



**HAL**  
open science

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

Kévin Robic, Euphrasie Munier, Géraldine Effantin, Joy Lachat, Delphine Naquin, Erwan Gueguen, Denis Faure

### ► To cite this version:

Kévin Robic, Euphrasie Munier, Géraldine Effantin, Joy Lachat, Delphine Naquin, et al.. Dissimilar gene repertoires of *Dickeya solani* involved in colonization of lesions and roots of *Solanum tuberosum*. *Frontiers in Plant Science*, 2023, 14, pp.1154110. <10.3389/fpls.2023.1154110>. <hal-04247985>

**HAL Id: hal-04247985**

**<https://hal.science/hal-04247985v1>**

Submitted on 18 Oct 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

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

**Short title: Versatile lifestyle of *Dickeya solani***

Kévin Robic<sup>1,2</sup>, Euphrasie Munier<sup>1</sup>, Géraldine Effantin<sup>3</sup>, Joy Lachat<sup>2</sup>, Delphine Naquin<sup>2</sup>, Erwan Gueguen<sup>3</sup>, Denis Faure<sup>2</sup>.

<sup>1</sup> French Federation of Seed Potato Growers (FN3PT/inov3PT), Paris, France

<sup>2</sup> Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France

<sup>3</sup> Univ Lyon, Université Claude Bernard Lyon1, CNRS, INSA Lyon, UMR5240 MAP, F-69622, Lyon, France

Corresponding author: Denis Faure

email: denis.faure@i2bc.paris-saclay.fr

## 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 oocydin (*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  $\Delta$ *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  $\beta$ -proteobacteria and  $\gamma$ -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*, *bcsA* 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<sup>R</sup>) 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<sup>R</sup> 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  $2.3 \times 10^{10}$  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<sub>600nm</sub> 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<sub>600nm</sub> 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<sub>600nm</sub> of the Tn-mutant  
208 library were injected into the stem in the axil of the 4<sup>th</sup> 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<sub>600nm</sub> 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<sup>R</sup>-Gm<sup>R</sup> 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<sup>R</sup>-Gm<sup>R</sup> 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<sup>R</sup> Gm<sup>R</sup>),  
307 root colonization assays were performed in the presence of the ancestral strain *D.*  
308 *solani* RNS 08.23.3.1A Rif<sup>R</sup>. Competition between the ancestor *D. solani* RNS  
309 08.23.3.1A Rif<sup>R</sup> and its derivative *D. solani* RNS 08.23.3.1A Rif<sup>R</sup>-Gm<sup>R</sup> was used as  
310 control to evaluate the potential bias caused by the Gm<sup>R</sup>-cassette.

311 The *D. solani* RNS 08.23.3.1A Rif<sup>R</sup> 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<sub>600nm</sub>  
313 was adjusted to 1 unit (10<sup>9</sup> CFU/mL). Each Rif<sup>R</sup> Gm<sup>R</sup> strain was mixed with the Rif<sup>R</sup>  
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<sup>R</sup> and  
323 *D. solani* RNS 08.23.3.1A Rif<sup>R</sup>-Gm<sup>R</sup> (mutant strains), CI values greater than one  
324 indicated a fitness advantage of the RNS 08.23.3.1A Rif<sup>R</sup>-Gm<sup>R</sup> mutant strain, and CI  
325 values below one indicated a fitness advantage of RNS 08.23.3.1A Rif<sup>R</sup>.

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<sup>R</sup>-Gm<sup>R</sup> 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<sup>R</sup>-Gm<sup>R</sup>. For all statistical  
349 analyses and coefficient calculation, we used GraphPad Prism version 9.3.0,  
350 GraphPad Software ([www.graphpad.com](http://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 oocycin (*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<sup>R</sup>). A control  
470 competition between RNS 08.23.3.1A Rif<sup>R</sup>-Gm<sup>R</sup> and RNS 08.23.3.1A Rif<sup>R</sup> 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<sup>R</sup> and RNS 08.23.3.1A Rif<sup>R</sup>-Gm<sup>R</sup> 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<sup>R</sup>-Gm<sup>R</sup> versus RNS 08.23.3.1A Rif<sup>R</sup>). 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<sup>R</sup>-Gm<sup>R</sup> versus  
484 RNS 08.23.3.1A Rif<sup>R</sup> (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<sup>R</sup>Gm<sup>R</sup> used as a reference) was inoculated separately  
490 by watering the roots with 10<sup>10</sup> 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<sup>R</sup>Gm<sup>R</sup> 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<sup>R</sup>Gm<sup>R</sup> 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<sup>R</sup>Gm<sup>R</sup> 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<sup>R</sup>Gm<sup>R</sup> (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 10<sup>5</sup> CFU/g of  
513 roots; a copiotroph lifestyle in lesions where *D. solani* proliferation reached 10<sup>9</sup> to  
514 10<sup>10</sup> 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 oocidin 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 (H<sub>2</sub>O<sub>2</sub>) and biofilm formation.

574 The role of *pst* genes is remarkable. In *D. solani*, we observed that a  $\Delta$ *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<sup>R</sup>  
604 S3 Appendix. Construction of the deletion mutant in *D. solani* RNS 08.23.3.1.A Rif<sup>R</sup> Gm<sup>R</sup>  
605 S4 Appendix. Construction of the *D. solani* RNS 08.23.3.1.A Rif<sup>R</sup> Gm<sup>R</sup>  
606 S5 Appendix. Calculation of competitive index in competition assays  
607

## 608 **Acknowledgements**

609 KR was supported by PhD and post-PhD grants from FN3PT/innov3P. This work has  
610 benefited from the facilities and expertise of the high-through-put sequencing  
611 platform and the plant culture facilities at I2BC (Gif-sur-Yvette). This work was  
612 supported by CNRS (SE2016-2017-2018-2019), French Federation of Seed Potato  
613 Growers (FN3PT/innov3PT) and LabEx Saclay Plant Sciences-SPS (ANR-10-LABX-  
614 0040-SPS). The LABGeM (CEA/Genoscope & CNRS UMR8030), the France  
615 Génomique and French Bioinformatics Institute national infrastructures (funded as  
616 part of Investissement d'Avenir program managed by Agence Nationale pour la  
617 Recherche, contracts ANR-10-INBS-09 and ANR-11-INBS-0013) are acknowledged  
618 for support within the MicroScope annotation platform.

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 ;  
633 SAMN33482664 ; SAMN33482665 ; SAMN33482666 ; SAMN33482667 ; SAMN33482668 ;  
634 SAMN33482669 ; SAMN33482670 ; SAMN33482671 ; SAMN33482672 ; SAMN33482673 ;  
635 SAMN33482674 ; SAMN33482675 ; SAMN33482676 ; SAMN33482677 ; SAMN33482678 ;  
636 SAMN33482679 ; SAMN33482680 ; SAMN33482681 ; SAMN33482682 ; SAMN33482683 ;  
637 SAMN33482684 ; SAMN33482685 ; SAMN33482686 ; SAMN33482687 ; SAMN33482688 ;  
638 SAMN33482689 ; SAMN33482690 ; SAMN33482691 ; SAMN33482692 ; SAMN33482693 ;  
639 SAMN33482694 ; SAMN33482695 ; SAMN33482696 ; SAMN33482697). All the annotation data  
640 are available in Supporting Information for the article (tables S1 and S2).

641

642 **ORCID**

643 Denis Faure <https://orcid.org/0000-0002-5379-8867>

644 Erwan Gueguen <https://orcid.org/0000-0001-8784-1491>

645 Delphine Naquin <https://orcid.org/0000-0001-9174-4686>

646

647 **Declarations**

648 The funders had no role in study design, data collection and analysis, decision to  
649 publish, or preparation of the manuscript.

650 The authors have no conflicts of interest to declare.

651

652 **References**

- 653 Al-Karablieh, N., Weingart, H., Ullrich, M.S. (2009). The outer membrane protein TolC is  
654 required for phytoalexin resistance and virulence of the fire blight pathogen *Erwinia amylovora*.  
655 *Microb. Biotechnol.* 2, 465–475. doi:10.1111/j.1751-7915.2009.00095.x
- 656 Blin, K., Shaw, S., Kloosterman, A.M., Charlop-Powers, Z., Wezel, G.P.V., Medema, M.H., et al.  
657 (2021). AntiSMASH 6.0: Improving cluster detection and comparison capabilities. *Nucleic Acids*  
658 *Research* 49: W29–W35. doi:10.1093/nar/gkab335
- 659 Blin, P., Robic, K., Khayi, S., Cigna, J., Munier, E., Dewaegeneire, P., et al. (2021). Pattern and  
660 causes of the establishment of the invasive bacterial potato pathogen *Dickeya solani* and of the  
661 maintenance of the resident pathogen *D. dianthicola*. *Mol. Ecology* 30, 608–624.  
662 doi:10.1111/mec.15751
- 663 Bolger, A.M., Lohse, M., Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina  
664 sequence data. *Bioinformatics* 30, 2114–2120. doi:10.1093/bioinformatics/btu170
- 665 Bouvier, J.T., Sernova, N.V., Ghasempur, S., Rodionova, I.A., Vetting, M.W., Al-Obaidi, N.F., et  
666 al. (2019). Novel metabolic pathways and regulons for hexuronate utilization in Proteobacteria. *J.*  
667 *Bacteriol.* 201(2), e00431-18. doi:10.1128/JB.00628-18
- 668 Cole, B.J., Feltcher, M.E., Waters, R.J., Wetmore, K.M., Mucyn, T.S., Ryan, E.M., et al. (2017).  
669 Genome-wide identification of bacterial plant colonization genes. *PLoS Biology* 15, 1–24.  
670 doi:10.1371/journal.pbio.2002860
- 671 Czajkowski, R., de Boer, W.J., Velvis, H., van der Wolf, J.M. (2010). Systemic colonization of  
672 potato plants by a soilborne, green fluorescent protein-tagged strain of *Dickeya* sp. Biovar 3.  
673 *Phytopathology* 100, 134-142. doi: 10.1094/PHYTO-100-2-0134
- 674 Czajkowski, R., Fikowicz-Krosko, J., Maciag, T., Rabalski, L., Czaplewska, P., Jafra, S., et al.  
675 (2020). Genome-wide identification of *Dickeya solani* transcriptional units up-regulated in response to  
676 plant tissues from a crop-host *Solanum tuberosum* and a weed-host *Solanum dulcamara*. *Front. Plant*  
677 *Sci.* 11, 580330. doi:10.3389/fpls.2020.580330
- 678 Daigle, F., Fairbrother, J.M., Harel, J. (1995). Identification of a mutation in the *pst-phoU* operon  
679 that reduces pathogenicity of an *Escherichia coli* strain causing septicemia in pigs. *Infect Immun.* 63,  
680 4924–4927. doi:10.1128/iai.63.12.4924-4927
- 681 Duong, D.A., Jensen, R.V., Stevens, A.M. (2018). Discovery of *Pantoea stewartii* ssp. *stewartii*  
682 genes important for survival in corn xylem through a Tn-Seq analysis. *Mol. Plant Pathology* 19, 1929–  
683 1941. doi:10.1111/mpp.12669
- 684 Duprey, A., Nasser, W., Léonard, S., Brochier-Armanet, C., Reverchon, S. (2016).  
685 Transcriptional start site turnover in the evolution of bacterial paralogous genes - the *pelE-pelD*  
686 virulence genes in *Dickeya*. *FEBS J.* 283, 4192–4207. doi:10.1111/febs.13921
- 687 Edwards, R.A., Keller, L.H., Schifferli, D.M. (1998). Improved allelic exchange vectors and their  
688 use to analyze 987P fimbria gene expression. *Gene* 207, 149–157. doi:10.1016/S0378-  
689 1119(97)00619-7
- 690 Effantin, G., Brual, T., Rahbé, Y., Hugouvieux-Cotte-Pattat, N., Gueguen, E. (2021). *Dickeya*  
691 *solani* D s0432-1 produces an arsenal of secondary metabolites with anti-prokaryotic and anti-  
692 eukaryotic activities against bacteria, yeasts, fungi, and aphids. bioRxiv.  
693 doi:10.1101/2021.07.19.452942
- 694 Galperin, M.Y., Makarova, K.S., Wolf, Y.I., Koonin, E.V. (2015). Expanded microbial genome  
695 coverage and improved protein family annotation in the COG database. *Nucleic Acids Research* 43,  
696 D261–D269. doi:10.1093/nar/gku1223

697 Golanowska, M., Potrykus, M., Motyka-Pomagruk, A., Kabza, M., Bacci, G., Galardini, M., et al.  
698 (2018). Comparison of highly and weakly virulent *Dickeya solani* strains, with a view on the  
699 pangenome and panregulon of this species. *Front Microbiol.* 9, 1940. doi:10.3389/fmicb.2018.01940

700 Gonzalez-Mula, A., Lachat, J., Mathias, L., Naquin, D., Lamouche, F., Mergaert, P., et al.  
701 (2019). The biotroph *Agrobacterium tumefaciens* thrives in tumors by exploiting a wide spectrum of  
702 plant host metabolites. *New Phytologist* 222, 455–467. doi:10.1111/nph.15598

703 Hélias, V., Hamon, P., Huchet, E., Wolf, J.V.D., Andrivon, D. (2012). Two new effective  
704 semiselective crystal violet pectate media for isolation of *Pectobacterium* and *Dickeya*. *Plant*  
705 *Pathology* 61, 339–345. doi:10.1111/j.1365-3059.2011.02508.x

706 Helmann, T.C., Deutschbauer, A.M., Lindow, S.E. (2019). Genome-wide identification of  
707 *Pseudomonas syringae* genes required for fitness during colonization of the leaf surface and apoplast.  
708 Proceedings of the National Academy of Sciences of the United States of America. 116, 18900–18910.  
709 doi:10.1073/pnas.1908858116

710 Helmann, T.C., Filiatrault, M.J., Stodghill, P.V. (2022). Genome-wide identification of genes  
711 important for growth of *Dickeya dadantii* and *Dickeya dianthicola* in Potato (*Solanum tuberosum*)  
712 tubers. *Front Microbiol.* 13, 778927. doi:10.3389/fmicb.2022.778927

713 Hooven, T.A., Catomeris, A.J., Akabas, L.H., Randis, T.M., Maskell, D.J., Peters, S.E., et al.  
714 (2016). The essential genome of *Streptococcus agalactiae*. *BMC Genomics* 17, 406. doi:  
715 10.1186/s12864-016-2741-z.

716 Jackson, S.A., Fellows, B.J., Fineran, P.C. (2020). Complete genome sequences of the  
717 *Escherichia coli* donor strains ST18 and MFD *pir*. Putonti C, editor. *Microbiol Resour Announc.*  
718 9,e01014-20. doi:10.1128/MRA.01014-20

719 Johnston, I., Osborn, L.J., Markley, R.L., McManus, E.A., Kadam, A., Schultz, K.B., et al. (2021)  
720 Identification of essential genes for *Escherichia coli* aryl polyene biosynthesis and function in biofilm  
721 formation. *Biofilms Microbiomes.* 7, 56. doi:10.1038/s41522-021-00226-3

722 Khayi, S., Blin, P., Chong, T.M., Robic, K., Chan, K.G., Faure, D. (2018). Complete genome  
723 sequences of the plant pathogens *Dickeya solani* RNS 08.23.3.1.A and *Dickeya dianthicola* RNS04.9.  
724 *Genome Announcements* 6(4),e01447-17. doi: 10.1128/genomeA.01447-17

725 Khayi, S., Blin, P., Pédrón, J., Chong, T.M., Chan, K.G., Moumni, M., et al. (2015). Population  
726 genomics reveals additive and replacing horizontal gene transfers in the emerging pathogen *Dickeya*  
727 *solani*. *BMC Genomics.* 16, 788. doi:10.1186/s12864-015-1997-z

728 Koroney, A.S., Plasson, C., Pawlak, B., Sidikou, R., Driouich, A., Menu-Bouaouiche, L., et al.  
729 (2016). Root exudate of *Solanum tuberosum* is enriched in galactose-containing molecules and  
730 impacts the growth of *Pectobacterium atrosepticum*. *Ann Bot.* 118, 797–808. doi:10.1093/aob/mcw128

731 Kuivanen, J., Biz, A., Richard, P. (2019). Microbial hexuronate catabolism in biotechnology.  
732 *AMB Express.* 9(1), 16. doi: 10.1186/s13568-019-0737-1

733 Langmead, B., Trapnell, C., Pop, M., Salzberg, S.L. (2009). Ultrafast and memory-efficient  
734 alignment of short DNA sequences to the human genome. *Genome Biology* 10(3),R25. doi:  
735 10.1186/gb-2009-10-3-r25

736 Liu, Z., Beskrovnyaya, P., Melnyk, R.A., Hossain, S.S., Khorasani, S., O'Sullivan, L.R., et al.  
737 (2018). A genome-wide screen identifies genes in rhizosphere-associated *Pseudomonas* required to  
738 evade plant defenses. *mBio* 9(6), e00433-18. doi: 10.1128/mBio.00433-18

739 López-Solanilla, E., García-Olmedo, F., Rodríguez-Palenzuela, P. (1998). Inactivation of the  
740 *sapA* to *sapF* locus of *Erwinia chrysanthemi* reveals common features in plant and animal bacterial  
741 pathogenesis. *Plant Cell* 10, 917–924. doi:10.1105/tpc.10.6.917

- 742 Luneau, J.S., Baudin, M., Quiroz-Monnens, T., Carrère, S., Bouchez, O., Jardinaud, F., et al.  
743 (2022). Genome-wide identification of fitness determinants in the *Xanthomonas campestris* bacterial  
744 pathogen during early stages of plant infection. *New Phytologist* 236, 235-248. doi:  
745 10.1111/nph.18313
- 746 Macho, A.P., Guidot, A., Barberis, P., Beuzón, C.R., Genin, S. (2010). A competitive index  
747 assay identifies several *Ralstonia solanacearum* type III effector mutant strains with reduced fitness in  
748 host plants. *Mol. Plant-Microbe Interactions* 23, 1197–1205. doi:10.1094/MPMI-23-9-1197
- 749  
750 Matilla, M.A., Stöckmann, H., Leeper, F.J., Salmond, G.P.C. (2012). Bacterial biosynthetic gene  
751 clusters encoding the anti-cancer haterumalide class of molecules. *J. Biol. Chemistry*. 287, 39125–  
752 39138. doi:10.1074/jbc.M112.401026
- 753 Matilla, M.A., Monson, R.E., Murphy, A., Schicketanz, M., Rawlinson, A., Duncan, C., Mata, J.,  
754 Leeper, F., Salmond, G.P.C. (2022). Solanimycin: biosynthesis and distribution of a new antifungal  
755 antibiotic regulated by two quorum-sensing systems. *mBio* 13(6),e0247222. doi: 10.1128/mbio.02472-  
756 22.
- 757 Mee-Ngan, Y., Yang, C.H., Barak, J.D., Jahn, C.E., Charkowski, A.O. (2005). The *Erwinia*  
758 *chrysanthemi* Type III secretion system is required for multicellular behavior. *J Bacteriol.* 187, 639–  
759 648. doi:10.1128/JB.187.2.639-648
- 760 Morinière, L., Mirabel L, Gueguen E, Bertolla F. A comprehensive overview of the genes and  
761 functions required for lettuce infection by the hemibiotrophic phytopathogen *Xanthomonas hortorum*  
762 pv. *vitiensis*. *mSystems*. 2022;7: e01290-21. doi:10.1128/msystems.01290-21
- 763 Olson, E.R., Duniak, D.S., Jurss, L.M. (1991). Identification and characterization of *dppA*, an  
764 *Escherichia coli* gene encoding a periplasmic dipeptide transport protein. *J. Bacteriol.* 173(1), 234-44.  
765 doi: 10.1128/jb.173.1.234-244.1991
- 766 Pédrón, J., Schaerer, S., Kellenberger, I., Van Gijsegem, F. (2021). Early emergence of  
767 *Dickeya solani* revealed by analysis of *Dickeya* diversity of potato blackleg and soft rot causing  
768 pathogens in Switzerland. *Microorganisms*. 9, 1187. doi:10.3390/microorganisms9061187
- 769 Peters, J.M., Koo, B.M., Patino, R., Heussler, G.E., Hearne, C.C., Qu, J., et al. (2019). Enabling  
770 genetic analysis of diverse bacteria with Mobile-CRISPRi. *Nature Microbiology* 4, 244–250.  
771 doi:10.1038/s41564-018-0327-z
- 772 Potrykus, M., Golanowska, M., Hugouvieux-Cotte-Pattat, N., Lojkowska, E. (2015). Regulators  
773 involved in *Dickeya solani* virulence, genetic conservation, and functional variability. *Mol. Plant*  
774 *Microbe Interactions* 1, 57-68. doi: 10.1094/MPMI-99-99-0003-R.
- 775 Potrykus, M., Hugouvieux-Cotte-Pattat, N., Lojkowska, E. (2018). Interplay of classic Exp and  
776 specific Vfm quorum sensing systems on the phenotypic features of *Dickeya solani* strains exhibiting  
777 different virulence levels. *Mol. Plant Pathology*. 19, 1238–1251. doi:10.1111/mp.12614
- 778 Prigent-Combaret, C., Zghidi-Abouzid, O., Effantin, G., Lejeune, P., Reverchon, S., Nasser, W.  
779 (2012). The nucleoid-associated protein Fis directly modulates the synthesis of cellulose, an essential  
780 component of pellicle–biofilms in the phytopathogenic bacterium *Dickeya dadantii*. *Mol. Microbiol.* 86,  
781 172–186. doi:10.1111/j.1365-2958.2012.08182.x
- 782 Pritchard, J.R., Chao, M.C., Abel, S., Davis, B.M., Baranowski, C., Zhang, YJ, et al. (2014).  
783 ARTIST: high-resolution genome-wide assessment of fitness using transposon-insertion sequencing.  
784 *PLoS Genetics* 10, e1004782. doi:10.1371/journal.pgen.1004782
- 785 Raoul des Essarts, Y., Pédrón, J., Blin, P., Van Dijk, E., Faure, D., Van Gijsegem, F. (2019).  
786 Common and distinctive adaptive traits expressed in *Dickeya dianthicola* and *Dickeya solani*  
787 pathogens when exploiting potato plant host. *Environ. Microbiol.* 21, 1004–1018. doi:10.1111/1462-  
788 2920.14519

- 789 Röder, J., Felgner, P., Hensel, M. (2021). Single-cell analyses reveal phosphate availability as  
790 critical factor for nutrition of *Salmonella enterica* within mammalian host cells. *Cellular Microbiology* 10,  
791 e13374. doi: 10.1111/cmi.13374
- 792 Royet, K., Parisot, N., Rodrigue, A., Gueguen, E., Condemine, G. (2019). Identification by Tn-  
793 seq of *Dickeya dadantii* genes required for survival in chicory plants. *Mol. Plant Pathology* 20, 287–  
794 306. doi:10.1111/mpp.12754
- 795 Runyen-Janecky, L.J., Boyle, A.M., Kizzee, A., Liefer, L., Payne, S.M. (2005). Role of the Pst  
796 system in plaque formation by the intracellular pathogen *Shigella flexneri*. *Infect. Immun.* 73, 1404–  
797 1410. doi:10.1128/IAI.73.3.1404-1410
- 798 Schöner, T.A., Gassel, S., Osawa, A., Tobias, N.J., Okuno, Y., Sakakibara, Y., et al. (2016).  
799 Aryl Polyenes, a highly abundant class of bacterial natural products, are functionally related to  
800 antioxidative carotenoids. *ChemBioChem.* 17, 247–253. doi:10.1002/cbic.201500474
- 801 Schütze A, Benndorf D, Püttker S, Kohrs F, Bettenbrock K. The impact of *ackA*, *pta*, and *ackA*-  
802 *pta* mutations on growth, gene expression and protein acetylation in *Escherichia coli* K-12. *Front*  
803 *Microbiol.* 2020;11: 233. doi:10.3389/fmicb.2020.00233
- 804 Sivakumar, R., Ranjani, J., Vishnu, U.S., Jayashree, S., Lozano, G.L., Miles, J., et al. (2019).  
805 Evaluation of INSeq to identify genes essential for *Pseudomonas aeruginosa* PGPR2 corn root  
806 colonization. *G3 (Bethesda)* 9,651-661. doi: 10.1534/g3.118.200928
- 807 Su, Y., Xu, Y., Liang, H., Yuan, G., Wu, X., Zheng, D. (2021). Genome-wide identification of  
808 *Ralstonia solanacearum* genes required for survival in tomato plants. *mSystems* 6, e00838-21.  
809 doi:10.1128/mSystems.00838-21
- 810 Tervo, C.J., Reed, J.L. (2012). FOCAL: an experimental design tool for systematizing metabolic  
811 discoveries and model development. *Genome biology*, 13, R116. doi:10.1186/gb-2012-13-12-r116
- 812 Torres, M., Jiquel, A., Jeanne, E., Naquin, D., Dessaux, Y., Faure D. (2021). *Agrobacterium*  
813 *tumefaciens* fitness genes involved in the colonization of plant tumors and roots. *New Phytologist*  
814 233,905-918. doi: 10.1111/nph.17810
- 815 Valecillos, A.M., Palenzuela, P.R., López-Solanilla, E. (2006). The role of several multidrug  
816 resistance systems in *Erwinia chrysanthemi* pathogenesis. *Mol. Plant-Microbe Interactions* 19, 607–  
817 613. doi:10.1094/MPMI-19-0607
- 818 van der Wolf, J.M., Acuña, I., De Boer, S.H., Brurberg, M.B., Cahill, G., Charkowski, A.O., et al.  
819 (2021). Diseases Caused by *Pectobacterium* and *Dickeya* Species Around the World. In: Van  
820 Gijsegem F, van der Wolf JM, Toth IK, editors. plant diseases caused by *dickeya* and *Pectobacterium*  
821 species. Cham: Springer International Publishing; pp. 215–261. doi:10.1007/978-3-030-61459-1\_7
- 822 van der Wolf, J.M., Nijhuis, E.H., Kowalewska, M.J., Saddler, G.S., Parkinson, N., Elphinstone,  
823 J.G., et al. (2014). *Dickeya solani* sp. nov., a pectinolytic plant-pathogenic bacterium isolated from  
824 potato (*Solanum tuberosum*). *Int. J. Syst. Evol. Microbiol.* 64, 768–774. doi:10.1099/ijs.0.052944-0
- 825 Van Gijsegem, F., Hugouvieux-Cotte-Pattat, N., Kraepiel, Y., Lojkowska, E., Moleleki, L.N.,  
826 Gorshkov, V., et al. (2021). Molecular Interactions of *Pectobacterium* and *Dickeya* with Plants. In: Van  
827 Gijsegem F, van der Wolf JM, Toth IK, editors. Plant diseases caused by *Dickeya* and *Pectobacterium*  
828 Species. Cham: Springer International Publishing; pp. 85–147. doi:10.1007/978-3-030-61459-1\_4
- 829 Wiles, T.J., Norton, J.P., Russell, C.W., Dalley, B.K., Fischer, K.F., Mulvey, M.A. (2013).  
830 Combining quantitative genetic footprinting and trait enrichment analysis to identify fitness  
831 determinants of a bacterial pathogen. *PLoS Genetics* 9(8), e1003716. doi:  
832 10.1371/journal.pgen.1003716

833           Zhou, J., Zhang, H., Wu, J., Liu, Q., Xi, P., Lee, J., et al. (2011). A novel multidomain polyketide  
834 synthase is essential for zeamine production and the virulence of *Dickeya zea*. *Mol. Plant-Microbe*  
835 *Interactions*. 24,1156–1164. doi:10.1094/MPMI-04-11-0087

836           Zobel, S., Benedetti, I., Eisenbach, L., De Lorenzo, V., Wierckx, N., Blank, L.M. (2015). Tn7-  
837 based device for calibrated heterologous gene expression in *Pseudomonas putida*. *ACS Synthetic*  
838 *Biology* 4, 1341–1351. doi:10.1021/acssynbio.5b00058

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 <sup>R</sup>	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 <sup>R</sup> -Gm <sup>R</sup>	RNS 08.23.3.1A Rif <sup>R</sup> strain with containing a Gm <sup>R</sup> 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 <sup>R</sup> -Gm <sup>R</sup> strain	This study
$\Delta dppA$	Deletion of gene <i>dppA</i> (DS0823_v1_2392) in the RNS 08.23.3.1A Rif <sup>R</sup> Gm <sup>R</sup> strain	This study
$\Delta pstA$	Deletion of gene <i>pstA</i> (DS0823_v1_2527) in the RNS 08.23.3.1A Rif <sup>R</sup> Gm <sup>R</sup> strain	This study
$\Delta apeH$	Deletion of gene <i>apeH</i> (DS0823_v1_2513) in the RNS 08.23.3.1A Rif <sup>R</sup> Gm <sup>R</sup> strain	This study
<i>Escherichia coli</i> MFD $\lambda$ pir	RP4-2-Tc::( $\Delta$ Mu1::aac(3)IV- $\Delta$ aphA-- $\Delta$ nic35- $\Delta$ Mu2::zeo) $\Delta$ dapA::erm-pir) $\Delta$ recA	Jackson et al., 2020
<i>Escherichia coli</i> DH5 $\lambda$ pir	$\lambda$ pir phage lysogen of DH5 $\alpha$	Laboratory collection
pSAM-Ec	Suicide mobilizable vector; Amp <sup>R</sup> , Km <sup>R</sup> ; Km-resistance gene bordered by mariner inverted repeat sequence containing MmeI restriction site; himar1-C9 transposase gene under the control of <i>P<sub>lac</sub></i> .	Wiles et al., 2013
pRE112	Suicide vector for allelic exchange in <i>D. solani</i> ; Cm <sup>R</sup> , <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 <sup>R</sup> , Gm <sup>R</sup> , <i>oriR6K</i> , <i>Tn7L</i> and <i>Tn7R</i> extremities, multiple cloning site, <i>oriT</i> RP4	Zobel et al., 2015

842

843

844

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<sup>R</sup> 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 Log<sub>10</sub> 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<sup>R</sup>-Gm<sup>R</sup> (10 infected stems). The strain *D. solani* RNS 08.23.3.1A Rif<sup>R</sup>-  
899 Gm<sup>R</sup> 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<sup>R</sup>-Gm<sup>R</sup>. 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 25<sup>th</sup> and 75<sup>th</sup> 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<sup>R</sup>-Gm<sup>R</sup>. 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: Competitivity 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<sup>R</sup>-Gm<sup>R</sup> (indicated as WT Rif-Gm) were challenged with  
916 their ancestor *D. solani* RNS 08.23.3.1A Rif<sup>R</sup> 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<sup>R</sup>-Gm<sup>R</sup>.  
919 Center lines show the medians; crosses, the mean value; box limits indicate the 25<sup>th</sup>  
920 and 75<sup>th</sup> 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<sup>R</sup>-  
924 Gm<sup>R</sup> (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<sup>R</sup>-Gm<sup>R</sup> (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<sup>R</sup>-  
932 Gm<sup>R</sup>. 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<sup>R</sup>-Gm<sup>R</sup> (post hoc Dunn's multiple comparison tests) are indicated on  
940 the graph.