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Title: Dissimilar gene repertoires of *Dickeya solani* involved in colonization of lesions and roots of *Solanum tuberosum*

Short title: Versatile lifestyle of *Dickeya solani*

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2 **Abstract**

3 *Dickeya* and *Pectobacterium* species are necrotrophic pathogens that macerate
4 stems (blackleg disease) and tubers (soft rot disease) of *Solanum tuberosum*. They
5 proliferate by exploiting plant cell remains. They also colonize roots, even if no
6 symptoms are observed. The genes involved in pre-symptomatic root colonization
7 are poorly understood. Here, transposon-sequencing (Tn-seq) analysis of *D. solani*
8 living in macerated tissues revealed 126 genes important for competitive colonization
9 of tuber lesions and 207 for stem lesions, including 96 genes common to both
10 conditions. Common genes included *acr* genes involved in the detoxification of plant
11 defense phytoalexins and *kduD*, *kdul*, *eda* (=kdgA), *gudD*, *garK*, *garL* and *garR*
12 genes involved in the assimilation of pectin and galactarate. In root colonization, Tn-
13 seq highlighted 83 genes, all different from those in stem and tuber lesion conditions.
14 They encode the exploitation of organic and mineral nutrients (*dpp*, *ddp*, *dctA* and
15 *pst*) including glucuronate (*kdgK* and *yeiQ*), and synthesis of metabolites: cellulose
16 (*celY* and *bcs*), aryl polyene (*ape*) and oocycin (*ooc*). We constructed in-frame
17 deletion mutants of *bcsA*, *ddpA*, *apeH* and *pstA* genes. All mutants were virulent in
18 stem infection assays, whereas they were impaired in competitive colonization of
19 roots. In addition, the Δ *pstA* mutant was impaired in its capacity to colonize progeny
20 tubers. Overall, this work distinguished two metabolic networks supporting either an
21 oligotrophic lifestyle on roots or a copiotrophic lifestyle in lesions. This work revealed
22 novel traits and pathways important for understanding how the *D. solani* pathogen
23 efficiently survives on roots, persists in the environment and colonizes progeny
24 tubers.

25

26 **Key words:** Tn-seq, blackleg, soft rot, *Solanum tuberosum*, root colonization

27 INTRODUCTION

28 *Dickeya solani* emerged in potato tuber cultivation (*Solanum tuberosum*) in Europe at
29 the beginning of the 2000s (van der Wolf et al. 2014). A retrospective analysis of
30 potato pathogen archives revealed that some *D. solani* had been isolated on rare
31 occasions in Switzerland in the 1990s (Pédron et al. 2021). *D. solani* as well as other
32 species of *Dickeya* and *Pectobacterium* genera (order of *Enterobacterales*) are the
33 causative agents of soft rot disease on tubers and blackleg disease on stems of
34 potato plants (van der Wolf et al. 2021). They also cause damage to a wide range of
35 plants of agronomical interest worldwide (van der Wolf et al. 2021). The necrotrophic
36 pathogens *Dickeya* and *Pectobacterium* secrete plant cell wall macerating enzymes
37 as their main virulence factors and then proliferate in lesions (macerated tissues) by
38 exploiting plant cell remains as nutrients (Van Gijsegem et al. 2021). How these
39 pathogens persist and disseminate in agrosystems (soil, surface waters, plant
40 reservoirs, insect vectors, propagation *via* progeny-tubers...) and more specifically,
41 how they survive by colonizing and exploiting roots of their host (underground
42 lifestyle) remain questions under investigation.

43

44 Comparative genomics has shown little genetic variation in *D. solani* isolates
45 collected in Europe consistent with a bottleneck associated with a recent spread in
46 potato and bulb plants (van der Wolf et al. 2014 ; Khayi et al., 2015; Golanowska et
47 al., 2018; Blin et al., 2021). The primary host(s) of *D. solani* remain(s) uncertain.
48 Horizontal gene transfer events have been observed in a few *D. solani* isolates,
49 replacing some *D. solani* genes by their orthologous counterparts acquired from
50 *Dickeya dianthicola* (Khayi et al., 2015). These transfer events suggest ecological
51 promiscuity between the two species. Variation in the degree of symptoms caused to

52 the potato host was also observed among *D. solani* isolates: these phenotypes were
53 tentatively associated with single nucleotide polymorphism in some genes (*fliC*, *fliN*,
54 *fhaB2* and *vfmB*) and the presence or absence of some other loci (*arcZ*, *mtgA* and
55 *hrpQ*) (Khayi et al., 2015; Golanowska et al., 2018; Blin et al., 2021). The
56 involvement of these genetic variations in virulence remains to be confirmed by
57 reverse genetics. Several studies compared *D. solani*, *D. dadantii* and *D. dianthicola*,
58 highlighting some distinctive traits in *D. solani* (Potrykus et al., 2015; Potrykus et al.,
59 2018; Raoul des Essarts et al. 2019). Notably, some specific regulatory sequences
60 trigger a higher expression of *pelD* and *pelE* genes, encoding macerating enzymes,
61 in *D. solani* compared to *D. dianthicola* (Blin et al., 2021; Duprey et al., 2016). High
62 expression of *pelD* and *pelE* would contribute to the capacity of *D. solani* to cause
63 rotting at a lower bacterial inoculum than *D. dianthicola* (Blin et al., 2021; Raoul des
64 Essarts et al. 2019). A comparative analysis of metabolic capabilities showed that *D.*
65 *solani* exploited a wider range of compounds as nitrogen source than *D. dianthicola*
66 (Raoul des Essarts et al. 2019). Transcriptomics of the pathogens recovered from
67 tuber lesions pinpointed higher expression of the glyoxylate shunt in *D. solani* than in
68 *D. dianthicola*, a metabolic pathway that contributes to exploitation of alternative
69 sources of carbon when sugar is limiting (Raoul des Essarts et al. 2019). All these
70 traits could facilitate settlement of *D. solani* in the potato plant host.

71
72 Invasion success of *D. solani* could also be related to its capacity to survive under
73 stressful conditions and to compete with the microbiota of the potato host. The *D.*
74 *solani* genome contains several polyketide synthase (PKS) and non-ribosomal
75 peptide synthetase (NRPS) clusters coding for different secondary metabolites: aryl-
76 polyene, oocydin, zeamine and solanimycin. Aryl-polyene synthesis is encoded by

77 the *ape* genes that are widespread in β -proteobacteria and γ -proteobacteria,
78 including several *Enterobacterales* such as *D. solani* and some strains of *Escherichia*
79 *coli* (Schöner et al. 2016; Johnston et al., 2021). Aryl-polyenes are pigments
80 anchored to the outer membrane of bacterial cells that may contribute to oxidative
81 stress response and biofilm formation (Johnston et al., 2021). A recent study
82 (Effantin et al., 2021), highlighted three other PKS/NRPKS clusters coding for
83 synthesis of oocycin, zeamine and solanimycin, which are present in all published
84 genomes of *D. solani*, but not all three together in the other *Dickeya* species. The *ooc*
85 cluster is highly similar to the *ooc* genes coding for synthesis of oocycin A, a
86 chlorinated macrolide present in some *Serratia* and *Dickeya* strains (Matilla et al.,
87 2012). In different *Dickeya* and *Serratia* strains, the *zms* cluster is responsible for
88 synthesis of zeamine, a cationic polyamine-polyketide (Zhou et al., 2011). The *sol*
89 cluster is present in different *Dickeya* species, and encodes solanimycin that is active
90 against a broad range of micro-eukaryotes (Effantin et al., 2021; Matilla et al., 2022).
91 As compared to a wild-type strain of *D. solani* Ds0432-1, the constructed mutants
92 $\Delta solG$, $\Delta zmsA$ and $\Delta oocL$, were impaired in antibiosis against yeasts, Gram-positive
93 bacteria and Ascomyceta, respectively (Effantin et al., 2021). The contribution of
94 these secondary metabolites to plant colonization by *D. solani* is not known.

95
96 *D. solani* colonizes stems, xylem vessels and leaves, as well as roots and stolons
97 from which develop progeny-tubers (Czajkowski et al., 2010). In greenhouse
98 conditions, when inoculated into pots, *D. solani* rapidly colonizes the roots (two
99 weeks post inoculation of soil) and, to a lesser extent, stems even if no symptoms
100 (blackleg lesions) were observed (Czajkowski et al., 2010). Several weeks later,
101 blackleg symptoms develop in stems with an incidence mainly depending on

102 pathogen abundance in the inoculum and wounding of roots; root wounding
103 facilitates entry of pathogens (Czajkowski et al., 2010; Blin et al. 2021). At the end of
104 the vegetative cycle, pathogens may cause soft rot in progeny-tubers or may
105 colonize them without symptoms. Transcriptomics revealed differentially expressed
106 genes in *D. solani* living in soft rot lesions in potato tubers (Raoul des Essarts et al.,
107 2019). In contrast, the lifestyle of *D. solani* and other pectinolytic *Dickeya* and
108 *Pectobacterium* when they colonize roots remains poorly investigated. In a recent
109 study, 10,000 Tn5-mutants carrying a promoterless *gusA* gene were screened to
110 identify *D. solani* genes differentially expressed in the presence of leaves, stems,
111 roots and tubers of *S. tuberosum* (Czajkowski et al., 2020). A single gene (*pstB*) was
112 identified as expressed in the presence of either root or tuber tissues, but not stem or
113 leaf (Czajkowski et al., 2020). The *pstABC* genes encode a transporter involved in
114 importing phosphate and phosphate-mediated gene regulation, including pathogeny,
115 in different *Enterobacterales* (Daigle et al., 1995; Runyen-Janecky et al., 2005; Röder
116 et al., 2021). Whether *pst* genes contribute to colonization and survive of *D. solani* on
117 roots is not known.

118

119 Transposon-sequencing (Tn-seq) identifies insertional mutations that confer an
120 advantage or a defect under a given condition. In several plant pathogens, Tn-seq
121 unveiled genes involved in competitive colonization of lesions caused by necrotrophic
122 and biotrophic pathogens (Duong et al., 2018; Helmann et al., 2019; Royet et al.,
123 2019; Su et al., 2021; Torres et al., 2021; Helmann et al., 2022; Luneau et al., 2022;
124 Morinière et al., 2022). Tn-seq analyses revealed a large set of genes associated
125 with colonization of lesions provoked by *D. dadantii* on chicory leaves and by *D.*
126 *dianthicola* and *D. dadantii* on potato tubers (Royet et al., 2019; Helmann et al.,

127 2022). Tn-seq also permitted discovery of genes associated with competitive
128 colonization of roots in pathogenic and beneficial bacteria, under gnotobiotic
129 conditions (Cole et al. 2017; Liu et al. 2018; Sivakumar et al., 2019). To our
130 knowledge, a unique Tn-seq study explored root colonization of a plant pathogen
131 under non-gnotobiotic conditions using a non-sterile substrate for plant growth
132 (Torres et al., 2021). Identification of genes associated with an underground lifestyle
133 of pathogens could be useful for preventing disease by targeting root colonization
134 traits of pathogens.

135

136 In this work, we constructed a Tn-mutant library in the necrotroph *D. solani* RNS
137 08.23.3.1.A (=PRI3337) to identify genes involved in colonization of *S. tuberosum*
138 roots under a non-gnotobiotic condition. We also searched for genes associated with
139 the colonization of the lesions of stems (blackleg disease) and tubers (soft rot
140 disease). In addition, we identified the *D. solani* genes contributing to growth on
141 different nutrients derived from plant cell wall: pectin, galacturonate, glucuronate and
142 galactarate, and compared them with those identified in lesions. Then, we focused on
143 four genes *bscA* (cellulose synthesis), *dppA* (peptide transport), *pstA* (phosphate
144 importation) and *apeH* (aryl polyene synthesis) that we identified by Tn-seq as
145 important for competitive colonization of roots. The *dppA*, *pstA*, *bscA* and *apeH*
146 genes had been characterized in some *Enterobacteriales*, especially in *E.coli* and *D.*
147 *dadantii* (Olson et al., 1991; Daigle et al., 1995; Mee-Ngan et al., 2005; Runyen-
148 Janecky et al. 2005; Prigent-Combaret et al., 2012; Schöner et al., 2016; Johnston et
149 al., 2021; Röder et al., 2021), but their role in root colonization is not known. We
150 constructed in frame deletions of these genes in *D. solani*, and we validated their
151 implication in the underground lifestyle of *D. solani*. We showed that the *pstA* gene is

152 also important for colonizing progeny-tubers. Overall, this work highlights a novel
153 landscape of genes and traits involved in *D. solani*-*S. tuberosum* interactions,
154 especially those involved in the underground lifestyle of this plant pathogen.

155

156 **MATERIAL AND METHODS**

157 **Bacteria and plant material**

158 Bacterial strains are described in Table 1 and culture conditions in the supporting
159 information SI1. Tubers (G0 generation, caliber 35-45 mm) of *Solanum tuberosum*
160 variety Bintje were provided by *Comité Nord Plants de Pomme de Terre* (CNPPT,
161 Achicourt, France).

162

163 **Construction of transposon library in *Dickeya solani* RNS 08.23.3.1.A**

164 The genome of *D. solani* RNS 08.23.3.1A contains a circular chromosome of
165 4,922,468 bp in which 169,132 TA dinucleotides are all potential sites for the
166 insertion of the *himar1* mariner transposon (Figure S1). Among them, 132 173 TA
167 sites are distributed in 99,5% of the 4,518 coding sequences. Only 22 genes do not
168 contain any TA dinucleotide in their sequence and could not be considered in this
169 study (listed in Table S1).

170 A library of Tn-mutants was constructed in a rifampicin-resistant (Rif^R) derivative of *D.*
171 *solani* RNS 08.23.3.1A using the plasmid pSAM-Ec, which harbors a modified
172 *Himar1* mariner associated to a kanamycin (Km) resistance cassette (Wiles et al.,
173 2013). Five hundred conjugation matings between *E. coli* MFDpir carrying pSAM-Ec
174 and *D. solani* RNS 08.23.3.1A Rif^R were performed as described by Gonzalez-Mula
175 et al. (2019) (experimental details in SI2). Around $1.8 \cdot 10^7$ independent Tn-mutant
176 colonies were recovered on agar TY medium supplemented with Rif and Km. This

177 corresponds to approximately 100 times the number of TA sites present in the
178 genome of *D. solani* RNS 08.23.3.1A (169,132 TA sites). All the Tn-mutant colonies
179 were collected, and the resulting Tn-mutant library was homogenized, aliquoted and
180 stored in 25% glycerol at -80°C. Each aliquot contained $2.3 \cdot 10^{10}$ CFU (colony forming
181 units) of Tn-mutants.

182

183 **Culture and analysis of the Tn-mutant library under different conditions**

184 An aliquot of the Tn-mutant library was thawed on ice and then incubated at 28 °C for
185 four hours in 10 ml of TY rich medium in order to reactivate the bacterial cells. Library
186 cells were centrifugated and washed with a solution at 0.8% NaCl, and used for
187 inoculating M9 minimal medium and plant materials. Four revived aliquots in TY rich
188 medium were used to analyze quality of the Tn-mutant library: these four replicates
189 correspond to TY rich medium condition. In the *in vitro* culture tests, the Tn-mutant
190 library was diluted to obtain a final OD_{600nm} of 0.02 in 50 ml of liquid M9 medium. M9
191 medium was supplemented with different carbon sources at 2g/L: sucrose (CAS 57-
192 50-1), galacturonate (CAS 91510-62-2), glucuronate (CAS 6556-12-3), galactarate
193 (CAS 526-99-8), which were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier,
194 France) and pectin Dipecta AG366 (Agdia EMEA, Soisy sur Seine, France). This
195 pectin is routinely used in crystal violet pectate medium for isolating *Dickeya* and
196 *Pectobacterium* from environmental samples (Hélias et al., 2012). Cultures were
197 incubated at 28°C for 24 hours under shaking (200 rpm). Bacterial cells were
198 centrifuged (4000 rpm, 4°C, 15 min) and stored at -20°C before DNA extraction.
199 Each culture condition was performed in four replicates.

200 In the *in planta* assays, the Tn-mutant library was inoculated in three conditions:
201 roots and stems of entire plants and potato tubers. Potato plants were cultivated

202 individually in 2-Litter pots containing horticultural compost (Floragard, Oldenburg,
203 Germany) under non-ghotobiotic conditions in a greenhouse (16 hour days and 8
204 hour nights at 24°C). In the root colonization assay, 1 mL at 6 units OD_{600nm} of the
205 Tn-mutant library was inoculated by watering ($5.5 \cdot 10^9$ CFU per pot). Forty plants
206 were inoculated when the aerial part reached about 30 cm in height (four weeks after
207 tuber planting). In the stem infection assay, 50 µL at 1 unit OD_{600nm} of the Tn-mutant
208 library were injected into the stem in the axil of the 4th leaf ($5 \cdot 10^7$ CFU per stem).
209 These plants were developed from axillary buds of tubers and 140 stems were
210 inoculated when they reached a size of about 20-30 cm in height. In the tuber
211 infection assay, 10 µL at 1 unit OD_{600nm} of the Tn-mutant library was injected into the
212 potato tubers (10^7 CFU per tuber). Forty tubers were inoculated and then placed at
213 24°C in the dark. Plant and tuber assays were described previously (Blin et al. 2021).
214 Six weeks post inoculation, the 40 root systems were collected and grouped into 8
215 lots that we designated as the 8 replicates of the root condition. Symptoms of
216 maceration were not observed on root systems. Roots were crushed in 25 ml of a
217 0.8% NaCl solution during 1 min using a laboratory blender (Waring, Stamford, USA).
218 The homogenate was filtered through miracloth (Millipore, Bedford, MA, USA) and
219 then plated onto TY agar medium supplemented with Rif, Km and cycloheximide.
220 After 48 hours at 28°C, the Tn-mutant colonies were collected and resuspended in
221 0.8% NaCl solution. For each replicate 2 mL of bacterial suspension was pelleted
222 and frozen prior to DNA extraction.

223 Five days post inoculation, macerated tissues of the stems and tubers were collected
224 and grouped into eight stem replicates (each from 15 stems) and four tuber replicates
225 (each from 10 tubers). Each replicate was crushed in 10 ml of 0.8% NaCl solution
226 and the Tn-mutant colonies were recovered using TY agar medium supplemented

227 with Rif, Km and cycloheximide, following the same protocol as for the root condition.
228 For each replicate 2 mL bacterial suspension was centrifugated and frozen prior to
229 DNA extraction.

230

231 **Analysis of the Tn-mutant library**

232 Overall 42 samples were analyzed. Four replicates were collected in the TY rich
233 medium condition, in which the Tn library was constructed. Four replicates were
234 collected in M9 medium for each of the five carbon sources: sucrose, pectin,
235 galacturonate, glucuronate and galactarate. In addition, four replicates were collected
236 in the macerated tuber condition, eight in the macerated stem condition and eight in
237 the root condition.

238 Total DNA from each replicate was extracted and then the Tn-insertion sites were
239 amplified (Primers in Table S3) and sequenced as described previously (Gonzalez-
240 Mula et al., 2019; Torres et al., 2021). Illumina NextSeq 500 instrument (Illumina,
241 San Diego, USA) was used in a single read 75 run at the I2BC-sequencing platform
242 (I2BC, Gif-sur-Yvette, France). Each population generated over 3 million reads after
243 specific cleaning of the Tn-seq data: transposon trimming with Trimmomatic (Bolger
244 et al., 2014), and then barcode removal.

245 The reads were mapped using Bowtie-1.1.2 (Langmead et al., 2009) to the *D. solani*
246 RNS 08.23.3.1A genome (Khayati et al., 2018; GenBank CP016928.1). Genome
247 annotation was generated at the LABGeM (CEA/Genoscope CNRS UMR8030, Evry,
248 France). Correspondence between the Genoscope annotation and NCBI annotation
249 (released in April 2022 [https://www.ncbi.nlm.nih.gov/data-](https://www.ncbi.nlm.nih.gov/data-hub/genome/GCA_000511285.2/)
250 [hub/genome/GCA_000511285.2/](https://www.ncbi.nlm.nih.gov/data-hub/genome/GCA_000511285.2/)) is provided in Table S1.

251 The mapping results (BAM files) were analyzed by the ARTIST pipeline (Pritchard et
252 al., 2014) using MATLAB software (MathWorks, Natick, USA). All .bam files of the 42
253 compared conditions were deposited as sequence read archives (SRA) in the
254 bioproject PRJNA939571 at the National Center for Biotechnology Information (Table
255 S4). When a high correlation ($r^2 \geq 0.80$) was observed in distribution and relative
256 abundance of Tn-insertions between the replicates of a same culture medium
257 condition, the sequencing data were pooled for further analyses using ARTIST. Two
258 ARTIST analyses were performed with a *P*-value cutoff of $P < 0.01$ in the Mann-
259 Whitney U test: EL-ARTIST and Con-ARTIST (Pritchard et al., 2014). By comparing
260 the number of Tn sequences at each AT insertion site in a given culture condition,
261 the EL-ARTIST pipeline predicts the genes that are 'non-essential' because they still
262 contain Tn insertions (class 1), 'essential' (class 2) because they contain no Tn
263 insertions, or an 'essential domain' (class 3) because no Tn- insertions are present in
264 a part of the gene. The 'essential' term is used in the ARTIST suite to indicate a
265 decrease ($P < 0.01$) in the relative abundance of Tn-mutants of a considered gene in a
266 given condition. The Con-ARTIST pipeline compares two conditions, a test condition
267 versus a reference condition. By comparing the number of Tn sequences at each AT
268 insertion site in the reference and test conditions, Con-ARTIST distinguishes the
269 genes with a conditionally 'essential domain' (class 1), conditionally 'essential genes'
270 (class 2), genes with a conditionally 'enriched domain' (class 3), conditionally
271 'enriched genes' (class 4) and genes conditionally 'non-essential, not enriched' (class
272 5). In this work, the Con-ARTIST class 2 'essential genes' were retained as
273 candidate genes that are important for growth and survival in a given growth
274 condition as compared to a reference condition. In the case of *in planta* culture
275 conditions (roots and macerated stems and tubers), each replicate was analyzed

276 separately and compared to the reference condition (TY rich medium) to provide a
277 list of fitness genes (class 2 'essential genes'). Then, those which had been assigned
278 to Con-ARTIST class 2 'essential genes' in at least half of the replicates of a
279 considered condition were retained in a final unique list of fitness genes, as
280 described by Torres *et al.* (2021).

281 The identified genes were categorized using COG classification (Clusters of
282 Orthologous Groups of proteins) by Galperin *et al.* (2015). Antismash was used for
283 identifying secondary metabolite biosynthetic gene clusters
284 (<https://antismash.secondarymetabolites.org>; Blin *et al.*, 2021). Some genes were
285 manually analyzed, such as those associated with carbon metabolism according to
286 the published data in *Enterobacteriales* (Tervo *et al.*, 2012; Jason *et al.*, 2019;
287 Kuivanen *et al.*, 2019).

288

289 **Construction of deletion mutants in *D. solani* by reverse genetics**

290 In frame deletions were constructed in four genes for which Tn-seq analysis
291 predicted a contribution to competitive fitness in root colonization: *bcsA*
292 (=DS0823_v1_2374) involved in cellulose synthesis, *dppA* (=DS0823_v1_2392)
293 involved in peptide transport, *pstA* (=DS0823_v1_2527) involved in phosphate
294 transport and *apeH* (=DS0823_v1_2513) involved in synthesis of an unknown aryl-
295 polyene compound. The mutated genes were chosen within a cluster coding for the
296 same function, therefore, the same behavior was expected, even in the case of a
297 potential polar effect.

298 The *D. solani* RNS 08.23.3.1A Rif^R-Gm^R strain was obtained by integration of a Gm-
299 resistance cassette at the unique *attTn7* site of the *D. solani* genome, as described in
300 SI3. The defective alleles were introduced in *D. solani* RNS 08.23.3.1A Rif^R-Gm^R by

301 conjugation (Effantin et al., 2021; Edwards et al., 1998). A detailed protocol is
302 presented in SI4. To validate the *in planta* behavior of the mutants, two independent
303 clones were retained per genotype and further analyzed in plant assays.

304

305 ***In planta* behavior of the constructed *D. solani* deletion mutants**

306 To measure the relative fitness of the four constructed deletion mutants (Rif^R Gm^R),
307 root colonization assays were performed in the presence of the ancestral strain *D.*
308 *solani* RNS 08.23.3.1A Rif^R. Competition between the ancestor *D. solani* RNS
309 08.23.3.1A Rif^R and its derivative *D. solani* RNS 08.23.3.1A Rif^R-Gm^R was used as
310 control to evaluate the potential bias caused by the Gm^R-cassette.

311 The *D. solani* RNS 08.23.3.1A Rif^R strain and its derivatives were grown overnight in
312 TY medium. The cells were rinsed twice in a 0.8% NaCl solution then their OD_{600nm}
313 was adjusted to 1 unit (10⁹ CFU/mL). Each Rif^R Gm^R strain was mixed with the Rif^R
314 strain at a 1: 1 ratio. For each mutant construction, two independent clones were
315 analyzed. Each clone was inoculated in 10 pots. Six weeks post-inoculation, roots of
316 each pot were sampled and 10 g were crushed in 30 ml of 0.8% NaCl solution
317 following the same procedure as described above for the Tn-infection assays. The
318 homogenate was diluted and spread on TY Rif and TY Rif-Gm agar medium, both
319 being supplemented with cycloheximide, to enumerate the total *Dickeya* population
320 and the mutant population. For each replicate, a competitive index (CI) was
321 calculated (Macho et al., 2010) following the formula presented in SI5. A CI value
322 equal to one indicated an equal fitness between *D. solani* RNS 08.23.3.1A Rif^R and
323 *D. solani* RNS 08.23.3.1A Rif^R-Gm^R (mutant strains), CI values greater than one
324 indicated a fitness advantage of the RNS 08.23.3.1A Rif^R-Gm^R mutant strain, and CI
325 values below one indicated a fitness advantage of RNS 08.23.3.1A Rif^R.

326 In addition to competition assays, each bacterial genotype was inoculated separately:
327 in the first assay, pathogens were injected directly in stems while in the second
328 assay, pathogens were inoculated as described for the root colonization assay. For
329 each mutant, two independent clones were analyzed. Each clone was inoculated on
330 10 plants. The strain RNS 08.23.3.1A Rif^R-Gm^R was used as a virulent reference and
331 uninoculated plants as negative control. In the stem inoculation assay, the length of
332 internal lesions was measured five days post-infection. Abundance of pathogens was
333 quantified in lesions (CFU/g of stem fresh weight) using enumerations on TY
334 supplemented with Rif, Gm and cycloheximide. In the root inoculation assay, eight
335 weeks post-infection, abundance of pathogens in pots was quantified by counting on
336 TY agar medium supplemented with Rif, Gm and cycloheximide. The number of
337 asymptomatic and symptomatic progeny-tubers was counted, and finally, pathogen
338 acquisition in tuber progeny was evaluated by testing asymptomatic progeny-tubers
339 for the presence of *D. solani* using qPCR (Blin et al., 2021).

340

341 **Statistics**

342 The correlation between the different replicates of the Tn-seq mapping results was
343 analyzed by calculating the linear regression coefficient (r^2). The traits measured in
344 plants inoculated by the different bacterial genotypes (the CI values, length of the
345 stem symptoms in cm, abundance of pathogens in rotten tissue or pots in CFU/g and
346 the number of symptomatic or asymptomatic plants) were compared by Kruskal–
347 Wallis tests ($\alpha = 0.05$). Then a Dunn's test was performed to compare traits of each
348 constructed mutant with the strain RNS 08.23.3.1A Rif^R-Gm^R. For all statistical
349 analyses and coefficient calculation, we used GraphPad Prism version 9.3.0,
350 GraphPad Software (www.graphpad.com).

351

352 **RESULTS**

353 **Characteristics of the Tn-library in *D. solani* RNS 08.23.3.1A**

354 Using the *himar1* mariner transposon (Tn), we constructed a library of Tn-mutants in
355 a rifampicin-resistant derivative of *D. solani* RNS 08.23.3.1A (Table 1). To examine
356 the distribution of Tn in the *D. solani* genome, we extracted total DNA from four Tn-
357 library aliquots that were grown in TY rich medium. By comparing relative abundance
358 of the Tn-insertions along the circular chromosome, a high correlation ($r^2 > 0.98$) was
359 observed between the four replicates. Sequencing data from the replicates were
360 pooled for further analysis. In the *D. solani* Tn-library, more than 70% of genes have
361 at least 80% of their TA sites with a Tn insertion (Figure 1), revealing good coverage
362 of the genome. In the next step, the EL-ARTIST analysis (Pritchard et al., 2014)
363 pinpointed 521 genes ('essential genes' according to the EL-ARTIST classification)
364 for which Tn-mutants had impaired growth in TY medium. These 521 genes
365 represented 11.5% of the total genes of *D. solani* RNS 08.23.3.1A. A similar
366 percentage had been observed in previous Tn-seq studies (Hooven et al., 2016;
367 Gonzalez-Mula et al., 2019 ; Royet et al., 2019). According to the COG classification,
368 these 521 genes are associated with four main functional categories: bacterial
369 metabolism (31,9%), information storage and processing (29,4%), cellular processes
370 and signalling (25,3%) and poorly characterized genes (13,4%) (Table S2). Most of
371 them encompass genes encoding for cell processes that are essential for bacterial
372 viability and optimal growth: for instance, the *nuo* cluster (aerobic respiration), *atp*
373 cluster (synthesis of ATP), *rpl* genes (50S ribosomal protein) and *dnaE* (DNA
374 polymerase) (Figure 1). The Tn-library was thereafter challenged to several growth

375 conditions to unveil and compare the different gene repertoires supporting the
376 *D. solani* lifestyles.

377

378 **Fitness genes associated with growth of *D. solani* on plant cell wall** 379 **components**

380 Because *D. solani* secretes plant cell wall macerating enzymes, we searched for
381 genes associated with growth of *D. solani* on nutrients derived from plant cell walls.
382 The Tn-library was cultivated in M9 synthetic medium supplemented with different
383 carbon sources: galacturonate, glucuronate, galactarate and pectin. M9 medium with
384 sucrose was used as a reference condition in comparative analyses. On average,
385 three million reads were obtained and analyzed per replicate to determine the relative
386 abundance of the Tn along the chromosome. We observed a good correlation
387 between the four replicates of each growth condition: sucrose ($r^2 > 0.94$),
388 galacturonate ($r^2 > 0.98$), glucuronate ($r^2 > 0.88$), galactarate ($r^2 > 0.97$), and pectin ($r^2 >$
389 0.99). Hence, we pooled sequencing data for each carbon source for further
390 comparisons.

391 Using EL-ARTIST, we analyzed the sequencing data obtained under sucrose growth
392 condition and then we used Con-ARTIST to compare distribution of the Tn-insertions
393 in sucrose versus other conditions (Table S2). We focused on the 'conditionally
394 essential genes' (class 2) according to the Con-ARTIST analysis and we retained
395 them as fitness genes. We identified 98 genes important for growth on pectin, 89 for
396 growth on galacturonate, 96 for growth on glucoronate and 87 for growth on
397 galactarate (Table S2). Our Tn-seq experiments revealed some common and some
398 nutrient-specific genes, which are important for competitive growth of *D. solani* in the
399 presence of plant cell wall components (Figure 2). Some common genes are involved

400 in the pentose phosphate pathway and upper glycolysis/gluconeogenesis (*tpiA*, *fbp*,
401 *pgi*, *zwf*, *pgl*, *gnd*). Some nutrient-specific genes are associated to importation and
402 degradation of distinctive plant compounds: pectin (*kdul*, *kduD*), glucuronate (one
403 unnamed MSF-transporter = DS0823_v1_1165, *yeiQ*, *uxuA*), galacturonate (*exuT*,
404 *uxaB*, *uxaA*), and galactarate (*gudP*, *gudD*, *gudX*, *garL*). The genes *kdgK* and *eda*
405 (= *kdgA*) were important for fitness in growth on pectin, glucuronate and
406 galacturonate, but not galactarate. These genes reflect the metabolic pattern of *D.*
407 *solani* in each of the tested growth conditions. Their identification was useful to
408 interpret the fitness genes in complex environments such as roots and macerated
409 stems and tubers.

410

411 **Fitness genes associated with colonization of macerated stems, tubers and** 412 **roots**

413 To identify the *D. solani* genes involved in proliferation in macerated plant tissues,
414 we inoculated 10^7 CFU of the Tn-library on 40 tubers and 120 stems. After a 5-day
415 incubation, *D. solani* reached $2.3 \cdot 10^{10} \pm 3.6 \cdot 10^{10}$ CFU/g of lesions in tubers and 2.0
416 $\cdot 10^9 \pm 5.5 \cdot 10^8$ CFU/g of lesions in stems. The macerated tubers and stems were
417 assembled in four lots (each from 10 tubers) and eight lots (each from 15 stems),
418 respectively. Each lot of macerated tissue is considered as a replicate. After DNA
419 extraction and sequencing of Tn-insertion sites, Con-ARTIST analysis was performed
420 using TY medium as a reference condition. We identified 126 fitness genes important
421 for competitive colonization of tuber lesions and 207 for stem lesions, including 96
422 genes common to both conditions (Table S2). Most of these 237 genes are involved
423 in metabolism (40%) and cellular processes and signalling (32%) categories
424 according to the COG classification (Table S2). These genes are scattered along the

425 *D. solani* genome (Figure 3). A noticeable exception is a large cluster of 39 genes
426 coding for motility (*fli*, *flg* and *flh*) and chemotaxis (*che* and *chp*), which were
427 important for competitive fitness in stem lesions (Figure 3 and table S2). Additional
428 operons associated to fitness in stem lesions are involved in the synthesis of purines
429 (*purCDEHLM*) and different amino acids such arginine (*argABCEG*), histidine
430 (*hisABCDFGHI*), leucine (*leuABCD*), valine and isoleucine (*ilvCYDEM*). Some
431 remarkable genes were important for fitness in both conditions, tuber and stem
432 lesions: this category includes genes involved in upper glycolysis/gluconeogenesis
433 (*fbp*, *zwf*, *gnd*), and assimilation of pectin (*kduI*, *kduD*, *eda*) or galactarate (*gudD*,
434 *garL*, *garR*, *gark*) (Table S2).. Other common genes between macerated tuber and
435 stem conditions were associated with detoxification (*acrAB* genes coding for an efflux
436 system) and aerobic respiration (*cyoABCD* encoding the cytochrome o ubiquinol
437 oxidase).

438 In the root colonization condition, no symptoms on roots were observed: pathogens
439 reached $1.9 \cdot 10^5 \pm 1.6 \cdot 10^5$ CFU/g of roots. Forty root systems were grouped into eight
440 lots that represent eight replicates. Tn-seq analysis revealed 83 genes (Table S2),
441 which are clustered in four genomic regions (Figure 3). None of them was in common
442 with those identified in the macerated stem and tuber conditions (Figures 3 and 4). In
443 the first identified cluster, 11 of 13 adjacent genes were predicted to be involved in
444 the synthesis of oocydin (*oocJKLMNRSTUVW*). In the second cluster, 31 adjacent
445 genes code for C4-dicarboxylic acid transporter (*dctA*), cellulose biosynthesis (*celY*-
446 *bcsABCDQO*), a putative dipeptide ABC-transporter (*dppABCDF*), conversion of 2-
447 keto-3-deoxy-gluconate (*kdgK*) and some other less characterized functions. In the
448 third cluster (21 adjacent genes), some genes are associated with biosynthesis of an
449 aryl polyene (*apeDEFGHIJKLMNOPQR*) and a phosphate ABC-transporter

450 (*pstABCS*). In the fourth cluster, 18 adjacent genes code for another putative
451 dipeptide ABC-transporter (*ddpABC*), conversion of fructuronate (*yeiQ*) and some
452 other poorly characterized functions. Among the genes pinpointed by Tn-seq in the
453 root condition, two genes (*kdgK* and *yeiQ*) were also important in M9 medium
454 supplemented with glucuronate as a carbon source (Figure 2).

455

456 ***In planta* behavior of the *Dickeya solani* mutants $\Delta bcsA$, $\Delta ddpA$, $\Delta apeH$, $\Delta pstA$**

457 To analyze further some fitness genes associated with root colonization, we
458 constructed in-frame deletion mutants of four genes: *dppA* (=DS0823_v1_2392)
459 encoding an oligopeptide transporter, *pstA* (= DS0823_v1_2527) a phosphate
460 transporter, *bcsA* (=DS0823_v1_2374) involved in cellulose synthesis and *apeH*
461 (=DS0823_v1_2513) involved in synthesis of an aryl-polyene.

462 We evaluated the capability of the constructed mutants in *D. solani* to provoke
463 blackleg symptoms when injected directly in the stem of the potato plant. The
464 mutants caused similar lesions to those of the wild-type strain (Figure 5A). Moreover,
465 mutants and wild-type strains colonized stem lesions at a similar abundance (Figure
466 5B). Thus, a deletion of these genes did not abolish the virulence program of *D.*
467 *solani* when injected directly in plant tissues.

468 Then, we evaluated the competitive fitness of these mutants in root colonization
469 when they were challenged by their ancestor (RNS 08.23.3.1A Rif^R). A control
470 competition between RNS 08.23.3.1A Rif^R-Gm^R and RNS 08.23.3.1A Rif^R was
471 performed to evaluate a potential bias caused by the presence of a Gm-resistance
472 cassette (strains are described in Table 1). Eight weeks post-inoculation, the
473 pathogens living on roots were enumerated on selective media and a competitive
474 index (CI) value was calculated for each replicate. CI values of the competitions

475 between RNS 08.23.3.1A Rif^R and RNS 08.23.3.1A Rif^R-Gm^R did not differ from one
476 (Dunn test, p-value >0.9999). By contrast, median CI of the $\Delta dppA$, $\Delta apeH$ and $\Delta pstA$
477 mutants was below one (0.002, 0.01 and 0.01, respectively), revealing a decrease in
478 the competitive fitness of the constructed mutants (Figure 6). These CI values
479 significantly differed from CI values of the reference competition (RNS 08.23.3.1A
480 Rif^R-Gm^R versus RNS 08.23.3.1A Rif^R). In the case of the $\Delta bcsA$ mutant, the median
481 CI was also below one (0.19), but the heterogeneity of the CI values between
482 replicates compromised statistical significance (p-value=0.503) when CI values were
483 compared to those of the reference competition RNS 08.23.3.1A Rif^R-Gm^R versus
484 RNS 08.23.3.1A Rif^R (Figure 6). This fitness variability suggests that multiple
485 environmental parameters could influence the behavior of the $\Delta bcsA$ mutant during
486 root colonization.

487 We further investigated how a deletion in the *bcsA*, *ddpA*, *apeH* and *pstA* genes
488 could modify the underground behavior of *D. solani*. Each pathogen (the four mutants
489 and strain RNS 08.23.3.1A Rif^RGm^R used as a reference) was inoculated separately
490 by watering the roots with 10¹⁰ CFU per pot. Eight weeks later, when tuber progeny
491 had developed, we observed that potato plants produced a similar number of
492 progeny-tubers (mean value 10.0 tubers \pm 0.4) when inoculated by the mutants and
493 RNS 08.23.3.1A Rif^RGm^R or not inoculated (Kruskal-Wallis test, statistic 7.635, p-
494 value= 0.1775). However, the plants inoculated by $\Delta bcsA$, $\Delta apeH$ and $\Delta pstA$ mutants
495 exhibited a lower soft-rot disease incidence (% of rotted progeny-tubers) when
496 compared to those inoculated by the RNS 08.23.3.1A Rif^RGm^R strain (Dunn test, p-
497 values <0.05; Figure 7A). In the same experiment, the $\Delta pstA$ mutant was significantly
498 impaired in its capacity to colonize asymptomatic progeny-tubers as compared to
499 RNS 08.23.3.1A Rif^RGm^R strain (Dunn test, p-values <0.0005; Figure 7B) while the

500 three other mutants did not show a difference compared to the parental strain.
501 Finally, we decided to evaluate the ability of the mutants to survive in the compost at
502 the vicinity of the roots: all four mutants were impaired in this trait, as compared to
503 strain RNS 08.23.3.1A Rif^RGm^R (Figure 7C).

504

505 **DISCUSSION**

506 In the pathogen *D. solani*, our Tn-seq analyses highlighted four genomic regions,
507 grouping 13, 18, 21 and 31 adjacent genes, respectively, which may contribute to the
508 pathogen's capacity to colonize roots of potato plants grown under non-ghotobiotic
509 conditions. These root colonization genes differed from those involved in the
510 competitive proliferation in lesions of tubers (soft-rot disease) and stems (blackleg
511 disease). Hence, this study revealed two contrasting lifestyles of *D. solani* colonizing
512 a potato plant host: an oligotroph lifestyle on roots where it survived at 10⁵ CFU/g of
513 roots; a copiotroph lifestyle in lesions where *D. solani* proliferation reached 10⁹ to
514 10¹⁰ CFU/g of macerated tissues. *D. solani* used different gene repertoires to survive
515 and proliferate under these contrasting conditions: roots and lesions. Because of
516 some limitations in the Tn-seq approach, which we discussed in Torres et al. (2021),
517 we could not exclude that additional genes contribute to an efficient colonization of
518 roots and lesions.

519 In stem and tuber lesions, carbon metabolism of *D. solani* is oriented towards
520 exploitation of plant cell debris. Comparison of the Tn-seq data acquired *in planta*
521 and in culture media showed that carbon metabolism *in planta* resembled but was
522 not identical to that observed in the presence of pectin and galactarate (Figure 2).
523 Notably, *kduD*, involved in the degradation of pectin, was also identified by Tn-seq as
524 a fitness gene in *D. dianthicola* and *D. dadantii* colonizing potato tubers (Helmann et

525 al., 2022). Catabolism of these cell wall derivatives is connected to the upper part of
526 glycolysis/gluconeogenesis (connecting glucose and glyceraldehyde-3P) and to the
527 pentose phosphate pathway (Figure 2). In the lower part of glycolysis, the Pta-AckA
528 pathway was also important under maceration conditions (Table S2). In *E. coli*, this
529 pathway is involved in ATP production from acetyl-CoA with acetate as a by-product
530 (Schütze et al., 2020). This pathway was also pinpointed in a Tn-seq analysis of *D.*
531 *dadantii* macerating chicory leaves (Royet et al., 2019). We observed that survival of
532 *D. solani* in lesions is also dependent on its capacity to detoxify plant compounds
533 using the Acr and Sap systems. The Sap transporter is involved in resistance to
534 snakin-1, which is the most abundant antimicrobial peptide in potato tubers (López-
535 Solanilla et al., 1998). In *Erwinia amylovora*, *acr* genes are involved in the resistance
536 to several plant phytoalexins and antibiotics (Al-Karablieh et al., 2009). The Acr efflux
537 pump is also important for colonization of chicory leaves by *D. dadantii* (Royet et al.,
538 2019). In *D. dadantii*, this efflux system is involved in resistance to a wide spectrum
539 of toxic compounds including the plant antimicrobial peptide thionin (Valecillos et al.,
540 2006). Two important characteristics of the *D. solani* behavior in stem lesions are
541 motility and synthesis of several amino acids (Fig. 4). Motility may be connected to
542 propagation of the pathogen in plant vessels causing a progressive lesion along the
543 stem. Requirement of amino acid synthesis would reflect an imbalance of carbon and
544 nitrogen resources in cell wall remains. Motility and amino acid synthesis were also
545 highlighted in Tn-seq analyses of *D. dadantii* and *D. dianthicola* proliferating in
546 progressive lesions of chicory leaves and in tubers (Royet et al., 2019; Helmann et
547 al., 2022).

548 In the root colonization condition, Tn-seq data revealed that *D. solani* coped with
549 another pattern of environmental constraints. Some oligopeptides, organic acids and

550 mineral phosphate constitute important resources for nutrients that could be imported
551 by the *dpp*, *ddp*, *dctA* and *pst* transporters (Figures 3 and 4). Some sugars
552 (glucuronate and its by-products) would also be assimilated (Figure 2). *S. tuberosum*
553 exudates contain a wide variety of molecules, among which glucuronate represents
554 around 20% of total sugars (Koroney et al., 2016). *D. solani* reallocated part of its
555 resources to synthesize carbon-rich molecules: cellulose (*celY* and *bcs* genes),
556 antibiotic oocydin A (*ooc* genes) and an aryl polyene (*ape* genes). This suggests a
557 trade-off between proliferation and response to biotic and abiotic stresses. In *D.*
558 *dadantii*, cellulose synthesis is associated with formation of biofilm at the air-liquid
559 interface in carbon-rich culture medium (Mee-Ngan et al., 2005). On roots, the
560 formation of a cellulose biofilm, hence the fitness associated to *bcs* genes, would
561 strongly depend on the abundance of carbon resources and moisture. Variations in
562 these environmental parameters could explain heterogeneity in CI values that we
563 observed when the $\Delta bcsA$ mutant challenged a wild type strain (Figure 6). In *D.*
564 *solani* Ds0432.1, *ooc* genes are involved in antibiosis against several Ascomyceta:
565 *Botrytis cinerea*, *Magnaporthe oryzae* and *Sclerotinia sclerotiorum* (Effantin et al.,
566 2021). Aryl polyenes are present in numerous bacterial genera throughout the
567 Proteobacteria and Bacteroidetes, often in host-associated bacteria (Schöner et al.,
568 2016). Aryl polyenes share a remarkably similar chemical scaffold, consisting of an
569 aryl head group conjugated to a polyene carboxylic acid tail. The biosynthetic
570 pathway is well characterized in several genera, including *Escherichia*, *Vibrio* and
571 *Xenorhabdus* (Schöner et al., 2016; Johnston et al., 2021). This pigment molecule is
572 exported to the outer membrane. In *E. coli*, it contributes to oxidative stress
573 resistance (H₂O₂) and biofilm formation.

574 The role of *pst* genes is remarkable. In *D. solani*, we observed that a Δ *apeH* mutant
575 was impaired in competitive colonization of roots and persistence in horticultural
576 compost (Figures 6 and 7). In addition, we observed that this locus was important for
577 colonization of progeny-tubers, hence vertical transmission of the pathogens from
578 plant host to its progeny (Figure 7). In different pathogens, *pst* genes are involved in
579 phosphate importation as well as in several phosphate-regulated processes including
580 virulence and adherence to surfaces (Daigle et al., 1995; Runyen-Janecky et al.,
581 2005; Röder et al., 2021). In *D. solani* IPO2222, *pstB* was shown to be expressed in
582 the presence of pieces of roots and tubers (Czajkowski et al., 2020), in agreement
583 with an important role in the underground lifestyle of this pathogen.

584

585 Overall, this work highlights the versatile lifestyle of *D. solani* through its interaction
586 with the potato host plant: a copiotrophic lifestyle in plant lesions exploits abundant
587 carbon sources and faces plant defense compounds while an oligotrophic lifestyle on
588 roots exploits less abundant, but more diverse carbon resources and competes with
589 microbiota. Several traits and genes related to the underground behavior and
590 proliferation in lesions remain to be investigated further (using complementation of
591 mutants, gene expression monitoring or structure-function analysis of the encoded
592 proteins) to understand how *D. solani* pathogens efficiently survive on roots, persist
593 in the environment and colonize progeny-tubers.

594

595

596 **Supporting information**

597 S1 Table. Genome annotation of the *D. solani* RNS 08.23.3.1A genome (Excel file)

598 S2 Table. Tn-seq analyses of the *D. solani* Tn-mutant library (Excel file)

599 S3 Table. Primers used for DNA amplification

600 S4 Table. Accession numbers of Tn-Seq data (.bam files) (Excel file)

601 S1 Fig. Position of TA sites in the genome of *Dickeya solani* RNS 08.23.3.1A
602 S1 Appendix. Media for bacterial cultures
603 S2 Appendix. Construction of the Tn-library in *D. solani* RNS 08.23.3.1.A Rif^R
604 S3 Appendix. Construction of the deletion mutant in *D. solani* RNS 08.23.3.1.A Rif^R Gm^R
605 S4 Appendix. Construction of the *D. solani* RNS 08.23.3.1.A Rif^R Gm^R
606 S5 Appendix. Calculation of competitive index in competition assays
607

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619

620 **Author contribution**

621 DF and KR conceived experiments; KR constructed the Tn-library in *D. solani* and
622 prepared the DNA samples that were sequenced by DN at the I2BC platform; KR,
623 GE and EG constructed the deletion mutants; KR performed inoculation of Tn-library
624 and deletion mutants in tuber, root and culture medium conditions; KR and EM in the
625 stem condition. KR performed statistical analyses; KR and DF analyzed the list
626 fitness genes and wrote the manuscript.

627

628 **Data accessibility**

629 Tn-seq .bam files of the 42 compared conditions were deposited as sequence read
630 archives (SRA) in the bioproject PRJNA939571 at the National Center for
631 Biotechnology Information (SAMN33482656 ; SAMN33482657 ; SAMN33482658 ;
632 SAMN33482659 ;SAMN33482660 ; SAMN33482661 ; SAMN33482662 ; SAMN33482663 ;
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640 are available in Supporting Information for the article (tables S1 and S2).

641

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646

647 **Declarations**

648 The funders had no role in study design, data collection and analysis, decision to
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651

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839

840

841 **Table 1 Bacterial strains and plasmids**

Bacterial strains and plasmids	Characteristics	Reference
RNS 08.23.3.1A	<i>Dickeya solani</i> wild type	Khayi et al., 2018
RNS 08.23.3.1A Rif ^R	RNS 08.23.3.1A stain with spontaneous mutation of the <i>rpoB</i> gene conferring resistance to Rif	This study
RNS 08.23.3.1A Rif ^R -Gm ^R	RNS 08.23.3.1A Rif ^R strain with containing a Gm ^R cassette at the Tn7 insertion site	This study
$\Delta bcsA$	Deletion of gene <i>bcsA</i> (DS0823_v1_2374) in the RNS 08.23.3.1A Rif ^R -Gm ^R strain	This study
$\Delta dppA$	Deletion of gene <i>dppA</i> (DS0823_v1_2392) in the RNS 08.23.3.1A Rif ^R Gm ^R strain	This study
$\Delta pstA$	Deletion of gene <i>pstA</i> (DS0823_v1_2527) in the RNS 08.23.3.1A Rif ^R Gm ^R strain	This study
$\Delta apeH$	Deletion of gene <i>apeH</i> (DS0823_v1_2513) in the RNS 08.23.3.1A Rif ^R Gm ^R strain	This study
<i>Escherichia coli</i> MFD _{pir}	RP4-2-Tc::(Δ Mu1::aac(3)IV- Δ aphA-- Δ nic35- Δ Mu2::zeo) Δ dapA::erm-pir) Δ recA	Jackson et al., 2020
<i>Escherichia coli</i> DH5 λ pir	λ pir phage lysogen of DH5 α	Laboratory collection
pSAM-Ec	Suicide mobilizable vector; Amp ^R , Km ^R ; Km-resistance gene bordered by mariner inverted repeat sequence containing MmeI restriction site; himar1-C9 transposase gene under the control of <i>P_{lac}</i> .	Wiles et al., 2013
pRE112	Suicide vector for allelic exchange in <i>D. solani</i> ; Cm ^R , <i>sacB</i> , <i>oriT</i> RP4	Edwards et al., 1998
pMobile-CRISPRi_1	Plasmid encoding mobilizable transposase Tn7	Peters et al., 2019
pTn7-M	Suicide vector for Gm-resistance gene insertion at Tn7 site; Km ^R , Gm ^R , <i>oriR6K</i> , <i>Tn7L</i> and <i>Tn7R</i> extremities, multiple cloning site, <i>oriT</i> RP4	Zobel et al., 2015

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845

846 **Figure legends**

847

848 **Figure 1. Characteristics of the Tn-library in *Dickeya solani*.**

849 The position and abundance of Tn insertions in the circular chromosome of *D. solani*
850 RNS 08.23.3.1A Rif^R under TY rich medium condition are shown. A) Number of
851 genes according to percentage of mutated TA sites per gene. B) From the outside to
852 the inside, the tracks represent: forward and reverse coding sequences in blue color;
853 Log₁₀ of number of Tn insertions per TA site for each gene in black; EL-
854 ARTIST analysis that classified genes in three categories: 'non-essential genes' in
855 red, 'genes with an essential domain' in yellow and 'essential genes' in green. In the
856 center, circle chart shows the total number of 'non-essential genes' (3,829), 'essential
857 genes' (521) and 'genes with an essential domain' (146). Examples of 'essential
858 genes' are indicated.

859

860 **Figure 2: *Dickeya solani* pathways associated with competitive growth on plant**
861 **cell wall components.**

862 This scheme exemplifies fitness genes pinpointed by Tn-seq (a complete list is
863 available in Table S2) when the *D. solani* Tn-library was grown in M9 minimal
864 medium with distinctive plant cell wall components as carbon and energy sources. In
865 the schematic pathways, enzymes encoded by the identified genes are indicated in
866 red; in the red box, crosses indicate for each enzyme in which growth condition they
867 were identified using Tn-seq: pectin, glucuronate, galacturonate and galactarate in
868 M9 minimal medium, as well as *in planta* conditions: roots and rotted tubers and
869 stems. DGHD: 3-deoxy-D-glycero-hexo-2,5 diulosonate; KDPG: 2-keto-3-deoxy-6-
870 phosphogluconate; KDG: 2-keto-3-deoxy-gluconate.

871

872 **Figure 3: Genome map of *Dickeya solani* genes associated with competitive**
873 **growth on roots and lesions in stems and tubers of *Solanum tuberosum*.**

874 From the outside to the inside, the tracks represent: forward and reverse coding
875 sequence (in blue) and fitness genes (complete lists available in table S2) when *D.*
876 *solani* grew in macerated stems (green circle), in macerated tubers (yellow circle)
877 and on roots (orange circle). The Venn diagram represents a comparison of fitness
878 genes between the three *in planta* conditions. Some examples of fitness genes are
879 indicated, such as a large cluster of motility genes that are important under
880 macerated stem conditions, *arcAB* and *cyoABCDE* genes under macerated stem and
881 tuber conditions, and the four clusters associated to fitness in roots condition,
882 including *ddp*, *ape*, *pst*, *bcs*, *dpp* and *ooc* genes.

883

884 **Figure 4: *Dickeya solani* pathways associated with competitive growth on roots**
885 **and lesions in stems and tubers of *Solanum tuberosum*.**

886 From left to right, pictures and arrows indicate the plant tissues that are colonized by
887 *D. solani* and analyzed by Tn-seq. For each condition, some genes and pathways
888 are indicated (complete lists of fitness genes are available in Table S2). NA indicates
889 that genes were not associated with a variation of fitness in a given condition
890 according to Tn-seq analyses.

891

892 **Figure 5: Virulence of the *Dickeya solani* constructed mutants $\Delta dppA$, $\Delta apeH$,**
893 **$\Delta pstA$ and $\Delta bcsA$.**

894 Five days after infection in *S. tuberosum* stems, we measured length of lesions in cm
895 (A) and enumerated *D. solani* pathogens (CFU/g of macerated tissues) (B). Two

896 independent mutants of each genotype $\Delta dppA$, $\Delta apeH$, $\Delta pstA$ and $\Delta bcsA$ were
897 analyzed (20 infected stems per genotype) and compared to *D. solani* RNS
898 08.23.3.1A Rif^R-Gm^R (10 infected stems). The strain *D. solani* RNS 08.23.3.1A Rif^R-
899 Gm^R is indicated as WT Rif-Gm in the graph. Uninoculated stems (T-) did not
900 develop symptoms. In A, Kruskal-Wallis test compared the six conditions (Kruskal-
901 Wallis statistic = 50.52; p-value <0,0001; DF=5) and then Dunn test compared
902 symptom severity of each mutant genotype with that of *D. solani* RNS 08.23.3.1A
903 Rif^R-Gm^R. The p-value of the Dunn test is shown: all *D. solani* genotypes caused
904 similar symptoms. In B, center lines show the medians; the crosses, the mean
905 values; box limits are the 25th and 75th percentiles; whiskers extend 1.5 times the
906 interquartile distance; outliers are represented by dots. The statistical analysis was
907 carried out by a Kruskal-Wallis test (Kruskal-Wallis statistic = 54.20; p-value <0.0001;
908 DF=5) followed by a Dunn test to compare each mutant genotype with *D. solani* RNS
909 08.23.3.1A Rif^R-Gm^R. The p-values of the Dunn test are indicated on the graph: all *D.*
910 *solani* genotypes reached a similar abundance in lesions.

911

912 **Figure 6: Competitiveness of the *D. solani* constructed mutants $\Delta dppA$, $\Delta apeH$,**
913 **$\Delta pstA$ and $\Delta bcsA$ in root colonization assays.**

914 Two independent clones of each genotype $\Delta dppA$, $\Delta apeH$, $\Delta pstA$ and $\Delta bcsA$ and *D.*
915 *solani* RNS 08.23.3.1A Rif^R-Gm^R (indicated as WT Rif-Gm) were challenged with
916 their ancestor *D. solani* RNS 08.23.3.1A Rif^R in root colonization assays. Competitive
917 index (CI) values were determined in 10 inoculated plants for each constructed clone
918 (hence 20 per mutant genotype) and 10 plants for the RNS 08.23.3.1A Rif^R-Gm^R.
919 Center lines show the medians; crosses, the mean value; box limits indicate the 25th
920 and 75th percentiles as determined by Prism software; whiskers extend from the

921 minimum to maximum values. Statistical analysis was performed with the
922 Kruskal-Walis test (Kruskal-Walis statistic = 37.64; p-value <0.0001; DF = 4) and
923 then CI values of the mutants were compared with those of RNS 08.23.3.1A Rif^R-
924 Gm^R (post hoc Dunn's multiple comparison tests).

925

926 **Figure 7: Underground behavior of the *Dickeya solani* constructed mutants**
927 **$\Delta dppA$, $\Delta apeH$, $\Delta pstA$ and $\Delta bcsA$.**

928 Two independent mutants of each genotype $\Delta dppA$, $\Delta apeH$, $\Delta pstA$ and $\Delta bcsA$ and *D.*
929 *solani* RNS 08.23.3.1A Rif^R-Gm^R (indicated as WT Rif-Gm) were inoculated into
930 potato plant pots by watering. Eight weeks later, progeny-tubers were harvested from
931 20 plants per mutant genotype and 10 plants inoculated by RNS 08.23.3.1A Rif^R-
932 Gm^R. The percentage (%) of plants exhibiting blackleg symptoms was determined
933 (A) as well as abundance of pathogens in asymptomatic progeny-tuber (B). In C,
934 pathogens were enumerated in compost in the vicinity of the roots. Statistical
935 analyses were performed with the Kruskal-Walis test : in A, Kruskal-Walis statistic =
936 16.09; p-value= 0.0066; DF = 5; in B, Kruskal-Walis statistic = 48.20; p-
937 value<0.0001; DF = 5; in C, Kruskal-Walis statistic = 48.97; p-value< 0.0001; DF = 5.
938 The p-values of the comparisons between each mutant genotype and RNS
939 08.23.3.1A Rif^R-Gm^R (post hoc Dunn's multiple comparison tests) are indicated on
940 the graph.