore uncovered a previously unrecognized battle between bacterial virulence (creating apoplast water soaking) and host defense (preventing apoplast water soaking), in part linked to MIN7 stability, to take control of apoplast water availability.

Reconstitution of *P. syringae* infection

The discovery of apoplast water soaking as a key process of bacterial pathogenesis

prompted us to investigate a new model in which PTI suppression and creation of

apoplast water soaking are two principal pathogenic processes sufficient for bacterial

infection of the phyllosphere. To test this hypothesis, we infected Col-0 and two PTI-

compromised mutant plants (i.e., *fec* and *bbc*) with DC3000D28E, DC3000D28E

(*avrPto*) or DC3000D28E (*hopM1/shcM*) and found that only DC3000D28E

(*hopM1/shcM*), but not DC3000D28E or DC3000D28E (*avrPto*), caused strong water

soaking, multiplied aggressively (almost to the *Pst* DC3000 level) and produced

prominent disease symptoms in the *fec* and *bbc* mutant plants (Fig. 4a-c) in a high

humidity-dependent manner (Fig. 4d). Furthermore, unlike PTI mutants, the *npr1-6*

mutant plant, which is defective in salicylic acid-dependent defense (Extended Data Fig.

6a-c), could not rescue the ability of DC3000D28E (*hopM1/shcM*) to multiply (Fig. 4a).

Thus, a combination of defective PTI and presence of an aqueous-apoplast-inducing

effector (HopM1) could almost fully convert a non-pathogenic mutant into a virulent

pathogen in the Arabidopsis phyllosphere.

If immune suppression and creation of apoplast water soaking are two principal

pathogenic processes sufficient for bacterial infection of the phyllosphere, we reasoned

that we might be able to construct a multi-host-target mutant that simulates the two

processes. Such mutant plant might allow an otherwise nonpathogenic mutant

bacterium (e.g., the *hrcC⁻* mutant) to colonize the phyllosphere, thereby reconstituting

basic features of a phyllosphere bacterial infection. For this purpose, we mutated the

MIN7 gene in PTI mutants (*fec* and *bbc*) and generated *min7/fls2/efr/cerk1* (*mfec*) and

min7/bak1-5/bkk1-1/cerk1 (*mbbc*) quadruple mutants using CRISPR technology (see

Methods; Extended Data Fig. 7a). The quadruple mutant plants display a similar

morphology as wild type Col-0 plants (Extended Data Fig. 7b) and have a tendency of

showing some water-soaking spots, especially in mature leaves, under high humidity

(Extended Data Fig. 7c, d). Excitingly, these mutants allow the nonpathogenic *hrcC⁻*

mutant to multiply aggressively under high (~95%) humidity, to a final population that

233 was ~100 fold higher than in Col-0 plants 5 days after inoculation, with the *mbbc* plants

234 showing a greater susceptibility than the *mfec* plants (Fig. 5a). In addition, in these

235 quadruple mutant plants, the *hrcC⁻* mutant induced prominent disease chlorosis and

236 necrosis (Fig. 5b, Extended Data Fig. 7e), which were not observed for the *hrcC⁻* strain

237 in Col-0, *min7* or PTI mutants. Thus, a dual disruption of MIN7 and PTI signaling is

238 sufficient to reconstitute the basic features of a model phyllosphere bacterial disease.

239 Consistent with this conclusion, transient water supplementation to the leaf apoplast

240 was sufficient to enhance the growth of the *hrcC⁻* mutant in the *bbc* triple mutant, but

241 not in Col-0 plants (Fig. 5c). To our knowledge, this is the first infectious model disease,

242 in plant or animal, for which basic pathogenesis has been reconstituted using

243 biologically relevant host target mutants.

244

Dyshomeostasis of commensal bacteria

246 The inability of the nonpathogenic *hrcC⁻* mutant to multiply aggressively in wild-type

247 phyllosphere resembles that of the commensal bacterial community that resides in the

248 apoplast of healthy leaves. Consistent with this, only low levels of the endophytic

249 phyllosphere bacterial community were detectable in wild type Col-0 plants (Fig. 5d).

250 However, after plants were shifted from regular growth conditions (~60% relative

251 humidity, day 0; Fig. 5d) to high humidity conditions (~95% relative humidity), the

252 *mfec* and *mbbc* quadruple mutant plants, but not Col-0 plants, allowed excessive

253 proliferation of the endogenous endophytic bacterial community (Fig. 5d, Extended

254 Data Table 1), in a high humidity dependent manner (Extended Data Fig. 8a).

255 Furthermore, the excessive proliferation of the endophytic bacterial community was

256 associated with mild tissue chlorosis and necrosis in some leaves (Extended Data Fig.

257 8b). We found this result intriguing as a recent study showed that overgrowth of a

258 beneficial root-colonizing fungus in immune-compromised (against fungal pathogens)

259 plants also led to harmful effects in Arabidopsis[28], illustrating a potentially common

260 theme that the levels of commensal and beneficial microbiota must be strictly controlled

261 by the host for optimal plant health. Future comprehensive *in planta* 16S rRNA

262 amplicon-based analysis will be needed to determine whether there are also humidity-

263 dependent changes in the composition of commensal bacterial communities in the Col-

264 0, *mfec* and *mbbc* plants.

265

266 **Discussion**

267 Results from this study suggest a new conceptual framework for understanding

268 phyllosphere-bacterial interactions (Fig. 5e). Specifically, we have identified PTI

269 signaling and MIN7, presumably via vesicle trafficking, as two key components of the

270 elusive host barrier that functions to limit excessive and potentially harmful proliferation

271 of nonpathogenic microbes (e.g., *hrcC*⁻ mutant) in the phyllosphere. Pathogenic

272 bacteria, like *Pst* DC3000, have evolved T3SS effectors not only to disarm PTI signaling,

273 but also to establish an aqueous living space in a humidity-dependent manner in order

274 to aggressively colonize the phyllosphere. This new conceptual framework integrates

275 host, pathogen and environmental factors, providing a critical insight into the enigmatic

276 basis of the profound effect of humidity on the development of numerous bacterial

277 diseases, consistent with the "disease triangle" dogma in plant pathology.

278

279 Prior to this study, humidity was commonly thought to promote bacterial movements on

280 the plant surface and invasion into plant tissues. Our study, however, revealed a

281 striking and previously unrecognized effect of high humidity on the function of bacterial

282 effectors inside the plant apoplast. An aqueous apoplast could potentially facilitate the

283 flow of nutrients to bacteria, promote the spread/ egression of bacteria, and/or affect

284 apoplastic host defense responses, the latter of which may explain some of the

285 previously observed effects of HopM1, AvrE and MIN7 on plant immunity[21,23,25] and

286 suggest a potential "cross-talk" between plant immune responses and water availability.

287

288 Most of our current knowledge on plant-pathogens and plant-microbiome interactions

289 are derived from studies under limited laboratory conditions. This study illustrates a

290 need for future research to consider the dynamic climate conditions in which plants and

291 microbes live in nature in order to uncover new biological phenomena involved in host-

292 microbe interactions. Research that unravels the molecular bases of environmental

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293 influences of disease development should help us understand the severity, emergence

294 and/or disappearance of infectious diseases in crop fields and natural ecosystems,

295 especially in light of the dramatically changing drought/humidity patterns associated

296 with global climate change.

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Methods

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Plant materials and bacterial strains

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301 *Arabidopsis thaliana* plants were grown in the "Arabidopsis Mix" soil (equal parts of

302 SUREMIX [Michigan Grower Products Inc., Galesburg, MI], medium vermiculate and

303 perlite; autoclaved once) or Redi-Earth soil (Sun Gro® Horticulture) in environmentally-

304 controlled growth chambers, with relative humidity at 60%, temperature at 22 °C and

305 12h light/12h dark cycle. Five-week-old plants were used for bacterial inoculation and

306 disease assays.

307

308 The *bak1-5/bkk1-1/cerk1* mutant plant was generated by crossing the *bak1-5/bkk1-1*

309 mutant[14] with the *cerk1* mutant[29]. PCR-based genotyping was performed in F_2 progeny

310 to obtain a homozygous triple mutant. The *npr1-6* (Fig. 4a) mutant was the

311 SAIL_708_F09 line ordered from the Arabidopsis Biological Resource Center, and

312 confirmed to be a knock-out mutant and defective in SA signaling (Extended Data Fig.

313 6).

314

Bacterial disease assays

315

316 Syringe-infiltration and dip-inoculation were performed. Briefly, *Pst* DC3000 and mutant

317 strains were cultured in Luria-Marine (LM[30]) medium containing 100mg/L rifampicin

318 (and/or other antibiotics if necessary) at 28°C to OD_{600} of 0.8 - 1.0. Bacteria were

319 collected by centrifugation and re-suspended in sterile water. Cell density was adjusted

320 to OD_{600} = 0.2 (~1×10^8 cfu/ml). For syringe-infiltration, bacterial suspension was

321 further diluted to cell densities of 1×10^5 to 1×10^6 cfu/ml. Unless stated otherwise,

infiltrated plants were first kept under ambient humidity for 1-2 h for water to evaporate, and, after the plant leaves returned to pre-infiltration appearance, plants were kept under high humidity (~95%; by covering plants with domes) or other specified humidity settings for disease to develop. For dip-inoculation, plants were dipped in the bacterial suspension of $OD_{600} = 0.2$, with 0.025% Silwet L-77 added, and then kept under high humidity (~95%) immediately for disease to develop.

Different humidity settings were achieved by placing a plastic dome over a flat (in which plants are grown) with different degrees of opening. A humidity/temperature Data Logger (Lascar) was placed inside the flat to record the humidity and/or temperature over the period of disease assay.

For quantification of *Pst* DC3000 bacterial populations, Arabidopsis leaves were surface-sterilized in 75% ethanol and rinsed in sterile water twice. Leaf disks were taken using a cork borer (9.5mm in diameter) and ground in sterile water. Colony-forming units were determined by serial dilutions and plating on LM plates containing 100mg/L rifampicin. Two leaf disks from two leaves were pooled together as one technical replicate, and 4 technical replicates are included in each biological experiment. Experiments were repeated at least three times.

CRISPR-Cas9-mediated mutation of the *MIN7* gene

The one-plasmid CRISPR-Cas9 cloning system[31] was used to mutate *MIN7* in the *fls2/efr/cerk1* and *bak1-5/bkk1-1/cerk1* plants. *MIN7*-sgRNA primers containing target mutation regions were as follows, with *MIN7* sequence underlined.

MIN7-sgRNA-F: GATTG<u>ATCATTTGGAAGGGGATCC</u>

MIN7-sgRNA-R: AAAC<u>GGATCCCCTTCCAAATGAT</u>C

The constructs containing *MIN7*-sgRNA and Cas9 were cloned in pCAMBIA1300, which were then mobilized into *Agrobacterium tumefaciens* for plant transformation. For genotyping of *MIN7*-mutated lines, total DNA was extracted from individual lines and

351 the regions containing the CRISPR target sites were amplified by PCR using the

352 following primers:

353 *MIN7*-sgRNA-F2: GATGCTGCTTTGGATTGTCTTC

354 *MIN7*-sgRNA-R2: AATGGCTCCCCATGCACTGCGATA

355 For genotyping, the PCR products were digested by the *Bam*HI restriction enzyme and

356 plant lines showing an (partially or completely) uncut band were chosen. The PCR

357 products of putative homozygous T_2 lines, identified based on a lack of cutting by

358 *Bam*HI, were sequenced. The lines showing a frame-shift mutation and an absence of

359 *Cas9* gene based on PCR using the following primers were identified as homozygous

360 lines. The T_3 and T_4 progeny of homozygous lines were used for disease assays.

361 Primers for PCR-amplifying *Cas9* gene:

362 *Cas9*-F: CCAGCAAGAAATTCAAGGTGC

363 *Cas9*-R: GCACCAGCTGGATGAACAGCTT

364

Imaging of bacterial colonization with luciferase assay

366 Four-week-old Arabidopsis Col-0 plants were dip-inoculated with *Pst* DC3000 or *Pst*

367 DC3000-*lux* strain. The infected plants were fully covered with plastic dome to maintain

368 high humidity. Leaves were excised from the infected plants 2 days post inoculation and

369 the light signals were captured by a charge-coupled device (CCD) using ChemiDoc[TM] MP

370 system (Bio-Rad).

371

MIN7 protein blot

373 Arabidopsis leaves were syringe-infiltrated with bacteria or H_2O and kept under high

374 humidity (~95%) for 24h. Leaf disks were homogenized in 2xSDS buffer, boiled for 5

375 min and centrifuged at 10,000 x g for 1 min. Supernatants containing the total protein

376 extracts were subjected to separation by SDS-polyacrylamide gel electrophoresis

377 (PAGE). A MIN7 antibody[22] was used in the western blot to detect the MIN7 protein.

378 Uncropped blot/gel images are included in Supplementary Figure 1.

379

Bacterial community quantification

381 Five-week old plants were sprayed with H_2O and covered with a plastic dome to keep

382 high humidity (~95%) for 5 days. To quantify the endophytic bacterial community,

383 leaves were detached, sterilized in 75% ethanol for 1 min (Extended Data Fig. 9) and

384 rinsed in sterile water twice. Leaves were weighed and ground in sterile water using a

385 TissueLyser (Qiagen; at the frequency of 30 times per second for 1 min) in the

386 presence of 3 mm Zirconium oxide grinding beads (Glen Mills; 5 beads in each tube).

387 After serial dilutions, bacterial suspensions were plated on R2A plates, which were kept

388 at 22°C for 4 days before colonies were counted. Colony-forming units were normalized

389 to tissue fresh weight.

390

16S rRNA amplicon sequence analysis of endophytic bacterial community

392 The Col-0, *mfec* and *mbbc* plants were sprayed with water and kept under high

393 humidity (~95%) for 5 days. Leaves were surface-sterilized in 75% ethanol for 1 min

394 and rinsed in sterile water twice. Leaves from four plants were randomly selected (2

395 leaves from each plants; 8 leaves in total) and were divided in 4 tubes (2 leaves in each

396 tube) and ground in sterile water. Bacterial suspensions were diluted (Col-0 samples

397 were diluted to 10^{-3} and *mfec* and *mbbc* samples were diluted to 10^{-5}) and, for each

398 genotype, 15 µl suspension from each tube of the right dilution (10^{-3} dilution for Col-0

399 and 10^{-5} for *mfec* and *mbbc*) were pooled together and plated on R2A plates, which

400 were kept at 22°C for 4 days. Fifty colonies from each genotype were randomly picked

401 and genomic DNA was extracted and PCR was performed with AccuPrime high-fidelity

402 Taq DNA polymerase (Invitrogen) and primers 799F/1392R[33] to amplify bacterial 16S

403 rRNA gene. The PCR product was sequenced and taxonomy of each bacterium (family

404 level) was determined by Ribosomal Database Project at Michigan State University

405 (https://rdp.cme.msu.edu/)[34].

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Data analysis, statistics and experimental repeats

The specific statistical method used, the sample size and the results of statistical analyses are described in the relevant figure legends. Sample size was determined based on experimental trials and in consideration of previous publications on similar experiments to allow for confident statistical analyses. The Student's two-tailed t-test was performed for comparison of means between two data points. One-way or two-way ANOVA with Tukey's test was used for multiple comparisons within a dataset, with p value set at 0.05. ANOVA analysis was performed with the GraphPad Prism software.

Data Availability

The bacterial 16S rRNA sequences in Extended Data Table 1 have been deposited in the National Center for Biotechnology Information (NCBI) GenBank database under accession numbers KX959313-KX959462. Other data that support the findings of this study are available from the corresponding author upon request.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Author Contributions

X-F.X, K.N, and S.Y.H designed the experiments. K.A performed the *Pst* DC3000-*lux* imaging experiment. A.C.V performed biological repeats of bacterial infection experiments shown in Fig. 1a. J.Y characterized an unpublished plant mutant line. X-F.X and K.N performed all other experiments, including bacterial infections, protein blotting

527 and generation of Arabidopsis *mfec* and *mbbc* mutant lines. F.B and C.Z contributed

528 unpublished plant mutant materials. J.H.C contributed unpublished *Pst* DC3000 effector

529 constructs. X-F.X and S.Y.H wrote the manuscript with input from all co-authors.

530

Author Information

536 **Figure 1:** Full-scale *Pst* DC3000 infection requires high humidity and is tightly

537 associated with apoplast "water soaking". See Methods for syringe-infiltration or dip-

538 inoculation of plants described in all figures. **a,** Bacterial populations in Col-0,

539 *fls2/efr/cerk1 (fec)*, *bak1-5/bkk1-1/cerk1 (bbc)* and *dde2/ein2/pad4/sid2 (deps)* leaves

540 2 days post infiltration with bacteria at 1x10^6 cfu/ml. Humidity: ~95%. Two-way ANOVA

541 with Tukey's test (p value set at 0.05) was performed. No significant differences were

542 found for DC3000 populations in different plant genotypes (indicated by the same letter

543 a), whereas differences were found for *hrcC* or DC3000D28E populations in different

544 plant genotypes, as indicated by different letters of the same type (a' vs. b' for *hrcC*

545 and a" vs. b" for DC3000D28E). *n*=4 technical replicates; error bars, mean$\pm$s.d.

546 Experiments were repeated three times with similar results. **b-c,** Bacterial populations

547 **(b)** and disease symptoms **(c)** 3 days post infiltration with *Pst* DC3000 at 1x10^5 cfu/ml.

548 * indicates a significant difference determined by Student's *t*-test (two-tailed); ***,

549 p=1.08x10^{-6}. *n*=4 technical replicates; error bars, mean$\pm$s.d. Experiments were

550 repeated four times with similar results. **d**, Bacterial populations in Col-0 leaves 3 days

551 post infiltration with bacteria at 1x10^5 cfu/ml. Statistical analysis was the same as in **a**.

552 Significant differences were found for DC3000 populations under different humidities, as

553 indicated by different letters (a, b, c and d). No significant differences were found in

554 *hrcC* populations (indicated by the same letter a'). *n*=3 technical replicates; error bars,

555 mean$\pm$s.d. Experiments were repeated three times with similar results. **e,** Pictures of

556 the abaxial sides of Col-0 leaves 24 h post infiltration with *Pst* DC3000 at 1x10^6 cfu/ml.

557 Humidity: ~95%. Dark spots on the leaf indicate water soaking spots. Red boxes

558 indicate "zoomed-in" regions. **f,** Picture of a tomato leaf (cv. Castle Mart) 3 days after

559 infiltration with *Pst* DC3000 at 1x10^4 cfu/ml. Humidity: ~95%. Yellow circles in **e** and **f**

560 indicate infiltration sites. Images were representative of water-soaked leaves from more

561 than four plants. **g,** Col-0 plants were dip-inoculated with bacteria at 2x10^8 cfu/ml.

562 Humidity: ~95%. Bacterial colonies in inoculated leaves were visualized 2 days later by

563 a charge-coupled device (upper panel) and pictures of leaves were taken to show water

564 soaking spots (middle panel). Bottom panel shows merged images, with the artificial

565 red color labeling *Pst* DC3000-*lux* bacteria. Experiments were repeated three times.

566 Images were representative of leaves from more than four plants.

567

568 **Figure 2:** Type III effectors AvrE and HopM1 are necessary and sufficient to cause

569 water soaking. **a,** Pictures of Col-0 leaves 24 h post infiltration with bacteria (1-2x10^8

570 cfu/ml). Humidity: ~95%. **b,** Pictures of leaves of transgenic 6xHis:HopM1[22],

571 6xHis:AvrE[23] or AvrPto[32] plants after spray with 10µM dexamethasone (DEX; to induce

572 effector gene expression). Humidity: ~95%. Col-0 or Col-0 *gl* plants were non-

573 transgenic parental controls. Images were representative of leaves from more than four

574 plants. **c,** Pictures of Col-0 leaves (left) and bacterial populations (right) 24 h post

575 infiltration with *Pst* DC3000 (1x10^6 cfu/ml) or the *avrE⁻/hopM1⁻* strain (1x10^7 cfu/ml).

576 Humidity: ~95%. Student's *t*-test (two-tailed) was performed; ns, not significant

577 (p=0.104). *n*=3 biological replicates; error bars, mean±s.d. Experiments were repeated

578 three times. **d,** Bacterial populations in Col-0 plants 3 days post infiltration with bacteria

579 at 2x10^5 cfu/ml. *** indicates a significant difference (p=1.07x10^{-6}, 8.07x10^{-7} and

580 5.95x10^{-7} for DC3000, the *avrE⁻* mutant and the *hopM1⁻* mutant, respectively) of

581 bacterial population between different humidities, as determined by Student's *t*-test

582 (two-tailed); ns, not significant (p=0.13). *n*=4 technical replicates; error bars,

583 mean±s.d. Experiments were repeated three times. **e-f,** Bacterial populations **(e)** and

584 leaf pictures **(f)** in Col-0 leaves 3 days post infiltration with bacteria at 1x10^5 cfu/ml. In

585 the "- H$_2$O" treatment, plants were air-dried normally (for ~2 h) and then kept under

586 high humidity (~95%). In the "+H$_2$O" treatment, plants were kept under high (80-

587 95%) humidity after syringe-infiltration to allow slow evaporation of water (for ~16 h,

588 until no visible apoplast water can be seen). ** (p=8.29x10^{-3} and 1.14x10^{-3} for DC3000

589 and *hrcC⁻*, respectively) and *** (p=7.61x10^{-7} and 9.82x10^{-4} for *avrE⁻/hopM1⁻* and

590 CUCPB5452, respectively) indicate significant differences between "- H$_2$O" and "+H$_2$O"

591 treatments as determined by Student's *t*-test (two-tailed). *n*=3 technical replicates;

592 error bars, mean±s.d. Experiments were repeated three times.

593

594 **Figure 3:** Effects of MIN7 and effector-triggered immunity on water soaking. **a,** The

595 *min7* leaves, but not Col-0 leaves, showed partial water soaking 48 h after dip-

596 inoculation with the *avrE⁻/hopM1⁻* mutant at 1×10^8 cfu/ml. Humidity: ~95%. Water

597 soaking disappeared after transition to low humidity (~25%) to allow evaporation of

598 apoplast water. Images were representative of leaves from more than four plants. **b-c,**

599 ETI blocks apoplast water soaking. Col-0 and *rps2* leaves were infiltrated with

600 *Pst* DC3000 (1×10^6 cfu/ml) or *Pst* DC3000 (*avrRpt2*) (1×10^7 cfu/ml for Col-0 and 1×10^6

601 cfu/ml for *rps2* plants). Plants were kept under high humidity (~95%) for 24 h to

602 observe water soaking and then shifted to low humidity (~50%) for 4 h to observe ETI-

603 associated tissue collapse. Pictures were taken before and after low humidity exposure

604 (**b**) and bacterial populations were determined 24 h post infiltration to show similar

605 population levels (**c**). Statistical analysis of data in **c** was performed by one-way ANOVA

606 with Tukey's test (p value set at 0.05), and no significant difference was detected. *n*=3

607 technical replicates; error bars, mean±s.d. Experiments were repeated three times. **d,**

608 MIN7 protein is stabilized during ETI revealed by immunoblot. Col-0 or *min7* leaves

609 were infiltrated with bacteria (1×10^7 cfu/ml[25]) or H_2O and kept under high humidity

610 (~95%) for 24 h before protein extraction. Asterisk indicates a non-specific band.

611 Coomassie blue staining shows equal loading. See Supplementary Figure 1 for cropping.

612 **Figure 4:** *hopM1/shcM* transform the non-pathogenic DC3000D28E mutant into a

613 highly virulent pathogen in PTI-deficient mutant plants in a humidity-dependent

614 manner. **a-c,** Bacterial populations **(a)** and disease symptoms **(b)** 3 days post

615 infiltration with bacteria indicated at 1×10^6 cfu/ml. Humidity: ~95%. Statistical analysis

616 was performed by one-way ANOVA with Tukey's test (p value set at 0.05). Bacterial

617 populations indicated by different letters (i.e., a, b and c) are significantly different (ab

618 is not significantly different from a or b). *n*=4 technical replicates; error bars,

619 mean±s.d. Experiments were repeated three times. Water-soaking symptom was

620 recorded 24 h post inoculation **(c). d,** Bacterial populations 3 days post infiltration with

621 DC3000D28E (*hopM1/shcM*) at 1×10^6 cfu/ml under indicated humidities. Statistical

622 analysis was the same as in **(a)**. Bacterial populations indicated by different letters (i.e.,

623 a, b and c) are significantly different. $n=4$ technical replicates; error bars, mean$\pm$s.d.

624 Experiments were repeated three times. Images were representative of leaves from at

625 least four plants.

626 **Figure 5:** Disease reconstitution experiments. **a-b,** The $hrcC^-$ bacterial populations 5

627 days **(a)** and disease symptoms 10 days post dip-inoculation **(b)** in Col-0, *fec, bbc,*

628 *min7, min7/fls2/efr/cerk1* (*mfec*) and *min7/bak1-5/bkk1-1/cerk1* (*mbbc*) plants.

629 Humidity: ~95%. Statistical analysis was performed by one-way ANOVA with Tukey's

630 test (p value set at 0.05). Bacterial populations indicated by different letters (i.e., a, b, c

631 and d) are significantly different (ad is not significantly different from a or d). $n=4$

632 technical replicates; error bars, mean$\pm$s.d. Experiments were repeated four times. **c,**

633 The $hrcC^-$ bacterial populations in Col-0 and *bbc* leaves 3 days post infiltration with

634 bacteria at 1×10^6 cfu/ml. The "- H$_2$O" and "+ H$_2$O" conditions are the same as in Fig.

635 2e. Statistical analysis was performed by one-way ANOVA with Tukey's test (p value set

636 at 0.05). Bacterial populations indicated by different letters (i.e., a, b and c) are

637 significantly different (ab is not significantly different from a or b). $n=3$ technical

638 replicates; error bars, mean$\pm$s.d. Experiments were repeated three times. **d,** The Col-0,

639 *fec, bbc, min7, mfec* and *mbbc* plants were mock-sprayed with H$_2$O and kept under

640 high humidity (~95%). On day 0 (before water spray) and day 5, total populations of

641 the endophytic bacterial community were quantified by counting colony-forming units

642 on R2A plates, after surface sterilization of leaves with 75% ethanol, leaf

643 homogenization and serial dilutions. Statistical analysis is the same as in **(a)**. Bacterial

644 populations indicated by different letters (i.e., a and b) are significantly different. $n=4$

645 technical replicates; error bars, mean$\pm$s.d. Experiments were repeated three times. **e,** A

646 new model for *Pst* DC3000 pathogenesis in Arabidopsis. Dashed arrows indicate a

647 possible interplay, at spatial and temporal scales, between "immune suppression" and

648 "wet apoplast" during pathogenesis.

Extended Data Table 1: Endophytic bacterial taxa in Col-0, *mfec* and *mbbc* plants. *, not detected (nd). See the Methods section for 16S rRNA amplicon sequencing procedures.

Extended Data Figure 1: Water soaking does not affect luminescence signal. Col-0 plants were dip-inoculated with bacteria at 2×10^8 cfu/ml, and kept under high humidity (~95%) for 2 days. Imaging was performed in the same way as in Fig. 1g. Water-soaked leaves were air-dried for about 2 h and imaged again (right panel). Images were representative of leaves from more than four plants.

Extended Data Figure 2: a-b, The virulence of the *avrE⁻/hopM1⁻* mutant is insensitive to humidity settings. **a,** Col-0 plants were syringe-infiltrated with indicated bacteria at 2×10^5 cfu/ml. Inoculated plants were kept under high (~95%) humidity, and pictures were taken 24 h post infiltration. **b,** Col-0 plants were syringe-infiltrated with *Pst* DC3000, the *avrE⁻* mutant, the *hopM1⁻* mutant or the *avrE⁻/hopM1⁻* mutant at 2×10^5 cfu/ml. Inoculated plants were kept under high (~95%) or low (20-40%) humidity. Pictures were taken 3 days post inoculation. Images were representative of leaves from more than four plants. **c-d,** The 6xHis:HopM1 transgenic plants were infiltrated with 0.1 nM DEX, the *avrE⁻/hopM1⁻* mutant (at 1×10^5 cfu/ml) or both. H_2O was infiltrated as control. Infiltrated plants were kept at high humidity (~95%). Leaf pictures were taken 24 h post infiltration (**c**) and bacterial populations were determined 3 days post infiltration (**d**). * indicates a significant difference, as determined by Student's *t*-test; (two-tailed); ***, $p=1.03\times10^{-5}$. *n*=6 technical replicates from there independent experiments (n=2 in each experiment); error bars, mean±s.d.

Extended Data Figure 3: Bacterial multiplication and water soaking in Col-0 and the *min7* mutant. **a,** The Col-0 and *min7* plants were dip-inoculated with *Pst* DC3000, the *avrE⁻/hopM1⁻* mutant or the *hrcC⁻* mutant at 1×10^8 cfu/ml. Bacterial populations were determined 4 days post inoculation. * indicates a significant difference between Col-0 and *min7* plants, as determined by Student's *t*-test (two-tailed); *, $p=1.61\times10^{-2}$ and

678   3.12×10^{-2} for DC3000 and $hrcC^-$, respectively; ***, p=1.41×10^{-4} for $avrE^-/hopM1^-$. n=4

679   technical replicates; error bars, mean$\pm$s.d. Experiments were repeated three times. **b-**

680   **c,** The Col-0 and *min7* plants were syringe-infiltrated with *Pst* DC3000, the *avrE*

681   */hopM1* mutant or the $hrcC^-$ mutant at 1×10^6 cfu/ml. Bacterial populations were

682   determined 3 days post inoculation (**b**) and leaf pictures were taken 38 h after

683   infiltration to show water soaking in *min7* leaves (**c**). * indicates a significant difference

684   between Col-0 and *min7* plants, as determined by Student's *t*-test (two-tailed); **,

685   p=1.63×10^{-3} for $avrE^-/hopM1^-$; ns, not significant (p=0.72 and 0.14 for DC3000 and

686    $hrcC^-$, respectively). n=3 technical replicates; error bars, mean$\pm$s.d. Experiments were

687   repeated three times. Images were representative of leaves from more than four

688   plants.

689

690   **Extended Data Figure 4:** *Pst* DC3000 delivers a total of 36 effectors into the plant

691   cell. Many effectors, including AvrPto, appear to suppress pattern-triggered immunity

692   (PTI). AvrPto inhibits pattern recognition receptor (PRR) function[8]. Two conserved

693   effectors, HopM1 and AvrE, create an aqueous apoplast in a humidity-dependent

694   manner. AvrE is localized to the host plasma membrane (PM)[23]; its host target is

695   currently unknown. HopM1 targets MIN7 (an ARF-GEF protein) in the trans-Golgi-

696   network/early endosome (TGN/EE), which is involved in recycling of PM proteins[26].

697

698   **Extended Data Figure 5: a,** Col-0 leaves were syringe-infiltrated with *Pst* DC3000

699   (1×10^6 cfu/ml) or *Pst* DC3000 (*avrRpt2*) (1×10^7 cfu/ml). Plants were kept under high

700   humidity (~95%) for 24 h to observe water soaking and then shifted to low humidity

701   (~25%) for 2 h to observe ETI-associated tissue collapse. Pictures were taken before

702   and after low humidity exposure (**a**) and bacterial populations were determined 24 h

703   post infiltration to show similar population levels (**b**). * indicates a significant difference

704   of bacterial population, as determined by Student's *t*-test (two-tailed); *, p=0.033. n=3

705   technical replicates; error bars, mean$\pm$s.d. Experiments were repeated three times. This

706   is an experimental replicate of Fig. 3**b** and 3**c** (without *rps2*).

707

708 **Extended Data Figure 6:** Characterization of the *npr1-6* mutant. **a,** A diagram

709 showing the T-DNA insertion site in the *npr1-6* mutant. Blue boxes indicate exons in the

710 *NPR1* gene. **b,** RT-PCR results showing that the *npr1-6* line cannot produce the full-

711 length *NPR1* transcript. Primers used (*NPR1* sequence is underlined): *NPR1*-F:

712 agaattc<u>ATGGACACCACCATTGATGGA</u>; *NPR1*-R: agtcgac<u>CCGACGACGATGAGAGARTTTAC</u>;

713 *UBC21*-F: TCAAATGGACCGCTCTTATC; *UBC21*-R: TCAAATGGACCGCTCTTATC.

714 Uncropped gel images are included in Supplementary Figure 1. **c,** The *npr1-6* line,

715 similar to *npr1-1,* is greatly compromised in benzothiadiazole (BTH)-mediated resistance

716 to *Pst* DC3000 infection. The Col-0, *npr1-1* and *npr1-6* plants were sprayed with 100μM

717 BTH and, 24 h later, dip-inoculated with *Pst* DC3000 at 1x10^8 cfu/ml. Bacterial

718 populations were determined 3 days post inoculation. * indicates a significant difference

719 between mock and BTH treatment, as determined by Student's *t*-test (two-tailed); *,

720 p=0.027; ***, p=1.6x10^{-4}; ns, not significant (p=0.19). *n*=3 technical replicates; error

721 bars, mean$\pm$s.d. Experiments were repeated three times.

722 **Extended Data Figure 7:** Construction and characterization of the *mfec* and *mbbc*

723 quadruple mutants. **a,** CRISPR-Cas9-mediated mutations in the 4th exon of the *MIN7*

724 gene (exons indicated by blue boxes) in the quadruple mutant lines used in this study.

725 The underlined sequence in the wild type (WT) indicates the region targeted by sgRNA.

726 The number "399" indicates the nucleotide position in the *MIN7* coding sequence. "+1"

727 and -1" indicate frame shifts in the mutant lines. **b,** Col-0 and various mutants used in

728 this study have similar growth, development and morphology. Four-week-old plants are

729 shown. **c**, The *mfec* and *mbbc* plants show a tendency of developing sporadic water

730 soaking under high humidity. Five-week-old regularly-grown (~60% relative humidity)

731 Col-0, *mfec* and *mbbc* plants were shifted to high humidity (~95%) for overnight and

732 pictures of mature leaves were taken after high humidity incubation. **d**, Even leaves of

733 *mfec* and *mbbc* plants that do not have sporadic water-soaking have a tendency to

734 develop some water soaking after *hrcC*⁻ inoculation. Five-week old Col-0, *mfec* and

735 *mbbc* plants were dip-inoculated with *hrcC*⁻ at 1x10^8 cfu/ml, and kept under high

736 humidity (~95%). Leaf pictures were taken 2 days post inoculation. Images were

737 representative of leaves from at least four plants. **e**, The non-pathogenic *hrcC⁻* mutant

738 causes significant necrosis and chlorosis in the quadruple mutant plants. Col-0, *mfec*

739 and *mbbc* plants were dip-inoculated with the *hrcC⁻* strain at 1x10^8 cfu/ml. Pictures

740 were taken 9 days post inoculation. This is one of the four independent experimental

741 repeats of the results presented in Fig. 5b.

742 **Extended Data Fig. 8: a**, Increased endophytic bacterial community in the *mfec* and

743 *mbbc* plants depend on high humidity. Col-0, *mfec* and *mbbc* plants were either

744 sprayed with H$_2$O and kept under high humidity (~95%) or kept under low humidity

745 (~50%). On day 5, total populations of the endophytic bacterial community were

746 quantified. Statistical analysis was performed by one-way ANOVA with Tukey's test (p

747 value set at 0.05). Bacterial populations indicated by different letters (i.e., a and b) are

748 significantly different. *n*=4 technical replicates; error bars, mean±s.d. Experiments were

749 repeated three times. **b,** Mild chlorosis and necrosis in leaves is associated with

750 increased endophytic bacterial community level in the *mfec* and *mbbc* quadruple mutant

751 plants. Plants were sprayed with H$_2$O and kept under high (~95%) humidity. Pictures

752 were taken 10 days after spray. Individual leaves are enlarged and shown in the lower

753 panel, showing mild chlorosis and necrosis in some of the *mfec* and *mbbc* leaves.

754 **Extended Data Fig. 9:** Validation of 1 min as an effective surface sterilization time.

755 Five-week old Col-0 plants were sprayed with H$_2$O and kept under high humidity

756 (~95%) for 5 days. Leaves were detached, surface sterilized in 75% ethanol for 20s,

757 40s, 1min or 2min and then rinsed in sterile water twice. No sterilization (0s) was used

758 as control. Leaves were ground in sterile water and bacterial numbers were determined

759 by serial dilutions and counting of colony-forming units on R2A plates. Statistical

760 analysis was performed by one-way ANOVA with Tukey's test (p value set at 0.05).

761 Bacterial populations indicated by different letters (i.e., a and b) are significantly

762 different. *n*=4 technical replicates; error bars, mean±s.d. Experiments were repeated
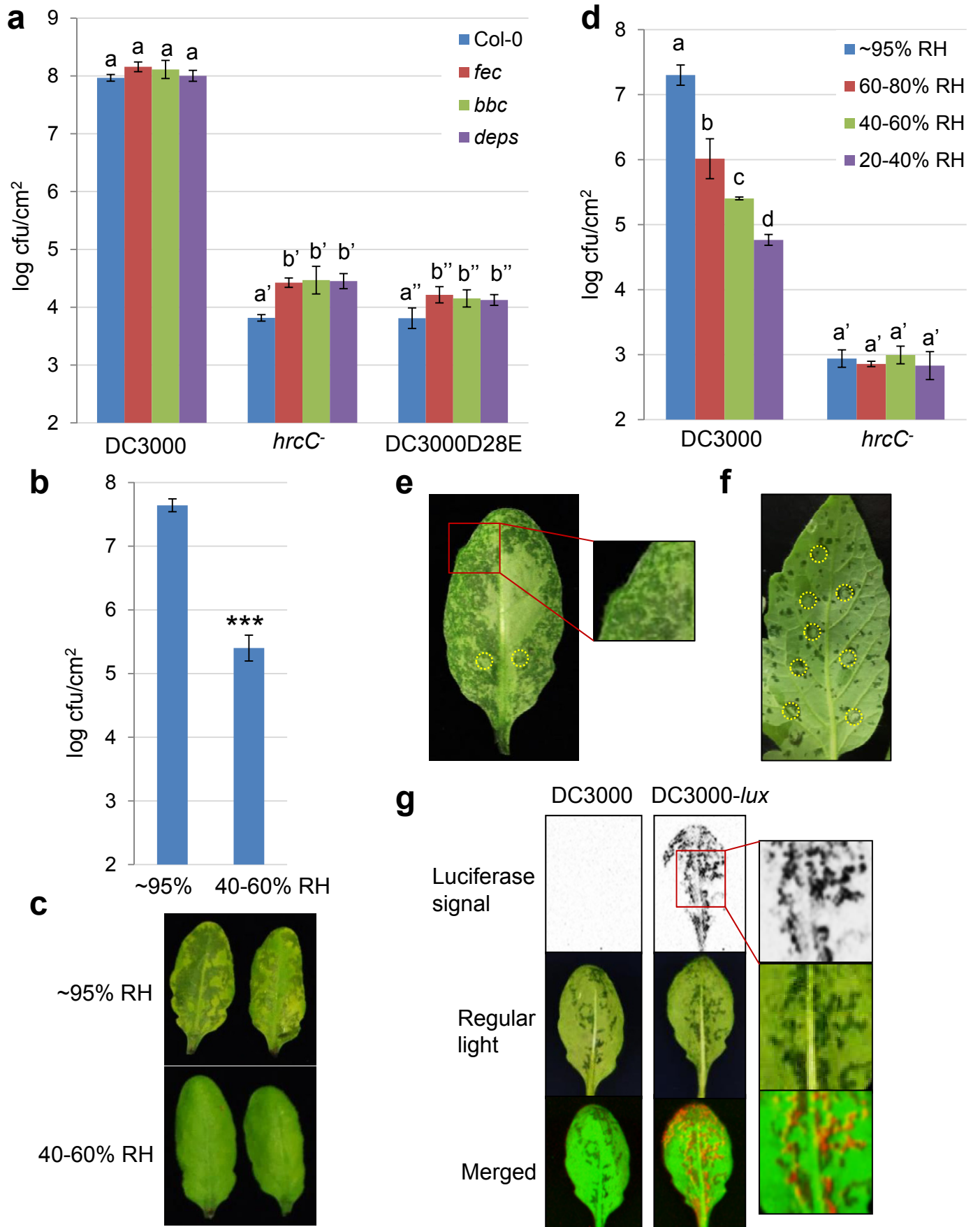
763 twice with similar results.

764

Supplementary Information

Supplementary Video 1: A movie showing the process of *Pst* DC3000 infection of Arabidopsis plants. Five-week-old Col-0 plants were dip-inoculated with *Pst* DC3000 at 1×10^8 cfu/ml. Plants were kept under high humidity (~95%) and the disease symptoms were recorded over 4 days. The process was sped up by 8,640-fold (24 h to 10 seconds). The recording started 7 h after inoculation and the red arrow indicates one leaf, as an example, that showed the transient appearance of water soaking.

Supplementary Figure 1: Uncropped gel/blot images. Red boxes indicate cropped sections that are used in the main or Extended Data figures. Diagram in **a** indicates how the two gel blots in **b** and **c** were generated.

Fig. 1

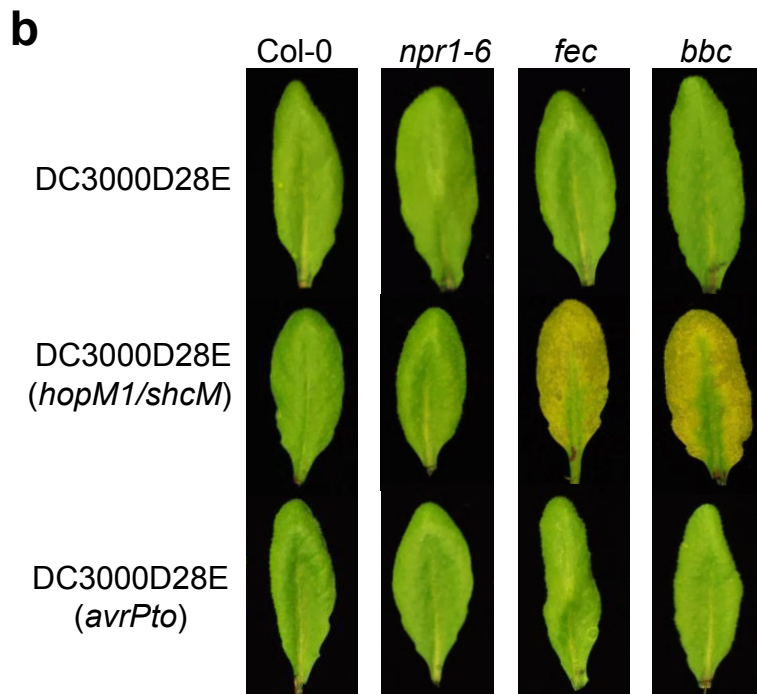# Fig. 2

a

−   (hopM1/shcM)   (avrE/avrF)   (avrPto)

DC3000D28E

b

Col-0 gl   6xHis:HopM1   AvrPto   Col-0   6xHis:AvrE

c

DC3000

avrE⁻/
hopM1⁻

ns

DC3000   avrE⁻/hopM1⁻

log cfu/cm²

d

■ ~95% RH
■ 20-40% RH

***   ***   ***   ns

DC3000   avrE⁻   hopM1⁻   avrE⁻/hopM1⁻

log cfu/cm²

e

■ − H₂O
■ + H₂O

**   ***   **   ***

DC3000   avrE⁻/hopM1⁻   hrcC⁻   CUCPB5452

log cfu/cm²

f

DC3000   avrE⁻/hopM1⁻   hrcC⁻   CUCPB5452

−H₂O

+H₂O

Fig. 3

a

~95% RH $\longrightarrow$ ~25% RH

min7 Col-0   *min7* Col-0

b

~95% RH $\longrightarrow$ ~50% RH

Col-0 *rps2*   Col-0 *rps2*

DC3000

DC3000(*avrRpt2*)

c

ns

log cfu / cm²

Col-0 *rps2*   Col-0 *rps2*

DC3000   DC3000(*avrRpt2*)

d

H₂O *avrE⁻/hopM1⁻* DC3000 DC3000(*avrRpt2*) H₂O/*min7*

kDa

250 ← MIN7

130 *

Coomassie blue staining

Fig. 4

a

b

c

DC3000D28E
(*hopM1/shcM*)

Col-0   *fec*   *bbc*

d

Fig. 5

a

b

Col-0   *fec*   *bbc*

min7   *m*   *mbbc*

c

d

e

Many effectors

PTI suppression

Wet apoplast

creation   *maintenance*

HopM1/AvrE   high humidity

Basic pathogenesis

Extended Data Table 1

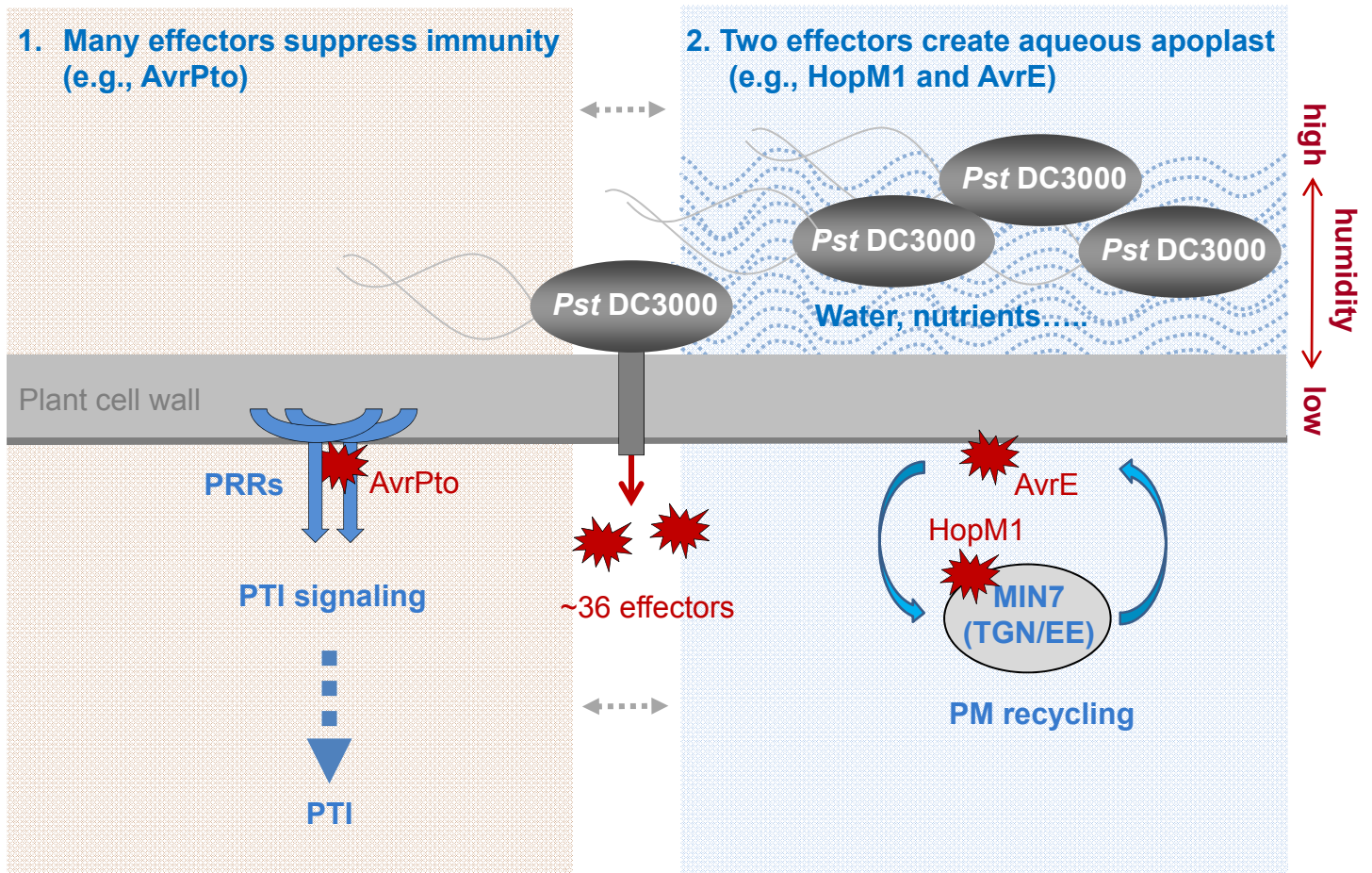| Order/Family	Col-0	*mfec*	*mbbc*
Bacillales			
Paenibacillaceae	15 (30%)	nd*	nd
Burkholderiales	15 (30%)	32 (64%)	42 (84%)
Comamonadaceae	8 (16%)	12 (24%)	9 (18%)
Burkholderiaceae	4 (8%)	1 (2%)	22 (44%)
Alcaligenaceae	3 (6%)	19 (38%)	12 (24%)
Flavobacteriales			
Flavobacteriaceae	6 (12%)	1 (2%)	1 (2%)
Xanthomonadales			
Xanthomonadaceae	4 (8%)	9 (18%)	nd
Sphingomonadales			
Sphingomonadaceae	3 (6%)	nd	1 (2%)
Sphingobacteriales			
Sphingobacteriaceae	3 (6%)	nd	nd
Chitinophagaceae	1 (2%)	nd	nd
Rhizobiales			
Rhizobiaceae	2 (4%)	5 (10%)	nd
Cytophagales			
Cytophagaceae	1 (2%)	nd	nd
Pseudomonadales			
Pseudomonadaceae	nd	1 (2%)	5 (10%)
Actinomycetales			
Microbacteriaceae	nd	2 (4%)	nd

Extended Data Fig. 1

~95% RH ⟶ ~50% RH

Luciferase signal

Regular light

Merged

DC3000   DC3000-*lux*   DC3000   DC3000-*lux*

Extended Data Fig. 2

a

DC3000   *avrE⁻*   *hopM1⁻*   *avrE⁻/hopM1⁻*

b

~95% RH

20-40% RH

DC3000   *avrE⁻*   *hopM1⁻*   *avrE⁻/hopM1⁻*

c

DEX   DEX   *avrE⁻/hopM1⁻*   H₂O
       +
       avrE⁻/hopM1⁻

d

Extended Data Fig. 3

a

log cfu / cm² — y-axis from 2 to 8

Legend: Col-0 (blue), *min7* (red)

DC3000 * · *avrE⁻/hopM1⁻* *** · *hrcC⁻* *

b

log cfu / cm² — y-axis from 2 to 8

Legend: Col-0 (blue), *min7* (red)

DC3000 ns · *avrE⁻/hopM1⁻* ** · *hrcC⁻* ns

c

Col-0 · *min7*

Extended Data Fig. 4

1. **Many effectors suppress immunity (e.g., AvrPto)**

2. **Two effectors create aqueous apoplast (e.g., HopM1 and AvrE)**

Pst DC3000

Pst DC3000

Pst DC3000

Pst DC3000

Water, nutrients.....

high ↕ humidity ↕ low

Plant cell wall

PRRs   AvrPto

PTI signaling

~36 effectors

PTI

AvrE

HopM1

MIN7 (TGN/EE)

PM recycling

Extended Data Fig. 5

a

~95% RH → ~25% RH

DC3000

DC3000 (*avrRpt2*)

b

Extended Data Fig. 6

a

ATG

npr1-6 (SAIL_708_F09)

NPR1

b

Col-0 npr1-6

NPR1

UBC21

c

Extended Data Fig. 7

a

MIN7

5' → 3'

WT	399	CATTGCCTATG<u>ATCATTTGGAAGGGG</u>-<u>ATCC</u>GGGGTT
mfec L1	399	CATTGCCTATGATCATTTGGAAGGGGAATCCGGGGTT	+1
mfec L2	399	CATTGCCTATGATCATTTGGAAGGGG--TCCGGGGTT	-1
mbbc L1	399	CATTGCCTATGATCATTTGGAAGGGG--TCCGGGGTT	-1

b

Col-0 *fec* *bbc*

min7 *mfec* L1 *mfec* L2 *mbbc*

c

Col-0 *mfec* *mbbc*

d

Col-0 *mfec* *mbbc*

e

Col-0 *mfec* *mbbc*

Extended Data Fig. 8

a

b

Extended Data Fig. 9