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1 ***Agrobacterium tumefaciens* fitness genes involved in the colonization of plant**
2 **tumors and roots**

3

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27 **Summary**

28 • *Agrobacterium tumefaciens* colonizes the galls (plant tumors) it causes, and the roots
29 of host and non-host plants.

30 • Transposon-sequencing (Tn-Seq) was used to discover *A. tumefaciens* genes
31 involved in reproductive success (fitness genes) on *Solanum lycopersicum* and *Populus*
32 *trichocarpa* tumors and *S. lycopersicum* and *Zea mays* roots.

33 • The identified fitness genes represent 3 to 8% of *A. tumefaciens* genes and contribute
34 to carbon and nitrogen metabolism, synthesis and repair of DNA, RNA and proteins and
35 envelope-associated functions. Competition assays between twelve knockout mutants and
36 wild-type confirmed the involvement of ten genes (*trpB*, *hisH*, *metH*, *cobN*, *ntrB*, *trxA*, *nrdJ*,
37 *kamA*, *exoQ*, *wbbL*) in *A. tumefaciens* fitness under both tumor and root conditions. The
38 remaining two genes (*fecA*, *noxA*) were important in tumors only. None of these mutants was
39 non-pathogenic, but four (*hisH*, *trpB*, *exoQ*, *ntrB*) exhibited impaired virulence. Finally, we
40 used this knowledge to search for chemical and biocontrol treatments that target some of the
41 identified fitness pathways and report reduced tumorigenesis and impaired establishment of
42 *A. tumefaciens* on tomato roots using tannic acid or *Pseudomonas protegens*, which affect
43 iron assimilation.

44 • This work revealed *A. tumefaciens* pathways that contribute to its competitive survival
45 in plants and highlights a strategy to identify plant protection approaches against this
46 pathogen.

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48

49 **Keywords:** *Agrobacterium*, biocontrol, maize, poplar, root, Tn-Seq, tomato, gall

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51

52 **Introduction**

53 The pathogen *Agrobacterium tumefaciens* provokes irreversible damage on plants via
54 genetic modification of the host leading to formation of galls, also called plant tumors
55 (Pitzschke & Hirt, 2010; Gordon & Christie, 2014). *A. tumefaciens* pathogenesis requires two
56 successive steps: host transformation and gall development, also called tumorigenesis
57 (Escobar & Dandekar, 2003). Under favorable conditions (plant wounding), the pathogen
58 transfers part of its tumor inducing (Ti) plasmid, the T-DNA, into the genome of infected plant
59 cells (Pitzschke & Hirt, 2010; Gordon & Christie, 2014; Gelvin, 2017). This DNA transfer
60 involves a type IV secretion system encoded by the *vir* (virulence) genes of the Ti plasmid
61 (Gordon & Christie, 2014) . Several chromosomal virulence (*chv*) genes of *A. tumefaciens*
62 contribute to severity of the symptoms (Garfinkel & Nester, 1980). In the plant host, genetic
63 screens revealed loci that modulate tumor formation (Nam *et al.*, 1999; Gelvin, 2017). The
64 precise mechanisms by which T-DNA is integrated into host DNA are still under investigation
65 (Gelvin, 2021).

66 Once T-DNA is integrated and expressed, the host cells are functionally
67 reprogrammed to proliferate (hence causing a gall) and to produce specific metabolites,
68 called opines, resulting from the enzymatic condensation of sugars and organic and amino
69 acids. Omics analyses of plant tumors provoked by *A. tumefaciens* on *Arabidopsis thaliana*
70 revealed extensive reshaping of the gene expression profile in host and pathogen cells
71 (Deeken *et al.*, 2006; Efetova *et al.*, 2007; Lee *et al.*, 2009; Gohlke *et al.*, 2013; González-
72 Mula *et al.*, 2018). In galls, opines (e.g. octopine, nopaline) contribute to the maintenance
73 and dissemination of the Ti plasmid in *Agrobacterium* populations in two complementary
74 ways (reviewed by Barton *et al.*, 2018; Dessaux & Faure, 2018): (i) opines are carbon and
75 nitrogen sources used by agrobacteria carrying the Ti plasmid, hence they confer a selective
76 advantage to pathogenic agrobacteria; and (ii) some opines (called conjugative opines) act
77 as signals to activate horizontal transfer of the Ti plasmid by bacterial conjugation, hence
78 disseminating the virulence genes. In addition to opines, plant tumors accumulate a wide
79 spectrum of primary metabolites, including sugars (e.g. sucrose, fructose, glucose) and

80 organic and amino acids (e.g. pyruvate, glutamine, proline) that may also be used as
81 nutrients by *A. tumefaciens* (Deeken *et al.*, 2006; Gonzalez-Mula *et al.*, 2019). Some plasmid
82 and chromosomal genes involved in the assimilation of opines and other tumor metabolites
83 contribute to reproductive success (fitness) of *A. tumefaciens* in plant tumors (Lang *et al.*,
84 2014, 2017; Gonzalez-Mula *et al.*, 2019; Meyer *et al.*, 2019).

85 *Agrobacterium* populations are common although not abundant within the root
86 microbiota of hosts and non-hosts (Bednářová *et al.*, 1979; Bouzar *et al.*, 1993; Gomes *et al.*,
87 2001; Krimi *et al.*, 2002; Vogel *et al.*, 2003; Sanguin *et al.*, 2006). Some of the cell surface
88 components and regulatory pathways that promote attachment and biofilm formation of *A.*
89 *tumefaciens* on roots and inanimate surfaces have been characterized extensively (Heindl *et*
90 *al.*, 2014; Matthyse, 2018; Thompson *et al.*, 2018; Onyeziri *et al.*, 2021). Research on
91 *Agrobacterium* biofilm formation and surface attachment are relevant to develop methods
92 preventing root colonization (Heindl *et al.*, 2014).

93 Almost 400 plant species are listed as hosts for agrobacteria, which rank amongst the
94 most promiscuous plant pathogenic clades (Bradbury, 1986). Millions of dollars of economic
95 losses are associated with crown gall disease every year (Puławska, 2010). Some disease
96 control procedures exist (Escobar & Dandekar, 2003) for limiting sensitivity of the plant host,
97 eliminating pathogenic *A. tumefaciens* using biocontrol agents such as *Agrobacterium* K84
98 and preventing environmental conditions conducive to infection (mostly preventing plant
99 wounding). Still, pathogenic *A. tumefaciens* strains that are resistant to the K84 biocontrol
100 agent are not uncommon (Moore & Warren, 1979; Burr *et al.*, 1993). In the future, crown-gall
101 disease management is expected to progress thanks to an increased knowledge of plant
102 defense mechanisms (a recent example by Fürst *et al.*, 2020) and pathogen traits involved in
103 plant colonization.

104 Decades of research on *A. tumefaciens* have produced a solid corpus of knowledge
105 on T-DNA transfer, tumorigenesis and root colonization. Yet, a global, comparative picture of
106 the *A. tumefaciens* fitness genes involved in the competitive colonization of tumors and roots
107 of host and non-host plants is still missing. To address this question, we used transposon-

108 sequencing (Tn-Seq). Tn-Seq allows genome-wide screening of the microbial Tn-mutants
109 which are impaired in their relative fitness. Tn-Seq can pinpoint fitness genes and pathways
110 that allow the pathogen to competitively survive and proliferate in a given environment (Van
111 Opijnen & Camilli, 2013; Kwon *et al.*, 2016; Cain *et al.*, 2020). This approach was already
112 used in *A. tumefaciens* to determine essential and fitness genes in different culture media
113 (Curtis & Brun, 2014; Gonzalez-Mula *et al.*, 2019), but not yet in plant tumors and roots.

114 We report here inoculation of a Tn-mutant population of *A. tumefaciens* strain C58
115 into the roots and wounded stem of the host plant *Solanum lycopersicum* to identify and
116 compare fitness genes involved in the colonization of roots and tumors. In order to identify a
117 common set of fitness genes allowing *A. tumefaciens* to cope with diverse plant conditions,
118 we extended our Tn-Seq approach to tumors of a ligneous host, *Populus trichocarpa*, and
119 roots of a non-host plant, *Zea mays*. We then constructed knockout (KO) mutants for twelve
120 identified fitness genes to measure the effects on fitness under four plant conditions: tomato
121 roots, tomato tumors, poplar tumors and maize roots. Finally, we focused on the tomato plant
122 host to discuss genes and pathways involved in competitive colonization of roots and tumors
123 to evaluate the potential link between the identified fitness genes and virulence and to test
124 whether fitness genes and pathways can provide targets for developing plant protection
125 strategies.

126

127 **Materials and methods**

128 *Bacterial strains and culture conditions*

129 *A. tumefaciens* strain C58 (also named *A. fabrum* C58), whose genome sequence is
130 available (Goodner *et al.*, 2001; Wood *et al.*, 2001), was cultivated at 28°C in tryptone yeast
131 (TY) medium (Beringer, 1974). *Escherichia coli* strains were cultivated at 37°C in lysogenic
132 broth (Sambrook *et al.*, 1989) modified medium (LBm; 5 g l⁻¹ sodium chloride instead of 10 g
133 l⁻¹). The biocontrol *Pseudomonas protegens* (formerly *P. fluorescens*) CHA0 and its
134 derivative CHA400, which is defective for the synthesis of the siderophore pyoverdine (Stutz
135 *et al.*, 1986; Keel *et al.*, 1989), were grown at 28°C in LBm. Media were supplemented when

136 appropriate with gentamicin (Gm; 25 $\mu\text{g ml}^{-1}$), kanamycin (Km; 50 $\mu\text{g ml}^{-1}$), ampicillin (Amp;
137 50 $\mu\text{g ml}^{-1}$), rifampicin (Rif; 100 $\mu\text{g ml}^{-1}$) and cycloheximide (200 $\mu\text{g ml}^{-1}$). We used a *Himar1*-
138 based transposon mutant library that was constructed in a Rif^R derivative of *A. tumefaciens*
139 C58 grown in TY medium by Gonzalez-Mula et al. (Gonzalez-Mula *et al.*, 2019). Aliquots of
140 the library (stored at -80°C) were thawed in 5 ml of liquid TY medium for 4 h at 28°C.
141 Bacteria were centrifuged (3,000 $\times g$ for 10 minutes) and washed twice with the same
142 volume of a 0.8% NaCl solution, and then optical density (OD₆₀₀) was adjusted to 1 (*ca.* 10⁹
143 cells per ml) before plant infection.

144

145 *Plant materials and infection with the transposon library*

146 *Solanum lycopersicum* (seeds provided by Vilmorin, France), *Zea mays* (seeds provided by
147 Arvallis, France) and *Populus trichocarpa* (all cuttings were prepared from a single tree
148 growing in the CNRS campus garden, Gif-sur-Yvette, France) were cultivated in 2-liter pots
149 filled with non-sterile Floradur B horticultural substrate (Floragard, Oldenburg, Germany).
150 Before planting, the horticultural substrate contained $2.4 \times 10^7 \pm 0.8 \times 10^7$ CFU g⁻¹ of total
151 bacteria, according to bacterial counting on TY medium supplemented with cycloheximide.
152 The pots were placed in a non-sterile greenhouse (biosafety level 2) under long day (16 h)
153 conditions and controlled temperature (24-26°C). Fifty 4-week-old tomato plants and fifty 3-
154 week-old maize plants were infected in the soil by *ca.* 10⁹ *A. tumefaciens* cells. Roots were
155 inoculated by watering the soil with 1 ml of the bacterial suspension at OD₆₀₀=1 diluted in 30
156 ml of sterile water. Two hundred 4-week-old tomato plants and two hundred and thirty 12-
157 week-old poplar cuttings were incised with a scalpel between the first and second nodes (1
158 cm-long incision). The wounded stems were inoculated with a cell suspension (~10 μl)
159 obtained after centrifugation of 1 ml of the initial bacterial suspension at OD₆₀₀=1, hence by
160 *ca.* 10⁹ *A. tumefaciens* cells.

161

162 *Recovery and enumeration of Tn-populations from plants*

163 Five weeks after infection, tumors and roots were collected and weighed. Roots and tumors
164 were crushed in 20 ml of a 0.8% NaCl solution during 1 min using a laboratory blender
165 (Waring, Stamford, USA). The homogenate was filtered through miracloth (Millipore, Bedford,
166 USA) and then plated on TY agar plates supplemented with Rif, Gm and the fungicide
167 cycloheximide for enumerating Tn-mutants and on TY agar with cycloheximide for
168 enumerating total bacteria (Table **S1**). The *A. tumefaciens* Tn-mutants reached $3.8 \times 10^6 \pm$
169 0.5×10^6 and $7.9 \times 10^5 \pm 4.1 \times 10^5$ CFU g⁻¹ of tumor fresh weight (FW) in tomato and poplar
170 tumors, respectively. In the roots, the *A. tumefaciens* Tn-mutants reached $8.5 \times 10^3 \pm 3.6 \times 10^3$
171 and $4.6 \times 10^4 \pm 0.3 \times 10^4$ CFU g⁻¹ of root FW of maize and tomato plants, respectively. The *A.*
172 *tumefaciens* Tn-mutants represented a minority fraction (~0.5%) of the total culturable
173 bacteria collected from maize and tomato roots and counted on TY agar plates
174 supplemented with cycloheximide only. In contrast, *A. tumefaciens* Tn-mutants represented
175 almost all the cultured bacteria in tomato and poplar tumors.

176 To enlarge the size of the collected Tn-populations, tomato tumors were grouped into
177 9 replicates, poplar tumors into 10 replicates, tomato roots into 8 replicates and maize roots
178 into 9 replicates. Each replicate consisted of 20-25 tumors (hence 10-20 g) or 15-20 g of root
179 systems which were crushed together. Cell suspension was spread onto ten TY agar plates
180 (plates 12 × 12 cm) supplemented with Gm and Rif and cycloheximide. After growth at 28°C
181 for 48 h, all the bacterial cells were recovered from the surface of the TY plates, centrifuged
182 (3,000 × g for 3 minutes) and stored at -20°C for further DNA manipulation. Each of these
183 replicates represented $3.8 \times 10^7 \pm 0.8 \times 10^7$ CFU of Tn-mutants for tomato tumors, $7.3 \times 10^6 \pm$
184 3.3×10^6 CFU for poplar tumors, $4.5 \times 10^5 \pm 0.6 \times 10^5$ CFU for tomato roots and $1.1 \times 10^5 \pm$
185 0.5×10^5 CFU for maize roots (Table **S1**). All these values were at least equal to the threshold
186 value of 1.1×10^5 , which is given by the total number of Tn-insertion sites along the *A.*
187 *tumefaciens* C58 genome, therefore limiting a potential bottleneck effect.

188

189 *DNA isolation, transposon library sequencing and analysis*

190 Experimental procedures, primers and sequence analysis were described previously
191 (Gonzalez-Mula *et al.*, 2019; see Notes **S1**).

192

193 *Competition and virulence assays with the constructed KO mutants*

194 A Gm-resistance cassette (Dennis & Zylstra, 1998) was used for construction of knockout
195 (KO) mutants in *A. tumefaciens* C58 (Notes **S2**). Competition between *A. tumefaciens* C107-
196 Km^R Rif^R and each of the constructed KO mutants (Gm^R Rif^R) was performed in TY liquid
197 medium and *in planta*. Competitive index (CI) values were calculated using at least 16
198 replicates per condition following the formula described by Macho *et al.* (Macho *et al.*, 2010)
199 and normalized by the colonization ratio of the control strains *A. tumefaciens* C107-Gm^R Rif^R
200 and C107-Km^R Rif^R.

201 Virulence assays were carried out on tomato plants and quantified by weighing the
202 tumors (g). Bacterial abundance (CFU per g of tumor FW) was also measured. At least eight
203 plants were infected by each bacterial genotype as described previously (Gonzalez-Mula *et*
204 *al.*, 2019).

205

206 *Treatments to prevent host colonization and tumorigenesis*

207 Chemical, biological and combined antagonistic assays against *A. tumefaciens* were
208 conducted *in vitro* and *in planta*. Potential antagonistic compounds (levulinic acid, 3-
209 indoleacrylic acid, tannic acid and aminoethylcysteine) and biocontrol strain *P. protegens*
210 CHA0 targeting some of the plant colonization pathways found in this work were identified by
211 literature review as indicated in the Notes **S3** (Fildes, 1941; Meller & Gassman, 1981; Stutz
212 *et al.*, 1986; Keel *et al.*, 1989; Miller *et al.*, 2001; Henry *et al.*, 2016). To challenge these
213 compounds, root colonization and tumorigenesis assays were performed (Notes **S3**).

214

215 **Results and discussion**

216 *Genome-wide scan of fitness genes in A. tumefaciens living on plants*

217 We used a genome-wide scan by Tn-Seq to discover genes involved in the fitness of *A.*
218 *tumefaciens* C58 when it colonizes plants cultivated under non-gnotobiotic conditions. In the
219 tomato host, we identified 216 fitness genes important for root colonization and 428 for gall
220 colonization, 159 being common between the two conditions (lists of the fitness genes in
221 Table **S2**). Because *A. tumefaciens* colonizes a wide spectrum of host and non-host plants,
222 we searched for common fitness genes involved in the exploitation of a wider spectrum of
223 plants. Tn-Seq revealed 155 fitness gene involved in maize root colonization and 214 in
224 poplar tumor colonization, giving a sum of 560 genes whatever the condition (tomato root
225 and tumor, poplar tumor and maize root). Among them, 62 genes were important for *A.*
226 *tumefaciens* fitness under the four investigated conditions, highlighting key-pathways for
227 exploiting roots or galls of diverse plants (Table **S2**).

228 In *A. tumefaciens* C58, most of the identified fitness genes were located on the
229 circular and linear chromosomes and a few (18 out of 560) on At and Ti plasmids (Fig. 1 and
230 Table **S2**). To identify the pathways involved in plant colonization, we used the NCBI, UniProt
231 and KEGG annotations of the *A. tumefaciens* C58 genome (Goodner *et al.*, 2001; Wood *et*
232 *al.*, 2001). Out of the 560 identified fitness genes, 466 (83%) were annotated in databases,
233 while the 94 remaining genes (17%) coded for hypothetical proteins. Considering each of the
234 four studied conditions (tomato tumors, tomato roots, poplar tumors and maize roots), fitness
235 genes represented 3 to 8% of genes in the *A. tumefaciens* C58 genome. These percentages
236 were in the same range (1 to 7%) as those found in the literature for other plant-colonizing
237 bacteria: competitive nodulation by *Rhizobium leguminosarum* (Wheatley *et al.*, 2020),
238 colonization of leaf surface and apoplast by *Pseudomonas syringae* (Helmann *et al.*, 2019,
239 2020), root by beneficial *Pseudomonas* (Cole *et al.*, 2017; Liu *et al.*, 2018; Sivakumar *et al.*,
240 2019), soil and root by plant growth-promoting *Azoarcus olearius* and *Herbaspirillum*
241 *seropedicae* (do Amaral *et al.*, 2020), xylem by *Pantoea stewartii* (Duong *et al.*, 2018) and
242 macerated chicory leaves by *Dickeya dadantii* (Royet *et al.*, 2019).

243 As with any global approach, the Tn-Seq method encounters some limitations. It
244 cannot consider genes coding for redundant functions and those coding for the synthesis of

245 secreted components or activities that could be shared as public goods by the Tn-population.
246 In *A. tumefaciens*, potential public goods could encompass biosynthesis of soluble
247 compounds (e.g. siderophore) and polymers for building extracellular matrix (e.g. cellulose),
248 as well as any processes that engineer the surrounding environment of the Tn-population
249 such as detoxification (e.g. ferulic acid and H₂O₂) and tumorigenesis (Matthysse & McMahan,
250 1998; Xu & Pan, 2000; Escobar & Dandekar, 2003; Rondon *et al.*, 2004; Campillo *et al.*,
251 2014). The Tn-Seq approach excludes essential genes (non-viable Tn-mutants), which were
252 estimated by Tn5-mutagenesis to be 360 in *A. tumefaciens* C58 growing in LB rich medium
253 (Curtis & Brun, 2014). In addition, identification of a given fitness gene in a tested growth
254 condition may be biased if fitness of its Tn-mutants is already impaired in the initial culture
255 condition (here, TY medium). In *A. tumefaciens* C58, Tn-mutants of ≈500 genes are impaired
256 in their viability or fitness in TY rich medium (Gonzalez-Mula *et al.*, 2019). Hence, Tn-Seq
257 underestimates the total number of fitness genes in a microorganism. Additional biases may
258 be caused by polar effects of transposon insertions within an operon, although it has been
259 shown that such biases are negligible in a number of microorganisms (Van Opijnen *et al.*,
260 2009; Rubin *et al.*, 2015; Hutchison *et al.*, 2019).

261 To validate candidate fitness genes identified by Tn-Seq, we constructed KO mutants
262 for twelve of them: *trpB*, *hisH*, *ntrB*, *metH*, *cobN*, *noxA*, *trxA*, *nrdJ*, *kamA*, *exoQ*, *fecA* and
263 *wbbL*. Then, we experimentally evaluated the relative fitness of these KO mutants and
264 calculated a competitive index (CI) in TY culture medium and after plant inoculations (Fig. 2
265 and Notes S4). From these data, we conclude that our Tn-Seq experimental data represent
266 quite a solid overview of the main fitness genes that contribute to survival of *A. tumefaciens*
267 in plants.

268 In the following paragraphs, we focus on tomato plant to compare root and tumor
269 conditions in a same plant host. In order to avoid an over interpretation of our Tn-Seq data,
270 we highlight pathways that were revealed by a coherent set of fitness genes. For each of the
271 main processes, which are (i) carbon and nitrogen metabolism, (ii) synthesis and repair of

272 DNA, RNA and proteins, and (iii) bacterial envelope functions, the common (tomato root and
273 tumor conditions) and then tumor- and root-specific genes and pathways are discussed.

274

275 *Fitness genes involved in nutrient exploitation in tomato host plant*

276 The common fitness genes associated with carbon and nitrogen metabolism (Table 1, Fig. 3,
277 Fig. S1) reflect the capacity of the biotroph *A. tumefaciens* to exploit the tomato plant as a
278 resource (nutrient and energy), especially via the catabolism of some sugars, organic acids
279 and amino acids, as well as uptake of essential metals.

280 The identified common fitness genes are implicated in import (*ptsH=atu0030* and
281 *atu0031*) and conversion (*scrK=atu0401* and *pgi=atu0404*) of sugars that are directed to the
282 pentose phosphate and Entner-Doudoroff pathways (*pgl=atu0599*, *zwf=atu0600* and
283 *tal=atu4464*). The Entner-Doudoroff pathway represents the main route for the assimilation of
284 sugars in *A. tumefaciens* (Fuhrer *et al.*, 2005). Glucose (13 nmol mg⁻¹ FW), fructose (9 nmol
285 mg⁻¹ FW) and sucrose (3 nmol mg⁻¹ FW) are the most abundant sugars in tomato plant
286 (Gonzalez-Mula *et al.*, 2019). The identification of *gapA=atu3737* and *atu3740* as common
287 fitness genes could reflect the capacity of *A. tumefaciens* to redirect part of its metabolism to
288 gluconeogenesis by exploiting a diversified range of plant-derived carbon skeletons such as
289 organic acids and amino acids.

290 Metabolic pathways of amino acids, which were important for *A. tumefaciens* fitness
291 in roots and tumors, were involved in either their synthesis (histidine, isoleucine, leucine,
292 methionine, serine, threonine, tryptophan and valine) or degradation (aspartate, asparagine,
293 glutamate and glutamine as precursors of nucleotides, hemes and other amino acids). This
294 synthesis/degradation trade-off could be better understood in the light of the availability of
295 amino acids. The abundant amino acids may be used as preferred nutrients, while less
296 abundant ones need to be synthesized by the pathogen. Our previous metabolomic analyses
297 revealed glutamine (1.93 nmol mg⁻¹ FW), glutamate (0.95 nmol mg⁻¹ FW), asparagine (0.52
298 nmol mg⁻¹ FW) and aspartate (0.44 nmol mg⁻¹ FW) as the most abundant amino acids in
299 tomato tumors. In contrast, serine, threonine, valine, isoleucine, leucine, tryptophan, histidine

300 and methionine ranged from 0.27 to 1.6 10⁻² nmol mg⁻¹ FW, the least abundant being
301 methionine (Gonzalez-Mula *et al.*, 2019). Methionine is also a precursor of an important
302 metabolite, S-adenosylmethionin (SAM). Biosynthesis of methionine by MetH and that of
303 dNTP by NrdJ requires the co-factor cobalamin, containing cobalt. Tn-Seq analysis showed
304 that the genes involved in the biosynthesis of cobalamin were also important for the fitness of
305 *A. tumefaciens* in tumor and root conditions, highlighting a coherent requirement for
306 cobalamin and the two cobalamin-dependent traits. Other rhizobiaceae, such as
307 *Sinorhizobium meliloti*, also require the synthesis of cobalamin and cobalamin-dependent
308 enzymes MetH and NdrJ for survival in plant nodules (Taga & Walker, 2010).

309 In plant tumors, Tn-Seq pinpointed the importance of the glyoxylate shunt
310 (*glcB=atu0047*). This is in agreement with transcriptomics data that we previously collected
311 from *A. tumefaciens* living in *A. thaliana* tumors (González-Mula *et al.*, 2018). The
312 importance of three other metabolites should be underlined as their exploitation required
313 tumor-specific fitness genes: proline, nopaline and spermidine (see Notes **S5**). Overall, the
314 convergence of data on fitness traits obtained by Tn-Seq with the metabolomics data on
315 tomato tumors was fruitful to understand plant exploitation by *A. tumefaciens*. Moreover,
316 galls in *A. thaliana* and *S. lycopersicum* plants exhibit a similar profile of metabolites,
317 including glucose and glutamine as the prominent sources of carbon and nitrogen (Deeken *et*
318 *al.*, 2006; Gonzalez-Mula *et al.*, 2019), supporting the hypothesis that *A. tumefaciens* could
319 use a common set of fitness genes for exploiting tumors in different host plants.

320 With regard to tomato roots, some root-specific fitness genes are involved in the
321 assimilation of organic acids such as pyruvate (*pycA=atu3762*) and malate (*maeB=atu1652*),
322 gluconeogenesis (*eno=atu1426*) and synthesis of glycogen (*glgA1=atu4075*) that may be
323 used in the storage of carbon. The greater importance of organic acids in the root
324 colonization process could reflect their higher abundance (around 10-fold excess) as
325 compared to sugars in tomato roots and root exudates (Kamilova *et al.*, 2006).

326

327 *Fitness genes involved in nucleotide, DNA and RNA processes during tomato plant*
328 *colonization*

329 Common fitness genes which are associated with both tomato root and gall colonization are
330 involved in nucleotide synthesis (Table 1): for instance, initiation of the pyrimidine and purine
331 pathways (*pyrE=atu0400*, *purF=atu1075*) and the last step (*purH=atu2823*) of inosine
332 monophosphate synthesis, a common precursor of GTP and ATP (Table 1, Fig. 3, Fig. S1).
333 Some other common fitness genes are involved in DNA, RNA and protein synthesis and
334 repair (Table 1, Fig. 4, Fig. S1). Among them, the genes *miaA*, *chvD*, *kamA* and *poxA* code
335 for factors that improve the quality and efficiency of translation: they are potential targets for
336 control of pathogen proliferation. The *miaA* gene codes for a tRNA:isopentenyltransferase
337 targeting an adenine near the tRNA anticodon loop (Gray *et al.*, 1992, 1996). Remarkably,
338 isopentenylated tRNA may be degraded to generate the cytokinin isopentenyladenine,
339 providing a small but significant part of the total amount of cytokinins secreted by *A.*
340 *tumefaciens* and contributing to virulence (Gray *et al.*, 1992, 1996). The translational factor
341 ChvD (also named EttA) binds the ribosomal E site and modulates ribosome-tRNA
342 interaction (Chen *et al.*, 2014). The *kamA* gene is involved in transforming L-lysine to β -
343 lysine, and *poxA* is required for β -lysine modification of the translational factor ChvH
344 (elongation factor P). The *chvD* and *chvH* genes were previously shown to be involved in the
345 virulence of *A. tumefaciens* (Peng *et al.*, 2001; Liu *et al.*, 2001) and their orthologs in the
346 virulence of *Salmonella enterica* (Navarre *et al.*, 2010), *Shigella flexneri* (Marman *et al.*,
347 2014) and *Erwinia amylovora* (Klee *et al.*, 2018).

348 Several tumor-specific genes were associated with synthesis of purines
349 (*purCEDKLMNQS=atu0647*, *atu1140*, *atu1141*, *atu1843-atu1845*, *atu1850*, *atu3754*,
350 *atu3755*). This may be explained by a rarefaction of purines and their precursors that are
351 imported from plant tissues into *A. tumefaciens*, and/or by a higher consumption of the
352 purines by *A. tumefaciens* in the tumor environment. For instance, the synthesis of cytokinins
353 by the adenosine phosphate-isopentenyltransferase pathway could represent a purine sink in
354 plant tissues and in *A. tumefaciens* cells (Aloni & Ullrich, 2008). Transcriptomes of

355 *A. tumefaciens*-*A. thaliana* tumors revealed that cytokinin pathways were expressed in both
356 plant and bacterial cells (Deeken *et al.*, 2006; Gonzalez-Mula *et al.*, 2019). Notably,
357 cytokinins contribute to tumorigenesis, as well as improving plant transformation (Sardesai *et*
358 *al.*, 2013). Purines are also used to synthesize co-factors, which could be more important for
359 surviving in tomato tumors than roots. In agreement with this hypothesis, Tn-Seq highlighted
360 two tomato tumor-specific genes involved in the synthesis of thiamine-phosphate
361 (*thiE=atu3750*) and cyclic-GMP/AMP (*cyaA=atu2580*).

362 Other DNA, RNA and protein modification and repair processes were identified as
363 tumor-specific fitness genes (*clpAS1=atu1363-atu1364*, *lonD=atu3697*, *deaD=atu4171*,
364 *rhIE=atu1833*, *pcrA=atu2057*, *uvrABC=atu1513*, *atu2001*, *atu1129*, *mutT=atu3719*). These
365 genes could be involved in the multi-stress (UV, acidic pH and oxidative stress) response of
366 *A. tumefaciens* when it colonizes plant tumors. A mutant of the *A. tumefaciens* protease
367 LonD was previously reported as impaired in cell morphology and virulence (Su *et al.*, 2006).

368 Some root-specific genes are *rbfA (=atu0086)* coding the ribosome factor A, *greA*
369 (*=atu2182*) coding for a transcriptional elongation factor and *ksgA (=atu1103)* coding for
370 methylation of the 16S RNAr.

371

372 *Fitness genes coding for cell envelope-related functions important for tomato plant*
373 *colonization*

374 Bacterial cell envelope protects from environmental stresses (e.g. osmotic stress), it
375 ensures particular functions (e.g. respiration) and its surface components interact with the
376 host cells. Some of the common fitness genes shared by the tomato tumor and root
377 conditions are exemplified in Table 1 (a complete list can be found in Table **S1**). Among
378 them, *chvB=atu2730* is involved in the synthesis of cyclic- β -(1,2)-glucan. A major role of
379 cyclic- β -(1,2)-glucans is to allow normal growth in low ionic strength conditions (Matthysse,
380 2018). It has been shown that an *A. tumefaciens chvB* mutant was impaired in attachment to
381 roots and virulence (Bash & Matthysse, 2002). The putative osmolarity sensor EnvZ,
382 encoded by the common fitness gene *atu2207*, could also contribute to the osmotic

383 response. In relation to exopolysaccharide synthesis, we showed that an *exoQ* (=atu3325)
384 mutant was affected in fitness and virulence (Fig. 2, Fig. 5). In several *Rhizobiaceae*
385 symbionts, *exo* and *ndvB* (ortholog of *chvB*) mutants were impaired in plant colonization and
386 symbiosis (Müller *et al.*, 1993; Gay-Fraret *et al.*, 2012; Mendis *et al.*, 2016), highlighting the
387 importance of these pathways for plant colonization by pathogenic and symbiotic bacteria of
388 the *Rhizobiaceae* family. The *wbbL* (=atu4614) gene codes for the addition of rhamnose to
389 O-antigen (Ma *et al.*, 2002). This gene was proved to be involved in *A. tumefaciens* fitness
390 for plant colonization (Fig. 2). The *wbbL* orthologs are also important for adhesion, virulence
391 and host colonization in the plant pathogen *Xylella fastidiosa* (Clifford *et al.*, 2013) and in the
392 bee symbiont *Snodgrassella alvi* (Powell *et al.*, 2016).

393 Remarkable tumor-specific fitness genes are those involved in siderophore
394 (*tonB*=atu0163, *exbBD*=atu0161-atu1602, *fecABCDEIR*=atu3687-atu3693) and sulfonate
395 (*ssuABC*=atu1884, *atu4155-atu4156*) import, highlighting the importance of these mineral
396 and nutritive resources in the tumor context. In the case of iron import, competitions *in planta*
397 confirmed the Fec system as a crucial pathway for iron exploitation in the tumors (Fig. 2).
398 The structure of this siderophore remains unknown (Rondon *et al.*, 2004). The *fec* genes
399 were shown to be expressed under iron limiting conditions in culture medium as well as in
400 the presence of ferulic acid and in plant tumors (Baude *et al.*, 2016; González-Mula *et al.*,
401 2018). On roots, *A. tumefaciens* could exploit the resource present in the horticultural
402 substrate that is enriched in oligo-elements including iron.

403 Some other tumor-specific fitness genes are associated with cell division
404 (*ftsEX*=atu3603-3607), peptidoglycan synthesis (*mtgA*=atu2720) and membrane ornithine
405 lipid synthesis (*plsC*=atu0355). Membrane ornithine lipids are bacteria-specific lipids that
406 increase as surrogates of phospholipids, allowing the bacteria to adjust membrane properties
407 under environmental stress conditions (Vences-Guzmán *et al.*, 2012). In *Pseudomonas*
408 *aeruginosa* they play an important role in biofilm formation, bacterial persistence and
409 interaction with the host models *Tenebrio molitor* and *Caenorhabditis elegans* (Kim *et al.*,
410 2018). In *Sinorhizobium meliloti* and *Rhizobium tropici*, ornithine lipid deficient mutants

411 showed a delay in nodule formation and symbiotic deficiency, respectively (Weissenmayer *et*
412 *al.*, 2002; Rojas-Jiménez *et al.*, 2005). Additionally, they seem to be required for optimal
413 steady-state amounts of c-type cytochromes in the α -proteobacterium *Rhodobacter*
414 *capsulatus* (Aygün-Sunar *et al.*, 2006). Interestingly, we showed that the genes involved in
415 the synthesis of this type of cytochrome are involved in plant colonization by *A. tumefaciens*
416 (*ctaG*=*atu0770*, *coxABC*=*atu0767-atu0768* and *atu0771*) (Fig. 3, Fig. S1).

417 Some root-specific genes involved in cell envelope-related functions were also
418 identified (Table S1), but they remain poorly characterized in *A. tumefaciens*. These include
419 *atu0188*, *atu0189* and *atu0190* coding for components of an ABC transporter and *atu0424*
420 (= *phoU*) coding for a putative regulatory protein of the phosphate transport system.

421

422 *Most of the fitness genes differed from virulence genes*

423 The Tn-Seq list (Table S2) revealed some fitness genes, such as *chvB* (= *atu2730*), *chvD*
424 (= *atu2125*) and tryptophan auxotrophs (*atu0017-atu0019*, *atu1687-atu1688*), which have
425 been reported as involved in the virulence of *A. tumefaciens* (Liu *et al.*, 2001; Bash &
426 Matthysse, 2002; Sastry *et al.*, 1986). This prompted us to test whether the 12 constructed
427 mutants used in fitness assays were also affected in virulence: gall weight was used to
428 evaluate degree of the pathogenesis. In addition, we measured pathogen abundance in the
429 galls. The *sbmA* mutant was also included in these assays. None of the mutants were non-
430 pathogenic (Fig. 5). Out of the 13 constructed mutants (including *sbmA*), only the *hisH*, *trpB*
431 and *exoQ* mutants induced symptoms exhibiting impaired tumor weight, and the *hisH*, *ntrB*
432 and *trpB* mutants colonized tumors at a lower pathogen abundance compared to wild-type. In
433 the case of the *hisH* and *trpB* auxotrophs, an impaired capacity to survive in plant tumors
434 correlated a decrease of virulence.

435 Noticeably, the *trxA* mutant showed increased bacterial abundance in plant tumors
436 when compared to the wild-type strain in mono-infection assays (Fig.5), but was less
437 competitive than the wild-type in co-infection assays (Fig. 2). An earlier report described the
438 pleiotropic phenotype of a *trxA* mutant in *A. tumefaciens*. It exhibits an increased resistance

439 to H₂O₂ and an increased sensitivity to menadione when compared to wild-type *A.*
440 *tumefaciens* (Vattanaviboon *et al.*, 2007). Because of the variable plant-associated
441 phenotypes associated with the KO mutant, TrxA would not be an appropriate target for
442 controlling *A. tumefaciens* infection.

443

444 *Fitness traits as targets of chemical and biocontrol treatments*

445 We hypothesized that chemical or biocontrol treatments aimed at the pathways
446 identified as important for fitness could limit plant colonization by *A. tumefaciens*. As a proof
447 of concept, the target pathways and potential antagonistic compounds were iron uptake,
448 using the iron chelator tannic acid (Henry *et al.*, 2016), cobalamin synthesis using levulinic
449 acid (Meller & Gassman, 1981), conversion of L-lysine to β-lysine using the specific inhibitor
450 aminoethylcysteine (Miller *et al.*, 2001) and synthesis of tryptophan using 3-indoleacrylic acid
451 (Fildes, 1941). Tannic acid chelates iron outside the bacterial cells, hence its action is not
452 specific to one of the iron importation systems of *A. tumefaciens*. The concentration of
453 inhibitors was chosen according to inhibition assays achieved in culture in AB (Chilton *et al.*,
454 1974) minimal medium and correspond to a growth inhibition of *A. tumefaciens* of 50-90%
455 (Fig. **S2**). Indoleacrylic acid and levulinic acid strongly impaired plant development (Fig. **S3**)
456 and were therefore eliminated from the study. All the other treatments had a moderate
457 impact on tomato plant growth, in comparison with the control (Fig. **S3**). A combination of
458 tannic acid and aminoethylcysteine resulted in a significant reduction (97%) of
459 *A. tumefaciens* root colonization (Fig. **6a**, Fig. **6c**).

460 To impair iron uptake, we also used the biocontrol agent *P. protegens* CHA0 (Stutz
461 *et al.*, 1986), which synthesizes two major siderophores: pyoverdine and enantio-pyochelin.
462 Pyoverdine is the most efficient for iron-scavenging in the soil environment (Drehe *et al.*,
463 2018). Growth inhibition assays using *P. protegens* CHA0 and its pyoverdine-deficient
464 mutant CHA400 confirmed iron scavenging by pyoverdine as a major mechanism of growth
465 inhibition of *A. tumefaciens* by *P. protegens* (Fig. **S4**). *P. protegens* CHA0 and CHA400 were
466 able to colonize tomato roots (Fig. **6b**), but only the siderophore-producer *P. protegens*

467 CHA0 reduced significantly (90%) tomato root colonization by *A. tumefaciens* (Fig. 6a) and
468 its relative abundance in root microbiota (Fig. 6c). The addition of tannic acid did not
469 enhance the biocontrol effect of *P. protegens* CHA0.

470 Finally, we tested whether treatment with *P. protegens*, tannic acid,
471 aminoethylcysteine or a combination of the two chemicals could affect tumorigenesis on
472 wounded roots of tomato plantlets. Tannic acid significantly reduced the percentage of
473 plantlets exhibiting tumors (Fig. 7). Under the tested condition (wounded young plantlets),
474 treatment with aminoethylcysteine was toxic for plant growth, and *P. protegens* CHA0 and
475 CHA400 did not reduce tumorigenesis (Fig. 7). Overall, reducing availability of iron seemed a
476 promising approach for controlling *A. tumefaciens*, either for limiting root colonization using a
477 siderophore-producing biocontrol agent such as *P. protegens* CHA0, or for limiting
478 tumorigenesis using an iron-chelating agent, such as tannic acid. Iron-chelation strategies
479 were previously proposed for control of a wide spectrum of pathogens (reviewed by Ahmed
480 and Holmström, 2014).

481

482 **Conclusions**

483 Although some genes are *a priori* excluded from Tn-seq analysis because of different
484 technical limitations, this approach highlighted a wide set of *A. tumefaciens* C58 fitness
485 genes involved in tomato gall and root colonization processes. Tn-Seq is complementary to
486 other omics approaches. Combination of Tn-Seq with metabolomics was fruitful to associate
487 abundant (e.g. sucrose, proline, glutamate) and scarce (e.g. spermidine, tryptophane,
488 histidine) resources with metabolic pathways involved in the *A. tumefaciens* biotrophic
489 lifestyle. Tn-Seq confirmed opine (nopaline) degradation as the most important Ti plasmid-
490 encoded pathway that contributes to the fitness of *A. tumefaciens* C58 in galls. Tn-Seq also
491 pinpointed envelope-associated functions contributing to metabolite exchange, respiration
492 and cell surface properties. A large set of fitness genes are involved in cell maintenance
493 (DNA, RNA and protein synthesis and repair), revealing the stress exerted by the plant
494 environment on pathogen survival and proliferation. Out of a set of twelve constructed KO

495 mutants of fitness genes, none of them were non-pathogenic and a minority were impaired in
496 their virulence (weight of the galls) and pathogen abundance in galls. The Tn-Seq approach
497 provides new insight on *A. tumefaciens* pathways involved in its interaction with plants. By
498 comparing tomato tumors and roots with poplar tumors and maize roots, this study revealed
499 a common set of fitness genes associated with colonization of different plants and tissue.
500 Other fitness genes appeared more specific to one condition; understanding this specificity
501 will be a future challenge. Finally, the identification of metabolic targets important for
502 pathogen proliferation *in planta* constitutes a potential applied development of the Tn-Seq
503 approach. As proof of concept, our study showed iron chelation to be an efficient approach
504 for impairing tomato root colonization in the presence of the biocontrol agent *P. protegens*
505 CHA0, and tumorigenesis in the presence of tannic acid. Further investigations should
506 evaluate the impact of these treatments on plant microbiota. From our work, additional
507 targets and chemicals may be investigated to control *A. tumefaciens* pathogens.

508

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518

519 **Competing interests**

520 The authors declare no competing financial or non-financial interests.

521

522 **Author contributions**

523 MT, YD and DF designed research; MT, AJ, DN and DF performed research; MT, AJ, EJ, YD
524 and DF analyzed data; MT, YD and DF wrote the paper.

525

526 **Data availability**

527 All the data about transposon sequencing results are available in Table **S2**. Other supporting
528 information for the article is also available online (Table **S1**, Fig. **S1** to **S4** and Notes **S1** to
529 **S5**).

530

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823

824 **Supporting information**

825 Fig. S1 *Agrobacterium tumefaciens* pathways involved in colonization of tomato tumors and
826 roots.

827 Fig. S2 *In vitro* inhibition growth of *Agrobacterium tumefaciens* C58 using antagonists
828 targeting fitness genes.

829 Fig. S3 Effect of antagonists on tomato plant growth and development.

830 Fig. S4 *In vitro* inhibition assay of *Agrobacterium tumefaciens* C58 by *Pseudomonas*
831 *protegens*.

832 Notes S1. DNA isolation, transposon library sequencing and analysis.

833 Notes S2. Mutant construction by reverse genetics.

834 Notes S3. Root colonization and tumorigenesis assays.

835 Notes S4. Validation of the fitness genes by reverse genetics

836 Notes S5. Proline, nopaline and spermidine pathways.

837 Table S1. Average bacterial population and percentage of TA-containing CDS with
838 transposon insertions in Tn-mutant populations recovered from plant assays.

839 Table S2. *Agrobacterium tumefaciens* C58 fitness genes involved in plant colonization (Excel
840 file).

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842

843 **Figure legends**

844

845 **Fig. 1 Genomic location and examples of *A. tumefaciens* fitness genes involved in**
846 **plant colonization.**

847 From outside to inside, the tracks represent: *A. tumefaciens* C58 circular map (in blue) of the
848 four replicons (circular chromosome, linear chromosome, At plasmid and Ti plasmid), forward
849 and reverse coding sequences (in dark blue) and fitness genes identified by Tn-Seq as
850 involved in tomato tumor colonization (in red), poplar tumor colonization (in green), tomato
851 root colonization (in orange) and maize root colonization (in purple). Some examples of
852 common fitness in all four conditions were indicated. A complete list of fitness genes
853 identified in each condition can be found in Table **S2**.

854

855 **Fig. 2 Competitive fitness of the constructed *A. tumefaciens* KO mutants.**

856 Thirteen KO mutants (Gm^R Rif^R) were constructed in *A. tumefaciens* C58: *atu0018* (=trpB),
857 *atu0022* (=trxA), *atu0041* (=hisH), *atu1445* (=ntrB), *atu1733* (=nrdJ), *atu2155* (=metH),

858 *atu2304* (=sbmA) *atu2555* (=kamA), *atu2804* (=cobN), *atu3325* (=exoQ), *atu3687* (=fecA),
859 *atu4614* (=wbbL) and *atu6019* (=noxA). Competitions between each KO mutants (Gm^R Rif^R)
860 and the *A. tumefaciens* C58 derivative C107-Km^R Rif^R carrying the wild-type allele
861 counterpart were performed in 5 conditions : TY medium (in blue color), tomato tumors (in
862 red color), poplar tumors (in green color), tomato roots (in orange color) and maize roots (in
863 violet color). Control condition consisted in competition between *A. tumefaciens* C58
864 derivatives C107-Gm^R Rif^R and C107-Km^R Rif^R. More details on these experiments are given
865 in Notes **S4**. For each condition, competitive index value (CI) was calculated. For boxplots,
866 each dot indicates an outlier beyond the first/third quartiles plus 1.5 times the interquartile
867 range. The central horizontal line indicates the median, the bottom and top edges of the box
868 represent the 25th and 75th percentiles, respectively, and the whiskers extend to the most
869 extreme non-outlier data point. Significant differences with the control were calculated using
870 a one-way ANOVA test coupled with a post-hoc Dunnett test and are shown with asterisks: *,
871 $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

872

873 **Fig. 3 A. *tumefaciens* pathways involved in nutrient exploitation in tomato host plant.**

874 Fitness genes related to carbon and nitrogen metabolism are represented in blue and yellow
875 boxes, respectively. Genes required for tomato tumor colonization are indicated in red, for
876 root colonization in dark orange and for both tomato tumors and roots, in blue. Not all the
877 fitness genes are represented, especially those coding for unknown functions are omitted
878 (see Table **S2** for a complete list). Dashed arrows indicate that several enzymatic steps exist
879 between the two connected metabolites.

880

881 **Fig. 4 A. *tumefaciens* pathways involved in nucleotide, DNA and RNA processes**
882 **during tomato host plant colonization.**

883 Fitness genes related to synthesis and repair of DNA, RNA and proteins are represented in
884 green boxes. Genes required for tomato tumor colonization are indicated in red, for root
885 colonization in dark orange and for both tomato tumors and roots, in blue. Not all the fitness

886 genes are represented, those coding for unknown functions are omitted (see Table **S2** for a
887 complete list). Dashed arrows indicate that several enzymatic steps exist between the two
888 connected metabolites.

889

890 **Fig. 5 Virulence and abundance in tumors of the constructed *A. tumefaciens* KO**
891 **mutants.**

892 Mono-infections of *A. tumefaciens* C58 derivative C107-Gm Rif^R (used as a control) and
893 constructed KO mutants were performed on incised tomato stems and then (a) tumor weight
894 (g of fresh tissues) and (b) colonization (CFU g⁻¹ of fresh tumor tissues) were measured 5
895 weeks post infection. Significant differences with the control were calculated using a one-way
896 ANOVA test coupled with a post-hoc Dunnett test and are shown with asterisks: *, $P < 0.05$;
897 ***, $P < 0.001$.

898

899 **Fig. 6 Limitation of tomato root colonization by targeting *A. tumefaciens* fitness traits.**

900 Tomato roots were infected by *A. tumefaciens* C58 derivative C107-Km^R Rif^R and treated
901 weekly by chemicals, either tannic acid (TA) or aminoethylcysteine (AE) or a mix of them, or
902 biocontrol agents, either *P. protegens* CHA0 or *P. protegens* CHA400 (a CHA0 derivative
903 that is defective for pyoverdine synthesis). A combination of biological and chemical (TA)
904 treatments was also tested. After 4 weeks, *A. tumefaciens* (a) and *P. protegens* (b)
905 populations were counted, as well as total culturable microbiota, to evaluate relative
906 abundance (%) of *A. tumefaciens* on roots (c). For boxplots (a, b), each dot indicates an
907 outlier beyond the first/third quartiles plus 1.5 times the interquartile range. The central
908 horizontal line indicates the median, the bottom and top edges of the box represent the 25th
909 and 75th percentiles, respectively, and the whiskers extend to the most extreme non-outlier
910 data point. In c, error bars show standard deviations. Significant differences with the
911 untreated control were calculated using a one-way ANOVA test coupled with a post-hoc
912 Dunnett test and are shown with asterisks: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

913

914 **Fig. 7 Limitation of tumorigenesis on wounded tomato roots.**

915 Wounded tomato roots (thirty per condition) were infected by *A. tumefaciens* C58 derivative
916 C107-Km^R Rif^R and then placed on half-strength MS medium supplemented with either tannic
917 acid (TA) or biocontrol agents, either *P. protegens* CHA0 or *P. protegens* CHA400.
918 Percentage of roots exhibiting tumors was measured after 4 weeks. Significant differences
919 with the untreated control were calculated using a Fisher's exact test and are shown with
920 asterisks: **, $P < 0.01$.

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932 **Table 1. Examples of common genes and pathways revealed by Tn-Seq as involved in**
933 **fitness of *Agrobacterium tumefaciens* in tomato tumors and roots.**

1. Carbon and nitrogen metabolism

Carbon metabolism

- Sugar metabolism (*scrK=atu0401*, *pgi=atu0404*, *pgl=atu0599*, *zwf=atu0600*, *tal=atu4464*)
 - Gluconeogenesis (*gapA=atu3737*, *atu3740*)
-

1.2. Nitrogen metabolism

- Synthesis of histidine (*hisF=atu0039*, *hisA=atu0040*, *hisH=atu0041*, *hisB=atu0043*, *hisD=atu0537*, *hisZ=atu0678*, *hisG=atu0679*)
- Synthesis of isoleucine/valine (*ilvC=atu2019*, *ilvD=atu3219*, *ilvI=atu2036*) and leucine (*leuA=atu2264*, *leuC=atu2709*, *leuD=atu2790*, *leuB=atu2791*)
- Synthesis of methionine (*hom=atu1588*, *metA=atu2719*, *metZ=atu0432*, *metH=atu2155*, *ahcY=atu0029*)
- Synthesis of serine (*serA=atu3706*, *serC=atu3707*, *serB=atu2040*)
- Synthesis of threonine (*thrB=atu0775*)
- Synthesis of tryptophan (*trpABF=atu0017*, *trpB=atu0018*, *trpF=atu0019*, *trpC=atu1687*, *trpD=atu1688*, *trpE=atu2289*)
- Synthesis of cobalamin (*cobA=atu2794*, *cobM=atu2796*, *cobL=atu2798*, *cobK=atu2799*, *cobJ=atu2800*, *cobI=atu2801*, *cobH=atu2802*, *cobG=atu2803*, *cobN=atu2804*, *cobW=atu2805*, *cobO=atu2807*, *cobQ=atu2808*)

2. Synthesis and repair of DNA, RNA and proteins

2.1. Nucleotide pathways

- Pyrimidine and purine pathways (*pyrE=atu0400*, *purF=atu1075*, *purH=atu2823*)
- Polyphosphate balance (*ppk=atu1144*, *ppx=atu1145*)
- Formation of deoxyribonucleotides (dNTP) from ribonucleotides by the cobalamin-dependant ribonucleotide reductase NrdJ (gene *atu1733*)
- Conversion of dCTP to dUTP (*dcd=atu0434*)
- Synthesis of co-factor molybdopterin (*moaC=atu1689*, *moeA=atu1690*) using GTP

2.2. DNA, RNA and protein synthesis and repair

- DNA synthesis (*polA=atu0110*) and repair (*ruvA=atu3722*, *ruvB=atu3723*, *ruvC=atu3724*, *uvrD=atu0023*)
- Modification and protection of coding and non-coding RNAs (*rluC=atu1471*, *miaA=atu2039*, *dusB=atu1444*, *rnhA=atu0776*)
- Synthesis and quality control of proteins (*kamA=atu2555*, *poxA=atu2554*, *chvD=atu2125*, *rimJ=atu0785*, *ppiD=atu1686*, *dop=atu0977*, *trxA=atu0022*)

3. Bacterial envelope functions

3.1. In/out exchange of components

- Import of mineral resources (*mnhA=atu0909*, *mnhB=atu0910*, *mnhC=atu0911*, *mnhD=atu0912*, *mnhE=atu09013*, *mnhF=atu0914*)
- Efflux of potential toxic compounds (*crcB=atu1470*, *atu2550*, *atu2551*)

3.2. Envelope-associated activities

- Synthesis of respiratory hemes (*ctaB=atu0769*, *ccmA=atu2686*, *ccmB=atu2687*, *ccmC=atu2688*, *ccmG=atu2690*, *ccmI=atu0973*, *ccmE=atu0974*, *ccmF=atu0975*, *ccmH=atu0976*)

3.3. Envelope and surface properties

- Transport of phospholipids to the outer membrane (*miaD=atu1645*, *miaE=1646*, *miaF=atu1647*)
- Exopolysaccharide (*exoN=atu4049*, *exoP=atu4050*, *exoQ=atu3325*, *exoF=atu3326*, *exoY=atu3327*)
- O-antigen (*rfaA=atu4615*, *rfaB=atu4615*, *rfaD=atu4617*, *wbbL=atu4614*)
- Cyclic- β -(1,2)-glucan (*chvB=atu2730*)