



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

Marta Torres, Audren Jiquel, Etienne Jeanne, Delphine Naquin, Yves Dessaux, Denis Faure

## **► To cite this version:**

Marta Torres, Audren Jiquel, Etienne Jeanne, Delphine Naquin, Yves Dessaux, et al.. Agrobacterium tumefaciens fitness genes involved in the colonization of plant tumors and roots. New Phytologist, In press, 10.1111/nph.17810 . hal-03411269

**HAL Id: hal-03411269**

**<https://hal.science/hal-03411269>**

Submitted on 30 Nov 2021

**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.

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

Marta Torres, Audren Jiquel, Etienne Jeanne, Delphine Naquin, Yves Dessaux and Denis Faure\*.

Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91190, Gif-sur-Yvette, France.

\*Corresponding author: Denis Faure. Tel: +33169823498. Email: denis.faure@i2bc.paris-saclay.fr

*Twitter accounts*

- Marta Torres: @martillatorres

- I2BC Paris-Saclay: @I2BCParisSaclay

## Summary

- *Agrobacterium tumefaciens* colonizes the galls (plant tumors) it causes, and the roots of host and non-host plants.
- Transposon-sequencing (Tn-Seq) was used to discover *A. tumefaciens* genes involved in reproductive success (fitness genes) on *Solanum lycopersicum* and *Populus trichocarpa* tumors and *S. lycopersicum* and *Zea mays* roots.
- The identified fitness genes represent 3 to 8% of *A. tumefaciens* genes and contribute to carbon and nitrogen metabolism, synthesis and repair of DNA, RNA and proteins and envelope-associated functions. Competition assays between twelve knockout mutants and wild-type confirmed the involvement of ten genes (*trpB*, *hisH*, *metH*, *cobN*, *ntrB*, *trxA*, *nrdJ*, *kamA*, *exoQ*, *wbbL*) in *A. tumefaciens* fitness under both tumor and root conditions. The remaining two genes (*fecA*, *noxA*) were important in tumors only. None of these mutants was non-pathogenic, but four (*hisH*, *trpB*, *exoQ*, *ntrB*) exhibited impaired virulence. Finally, we used this knowledge to search for chemical and biocontrol treatments that target some of the identified fitness pathways and report reduced tumorigenesis and impaired establishment of *A. tumefaciens* on tomato roots using tannic acid or *Pseudomonas protegens*, which affect iron assimilation.
- This work revealed *A. tumefaciens* pathways that contribute to its competitive survival in plants and highlights a strategy to identify plant protection approaches against this pathogen.

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

## Introduction

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

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

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

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

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

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

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

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

## Materials and methods

### *Bacterial strains and culture conditions*

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

appropriate with gentamicin (Gm; 25  $\mu\text{g ml}^{-1}$ ), kanamycin (Km; 50  $\mu\text{g ml}^{-1}$ ), ampicillin (Amp; 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*-based transposon mutant library that was constructed in a Rif<sup>R</sup> derivative of *A. tumefaciens* C58 grown in TY medium by Gonzalez-Mula et al. (Gonzalez-Mula *et al.*, 2019). Aliquots of the library (stored at -80°C) were thawed in 5 ml of liquid TY medium for 4 h at 28°C. Bacteria were centrifuged (3,000  $\times g$  for 10 minutes) and washed twice with the same volume of a 0.8% NaCl solution, and then optical density (OD<sub>600</sub>) was adjusted to 1 (ca. 10<sup>9</sup> cells per ml) before plant infection.

#### *Plant materials and infection with the transposon library*

*Solanum lycopersicum* (seeds provided by Vilmorin, France), *Zea mays* (seeds provided by Arvallis, France) and *Populus trichocarpa* (all cuttings were prepared from a single tree growing in the CNRS campus garden, Gif-sur-Yvette, France) were cultivated in 2-liter pots filled with non-sterile Floradur B horticultural substrate (Floragard, Oldenburg, Germany). Before planting, the horticultural substrate contained  $2.4 \times 10^7 \pm 0.8 \times 10^7$  CFU g<sup>-1</sup> of total bacteria, according to bacterial counting on TY medium supplemented with cycloheximide. The pots were placed in a non-sterile greenhouse (biosafety level 2) under long day (16 h) conditions and controlled temperature (24-26°C). Fifty 4-week-old tomato plants and fifty 3-week-old maize plants were infected in the soil by ca. 10<sup>9</sup> *A. tumefaciens* cells. Roots were inoculated by watering the soil with 1 ml of the bacterial suspension at OD<sub>600</sub>=1 diluted in 30 ml of sterile water. Two hundred 4-week-old tomato plants and two hundred and thirty 12-week-old poplar cuttings were incised with a scalpel between the first and second nodes (1 cm-long incision). The wounded stems were inoculated with a cell suspension (~10  $\mu\text{l}$ ) obtained after centrifugation of 1 ml of the initial bacterial suspension at OD<sub>600</sub>=1, hence by ca. 10<sup>9</sup> *A. tumefaciens* cells.

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

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

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

*DNA isolation, transposon library sequencing and analysis*



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

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

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

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

#### *Treatments to prevent host colonization and tumorigenesis*

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

## **Results and discussion**

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

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

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

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

secreted components or activities that could be shared as public goods by the Tn-population. In *A. tumefaciens*, potential public goods could encompass biosynthesis of soluble compounds (e.g. siderophore) and polymers for building extracellular matrix (e.g. cellulose), as well as any processes that engineer the surrounding environment of the Tn-population such as detoxification (e.g. ferulic acid and H<sub>2</sub>O<sub>2</sub>) and tumorigenesis (Matthysse & McMahan, 1998; Xu & Pan, 2000; Escobar & Dandekar, 2003; Rondon *et al.*, 2004; Campillo *et al.*, 2014). The Tn-Seq approach excludes essential genes (non-viable Tn-mutants), which were estimated by Tn5-mutagenesis to be 360 in *A. tumefaciens* C58 growing in LB rich medium (Curtis & Brun, 2014). In addition, identification of a given fitness gene in a tested growth condition may be biased if fitness of its Tn-mutants is already impaired in the initial culture condition (here, TY medium). In *A. tumefaciens* C58, Tn-mutants of ≈500 genes are impaired in their viability or fitness in TY rich medium (Gonzalez-Mula *et al.*, 2019). Hence, Tn-Seq underestimates the total number of fitness genes in a microorganism. Additional biases may be caused by polar effects of transposon insertions within an operon, although it has been shown that such biases are negligible in a number of microorganisms (Van Opijnen *et al.*, 2009; Rubin *et al.*, 2015; Hutchison *et al.*, 2019).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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



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

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

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

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

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

to H<sub>2</sub>O<sub>2</sub> and an increased sensitivity to menadione when compared to wild-type *A. tumefaciens* (Vattanaviboon *et al.*, 2007). Because of the variable plant-associated phenotypes associated with the KO mutant, TrxA would not be an appropriate target for controlling *A. tumefaciens* infection.

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

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

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

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

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

## Conclusions

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

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

## Acknowledgements

The authors wish to thank Antoine Cabon (University of Utah, United States) for his advice and guidance on statistical analyses, Christoph Keel (Université de Lausanne, Switzerland) for providing *Pseudomonas protegens* strains CHA0 and CHA400 and Peter Mergaert (I2BC, Gif-sur-Yvette, France) for helpful discussion and advice. This work has benefited from the facilities and expertise of the high throughput sequencing platform team and plant culture facilities (Guillaume Lucas and Véronique Couvreur) at I2BC (Gif-sur-Yvette, France) and was supported by the CNRS under Grant I2BC-SE2019, LabEx Saclay Plant Sciences-SPS (ANR-10-LABX-0040-SPS) and Fundación Ramón Areces under Grant BEVP-29A4757.

## Competing interests

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

## Author contributions

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

## Data availability

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

## ORCID

Marta Torres <https://orcid.org/0000-0002-0609-9123>

Audren Jiquel <https://orcid.org/0000-0002-7598-3369>

Etienne Jeanne <https://orcid.org/0000-0003-1380-4908>

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

Yves Dessaux <https://orcid.org/0000-0001-9702-783X>

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

## References

**Ahmed E, Holmström SJM. 2014.** Siderophores in environmental research: roles and applications. *Microbial Biotechnology* **7**:196–208.

**Aloni R, Ullrich CI. 2008.** Biology of Crown Gall Tumors. In: Tzfira T, Citovsky V, eds. *Agrobacterium: from biology to biotechnology*. New York, NY: Springer New York, 565–591.

**Aygun-Sunar S, Mandaci S, Koch HG, Murray IVJ, Goldfine H, Daldal F. 2006.** Ornithine lipid is required for optimal steady-state amounts of c-type cytochromes in *Rhodobacter capsulatus*. *Molecular Microbiology* **61**: 418–435.

**Barton IS, Fuqua C, Platt TG. 2018.** Ecological and evolutionary dynamics of a model facultative pathogen: *Agrobacterium* and crown gall disease of plants. *Environmental Microbiology* **20**: 16–29.

551 **Bash R, Matthysse AG. 2002.** Attachment to roots and virulence of a *chvB* mutant of  
552 *Agrobacterium tumefaciens* are temperature sensitive. *Molecular Plant-Microbe*  
553 *Interactions* **15**: 160–163.

554 **Baude J, Vial L, Villard C, Campillo T, Lavire C, Nesme X, Hommais F. 2016.**  
555 Coordinated regulation of species-specific hydroxycinnamic acid degradation and  
556 siderophore biosynthesis pathways in *Agrobacterium fabrum*. *Applied and Environmental*  
557 *Microbiology* **82**: 3515–3524.

558 **Bednářová M, Staněk M, Vančura V, Veselý D. 1979.** Microorganisms in the rhizosphere of  
559 wheat colonized by the fungus *Gaeumannomyces graminis* var. *tritici*. *Folia Microbiologica*  
560 **24**: 253–261.

561 **Beringer J. 1974.** R factor transfer in *Rhizobium leguminosarum*. *Microbiology* **84**: 188–198.

562 **Bouzar H, Ouadah D, Krimi Z, Jones JB, Trovato M, Petit A, Dessaux Y. 1993.**  
563 Correlative association between resident plasmids and the host chromosome in a diverse  
564 *Agrobacterium* soil population. *Applied and environmental microbiology* **59**: 1310–1317.

565 **Bradbury J. 1986.** *Guide to Plant Pathogenic Bacteria*. Wallingford, UK: CAB International.

566 **Burr T, Reid C, Katz B. 1993.** Failure of *Agrobacterium radiobacter* strain K-84 to control  
567 crown gall on raspberry. *HortScience* **28**: 1017–1019.

568 **Cain AK, Barquist L, Goodman AL. 2020.** A decade of advances in transposon-insertion  
569 sequencing. *Nature Reviews Genetics* **21**: 526–540.

570 **Campillo T, Renoud S, Kerzaon I, Vial L, Baude J, Gaillard V, Bellvert F, Chamignon C,**  
571 **Comte G, Nesme X, et al. 2014.** Analysis of hydroxycinnamic acid degradation in  
572 *Agrobacterium fabrum* reveals a coenzyme a dependent, beta-oxidative deacetylation  
573 pathway. *Applied and Environmental Microbiology* **80**: 3341–3349.

574 **Chen B, Boël G, Hashem Y, Ning W, Fei J, Wang C, Gonzalez RL, Hunt JF, Frank J.**  
575 **2014.** EttA regulates translation by binding the ribosomal E site and restricting ribosome-  
576 tRNA dynamics. *Nature Structural & Molecular Biology* **21**: 152–159.

577 **Chilton MD, Currier TC, Farrand SK, Bendich AJ, Gordon MP, Nester EW. 1974.**  
578 *Agrobacterium tumefaciens* DNA and PS8 bacteriophage DNA not detected in crown gall

579 tumors. *Proceedings of the National Academy of Sciences* **71**: 3672–3676.

580 **Clifford JC, Rapicavoli JN, Roper MC. 2013.** A rhamnose-rich O-antigen mediates  
581 adhesion, virulence, and host colonization for the xylem-limited phytopathogen *Xylella*  
582 *fastidiosa*. *Molecular Plant-Microbe Interactions* **26**: 676–685.

583 **Cole BJ, Feltcher ME, Waters RJ, Wetmore KM, Mucyn TS, Ryan EM, Wang G, Ul-**  
584 **Hasan S, McDonald M, Yoshikuni Y, et al. 2017.** Genome-wide identification of bacterial  
585 plant colonization genes. *PLOS Biology* **15**: 1–24.

586 **Curtis PD, Brun YV. 2014.** Identification of essential alphaproteobacterial genes reveals  
587 operational variability in conserved developmental and cell cycle systems. *Molecular*  
588 *Microbiology* **93**: 713–735.

589 **Deeken R, Engelmann JC, Efetova M, Czirjak T, Müller T, Kaiser WM, Tietz O, Krischke**  
590 **M, Mueller MJ, Palme K, et al. 2006.** An integrated view of gene expression and solute  
591 profiles of *Arabidopsis* tumors: a genome-wide approach. *The Plant Cell* **18**: 3617–3634.

592 **Dennis J, Zylstra G. 1998.** Plasposons: modular self-cloning minitransposon derivatives for  
593 rapid genetic analysis of gram-negative bacterial genomes. *Applied and environmental*  
594 *microbiology* **64**: 2710–2715.

595 **Dessaux Y, Faure D. 2018.** Niche construction and exploitation by *Agrobacterium*: how to  
596 survive and face competition in soil and plant habitats. *Curr Top Microbiol Immunol* **418**:  
597 55–86.

598 **Do Amaral FP, Tuleski TR, Pankievicz VCS, Melnyk RA, Arkin AP, Griffiths J, Tadra-**  
599 **Sfeir MZ, Maltempi de Souza E, Deutschbauer A, Monteiro RA, et al. 2020.** Diverse  
600 bacterial genes modulate plant root association by beneficial bacteria. *mBio* **11**: e03078-  
601 20.

602 **Drehe I, Simonetti E, Ruiz JA. 2018.** Contribution of the siderophores pyoverdine and  
603 enantio-pyochelin to fitness in soil of *Pseudomonas protegens* Pf-5. *Current Microbiology*  
604 **75**: 1560–1565.

605 **Duong DA, Jensen RV, Stevens AM. 2018.** Discovery of *Pantoea stewartii* ssp. *stewartii*  
606 genes important for survival in corn xylem through a Tn-seq analysis. *Molecular Plant*

607 *Pathology* **19**: 1929–1941.

608 **Efetova M, Zeier J, Riederer M, Lee C-W, Stingl N, Mueller M, Hartung W, Hedrich R,**  
609 **Deeken R. 2007.** A central role of abscisic acid in drought stress protection of  
610 *Agrobacterium*-induced tumors on *Arabidopsis*. *Plant Physiology* **145**: 853–862.

611 **El Sahili A, Li SZ, Lang J, Virus C, Planamente S, Ahmar M, Guimaraes BG, Aumont-**  
612 **Nicaise M, Vigouroux A, Soullère L, et al. 2015.** A pyranose-2-phosphate motif is  
613 responsible for both antibiotic import and quorum-sensing regulation in *Agrobacterium*  
614 *tumefaciens*. *PLOS Pathogens* **11**: e1005071.

615 **Escobar MA, Dandekar AM. 2003.** *Agrobacterium tumefaciens* as an agent of disease.  
616 *Trends in Plant Science* **8**: 380–386.

617 **Fildes P. 1941.** Inhibition of bacterial growth by indoleacrylic acid and its relation to  
618 tryptophan; an illustration of the inhibitory action of substances chemically related to an  
619 essential metabolite. *British Journal of Experimental Pathology* **22**: 293–298.

620 **Fuhrer T, Fischer E, Sauer U. 2005.** Experimental identification and quantification of  
621 glucose metabolism in seven bacterial species. *Journal of Bacteriology* **187**: 1581–1590.

622 **Fürst U, Zeng Y, Albert M, Witte AK, Fliegmann J, Felix G. 2020.** Perception of  
623 *Agrobacterium tumefaciens* flagellin by FLS2XL confers resistance to crown gall disease.  
624 *Nature Plants* **6**: 22–27.

625 **Garfinkel DJ, Nester EW. 1980.** *Agrobacterium tumefaciens* mutants affected in crown gall  
626 tumorigenesis and octopine catabolism. *Journal of Bacteriology* **144**: 732–743.

627 **Gay-Fraret J, Ardisson S, Kambara K, Broughton WJ, Deakin WJ, Quéré A. 2012.**  
628 Cyclic- $\beta$ -glucans of *Rhizobium* (*Sinorhizobium*) sp. strain NGR234 are required for hypo-  
629 osmotic adaptation, motility, and efficient symbiosis with host plants. *FEMS Microbiology*  
630 *Letters* **333**: 28–36.

631 **Gelvin SB. 2021.** Plant DNA repair and *Agrobacterium* T-DNA Integration. *International*  
632 *Journal of Molecular Sciences* **22**: 8458.

633 **Gelvin SB. 2017.** Integration of *Agrobacterium* T-DNA into the plant genome. *Annual Review*  
634 *of Genetics* **51**: 195–217.



635 **Gohlke J, Scholz CJ, Kneitz S, Weber D, Fuchs J, Hedrich R, Deeken R. 2013.** DNA  
636 methylation mediated control of gene expression is critical for development of crown gall  
637 tumors. *PLoS Genetics* **9**: e1003267.

638 **Gomes N, Heuer H, Schönfeld J, Costa R, Mendonça-Hagler L, Smalla K. 2001.** Bacterial  
639 diversity of the rhizosphere of maize (*Zea mays*) grown in tropical soil studied by  
640 temperature gradient gel electrophoresis. *Plant and Soil* **232**: 167–180.

641 **Gonzalez-Mula A, Lachat J, Mathias L, Naquin D, Lamouche F, Mergaert P, Faure D.**  
642 **2019.** The biotroph *Agrobacterium tumefaciens* thrives in tumors by exploiting a wide  
643 spectrum of plant host metabolites. *New Phytologist* **222**: 455–467.

644 **González-Mula A, Lang J, Grandclément C, Naquin D, Ahmar M, Soullère L, Queneau Y,**  
645 **Dessaux Y, Faure D. 2018.** Lifestyle of the biotroph *Agrobacterium tumefaciens* in the  
646 ecological niche constructed on its host plant. *New Phytologist* **219**: 350–362.

647 **Goodner B, Hinkle G, Gattung S, Miller N, Blanchard M, Quorllo B, Goldman B, Cao Y,**  
648 **Askenazi M, Halling C, et al. 2001.** Genome sequence of the plant pathogen and  
649 biotechnology agent *Agrobacterium tumefaciens* C58. *Science* **294**: 2323–2328.

650 **Gordon JE, Christie PJ. 2014.** The *Agrobacterium* Ti Plasmids. *Microbiology Spectrum* **2**:  
651 10.1128/microbiolspec.PLAS-0010-2013.

652 **Gray J, Gelvin SB, Meilan R, Morris RO. 1996.** Transfer RNA is the source of extracellular  
653 isopentenyladenine in a Ti-plasmidless strain of *Agrobacterium tumefaciens*. *Plant*  
654 *Physiology* **110**: 431–438.

655 **Gray J, Wang J, Gelvin SB. 1992.** Mutation of the *miaA* gene of *Agrobacterium tumefaciens*  
656 results in reduced *vir* gene expression. *Journal of Bacteriology* **174**: 1086–1098.

657 **Heindl JE, Wang Y, Heckel BC, Mohari B, Feirer N, Fuqua C. 2014.** Mechanisms and  
658 regulation of surface interactions and biofilm formation in *Agrobacterium*. *Frontiers in Plant*  
659 *Science* **5**: 176.

660 **Helmann TC, Deutschbauer AM, Lindow SE. 2019.** Genome-wide identification of  
661 *Pseudomonas syringae* genes required for fitness during colonization of the leaf surface  
662 and apoplast. *Proceedings of the National Academy of Sciences* **116**: 18900–18910.

663 **Helmann TC, Deutschbauer AM, Lindow SE. 2020.** Distinctiveness of genes contributing  
664 to growth of *Pseudomonas syringae* in diverse host plant species. *PLOS ONE* **15**:  
665 e0239998.

666 **Henry PM, Gebben SJ, Tech JJ, Yip JL, Leveau JHJ. 2016.** Inhibition of *Xanthomonas*  
667 *fragariae*, causative agent of angular leaf spot of strawberry, through iron deprivation.  
668 *Frontiers in Microbiology* **7**: 1589.

669 **Hutchison CA, Merryman C, Sun L, Assad-Garcia N, Richter RA, Smith HO, Glass JI.**  
670 **2019.** Polar effects of transposon insertion into a minimal bacterial genome. *Journal of*  
671 *Bacteriology* **201**: e00185-19.

672 **Kamilova F, Kravchenko LV, Shaposhnikov AI, Azarova T, Makarova N, Lugtenberg B.**  
673 **2006.** Organic acids, sugars, and L-tryptophane in exudates of vegetables growing on  
674 stonewool and their effects on activities of rhizosphere bacteria. *Molecular Plant-Microbe*  
675 *Interactions* **19**: 250–256.

676 **Keel C, Voisard C, Berling C, Kahr G, Défago G. 1989.** Iron sufficiency, a prerequisite for  
677 the suppression of tobacco black root rot by *Pseudomonas fluorescens* strain CHA0 under  
678 gnotobiotic conditions. *Phytopathology* **79**: 584–589.

679 **Kim SK, Park SJ, Li XH, Choi YS, Im DS, Lee JH. 2018.** Bacterial ornithine lipid, a  
680 surrogate membrane lipid under phosphate-limiting conditions, plays important roles in  
681 bacterial persistence and interaction with host. *Environmental Microbiology* **20**: 3992–  
682 4008.

683 **Klee SM, Mostafa I, Chen S, Dufresne C, Lehman BL, Sinn JP, Peter KA, McNellis TW.**  
684 **2018.** An *Erwinia amylovora yjeK* mutant exhibits reduced virulence, increased chemical  
685 sensitivity and numerous environmentally dependent proteomic alterations. *Molecular*  
686 *Plant Pathology* **19**: 1667–1678.

687 **Krimi Z, Petit A, Mougel C, Dessaux Y, Nesme X. 2002.** Seasonal fluctuations and long-  
688 term persistence of pathogenic populations of *Agrobacterium* spp. in soils. *Applied and*  
689 *Environmental Microbiology* **68**: 3358–3365.

690 **Kwon YM, Ricke SC, Mandal RK. 2016.** Transposon sequencing: methods and expanding

applications. *Applied Microbiology and Biotechnology* **100**: 31–43.

**Lang J, Vigouroux A, Planamente S, El Sahili A, Blin P, Aumont-Nicaise M, Dessaux Y, Moréra S, Faure D. 2014.** *Agrobacterium* uses a unique ligand-binding mode for trapping opines and acquiring a competitive advantage in the niche construction on plant host. *PLoS Pathogens* **10**: e1004444.

**Lang J, Vigouroux A, El Sahili A, Kwasiborski A, Aumont-Nicaise M, Dessaux Y, Shykoff JA, Moréra S, Faure D. 2017.** Fitness costs restrict niche expansion by generalist niche-constructing pathogens. *The ISME Journal* **11**: 374–385.

**Lee CW, Efetova M, Engelmann JC, Kramell R, Wasternack C, Ludwig-Müller J, Hedrich R, Deeken R. 2009.** *Agrobacterium tumefaciens* promotes tumor induction by modulating pathogen defense in *Arabidopsis thaliana*. *The Plant Cell* **21**: 2948–2962.

**Liu Z, Beskrovnaya P, Melnyk RA, Hossain SS, Khorasani S, O’Sullivan LR, Wiesmann CL, Bush J, Richard JD, Haney CH, et al. 2018.** A genome-wide screen identifies genes in rhizosphere-associated *Pseudomonas* required to evade plant defenses. *mBio* **9**: e00433-18.

**Liu Z, Jacobs M, Schaff DA, McCullen CA, Binns AN. 2001.** ChvD, a chromosomally encoded ATP-binding cassette transporter-homologous protein involved in regulation of virulence gene expression in *Agrobacterium tumefaciens*. *Journal of Bacteriology* **183**: 3310–3317.

**Ma Y, Pan F, McNeil M. 2002.** Formation of dTDP-rhamnose is essential for growth of mycobacteria. *Journal of Bacteriology* **184**: 3392–3395.

**Macho AP, Guidot A, Barberis P, Beuzón CR, Genin S. 2010.** A competitive index assay identifies several *Ralstonia solanacearum* type III effector mutant strains with reduced fitness in host plants. *Molecular Plant-Microbe Interactions* **23**: 1197–1205.

**Marman HE, Mey AR, Payne SM. 2014.** Elongation factor P and modifying enzyme PoxA are necessary for virulence of *Shigella flexneri*. *Infection and Immunity* **82**: 3612–3621.

**Matthysse AG. 2018.** Exopolysaccharides of *Agrobacterium tumefaciens*. In: Gelvin S, ed. *Agrobacterium* Biology. Current Topics in Microbiology and Immunology. Cham,

Switzerland: Springer, 111–141.

**Matthysse AG, McMahan S. 1998.** Root colonization by *Agrobacterium tumefaciens* is reduced in *cel*, *attB*, *attD* and *attR* mutants. *Applied and Environmental Microbiology* **64**: 2341–2345.

**Meller E, Gassman ML. 1981.** The effects of levulinic acid and 4,6-dioxoheptanoic acid on the metabolism of etiolated and greening barley leaves. *Plant Physiology* **67**: 728–732.

**Mendis HC, Madzima TF, Queiroux C, Jones KM. 2016.** Function of succinoglycan polysaccharide in *Sinorhizobium meliloti* host plant invasion depends on succinylation, not molecular weight. *mBio* **7**: e00606-16.

**Meyer T, Thiour-Mauprivez C, Wisniewski-Dyé F, Kerzaon I, Comte G, Vial L, Lavire C. 2019.** Ecological conditions and molecular determinants involved in *Agrobacterium* lifestyle in tumors. *Frontiers in Plant Science* **10**: 978.

**Miller J, Bandarian V, Reed GH, Frey PA. 2001.** Inhibition of lysine 2,3-aminomutase by the alternative substrate 4-thialysine and characterization of the 4-thialysyl radical intermediate. *Archives of Biochemistry and Biophysics* **387**: 281–288.

**Moore LW, Warren G. 1979.** *Agrobacterium radiobacter* strain 84 and biological control of crown gall. *Annual Review of Phytopathology* **17**: 163–179.

**Müller P, Keller M, Weng W, Quandt J, Arnold W, Pühler A. 1993.** Genetic analysis of the *Rhizobium meliloti* *exoYFQ* operon: ExoY is homologous to sugar transferases and ExoQ represents a transmembrane protein. *Molecular Plant-Microbe Interactions* **6**: 55.

**Nam J, Mysore KS, Zheng C, Knue MK, Matthysse AG, Gelvin SB. 1999.** Identification of T-DNA tagged *Arabidopsis* mutants that are resistant to transformation by *Agrobacterium*. *Molecular and General Genetics* **261**: 429–438.

**Navarre WW, Zou SB, Roy H, Xie JL, Savchenko A, Singer A, Edvokimova E, Prost LR, Kumar R, Ibba M, et al. 2010.** PoxA, YjeK, and elongation factor P coordinately modulate virulence and drug resistance in *Salmonella enterica*. *Molecular Cell* **39**: 209–221.

**Onyeziri MC, Natarajan R, Hardy GG, Xu J, Reynolds IP, Kim J, Merritt PM, Danhorn T, Hibbing ME, Weisberg AJ, Chang JH, Fuqua C. 2021.** Dual adhesive unipolar

polysaccharides synthesized by overlapping biosynthetic pathways in *Agrobacterium tumefaciens*. *BioRxiv* <https://doi.org/10.1101/2021.04.22.440995>.

**Peng W-T, Banta LM, Charles TC, Nester EW. 2001.** The *chvH* locus of *Agrobacterium* encodes a homologue of an elongation factor involved in protein synthesis. *Journal of Bacteriology* **183**: 36–45.

**Pitzschke A, Hirt H. 2010.** New insights into an old story: *Agrobacterium*-induced tumour formation in plants by plant transformation. *The EMBO Journal* **29**: 1021–1032.

**Powell JE, Leonard SP, Kwong WK, Engel P, Moran NA. 2016.** Genome-wide screen identifies host colonization determinants in a bacterial gut symbiont. *Proceedings of the National Academy of Sciences* **113**: 13887–13892.

**Puławska J. 2010.** Crown gall of stone fruits and nuts, economic significance and diversity of its causal agents: tumorigenic *Agrobacterium* spp. *Journal of Plant Pathology* **92**: 87–98.

**Rojas-Jiménez K, Sohlenkamp C, Geiger O, Martínez-Romero E, Werner D, Vinuesa P. 2005.** A CIC chloride channel homolog and ornithine-containing membrane lipids of *Rhizobium tropici* CIAT899 are involved in symbiotic efficiency and acid tolerance. *Molecular Plant-Microbe Interactions* **18**: 1175–1185.

**Rondon MR, Ballering KS, Thomas MG. 2004.** Identification and analysis of a siderophore biosynthetic gene cluster from *Agrobacterium tumefaciens* C58. *Microbiology* **150**: 3857–3866.

**Royet K, Parisot N, Rodrigue A, Gueguen E, Condemine G. 2019.** Identification by Tn-seq of *Dickeya dadantii* genes required for survival in chicory plants. *Molecular Plant Pathology* **20**: 287–306.

**Rubin BE, Wetmore KM, Price MN, Diamond S, Shultzaberger RK, Lowe LC, Curtin G, Arkin AP, Deutschbauer A, Golden SS. 2015.** The essential gene set of a photosynthetic organism. *Proceedings of the National Academy of Sciences* **112**: 6634–6643.

**Sambrook J, Fritsch E, Maniatis T. 1989.** *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press.

775 **Sanguin H, Remenant B, Dechesne A, Thioulouse J, Vogel TM, Nesme X, Moenne-**  
776 **Loccoz Y, Grundmann GL. 2006.** Potential of a 16S rRNA-based taxonomic microarray  
777 for analyzing the rhizosphere effects of maize on *Agrobacterium* spp. and bacterial  
778 communities. *Applied and Environmental Microbiology* **72**: 4302–4312.

779 **Sardesai N, Lee LY, Chen H, Yi H, Olbricht GR, Stirnberg A, Jeffries J, Xiong K, Doerge**  
780 **RW, Gelvin SB. 2013.** Cytokinins secreted by *Agrobacterium* promote transformation by  
781 repressing a plant Myb transcription factor. *Science Signaling* **6**: ra100.

782 **Sastry GRK, Miles CA, Miller IS, Borland PA, Saeed N, May CA. 1986.** Tryptophan  
783 auxotrophs for increasing safety of *Agrobacterium* Ti-based recombinant plasmid work.  
784 *Plant Mol Biol Rep* **4**: 93-97.

785 **Sivakumar R, Ranjani J, Vishnu US, Jayashree S, Lozano GL, Miles J, Broderick NA,**  
786 **Guan C, Gunasekaran P, Handelsman J, et al. 2019.** Evaluation of InSeq to identify  
787 genes essential for *Pseudomonas aeruginosa* PGPR2 corn root colonization. *G3*  
788 (*Genes/Genomes/Genetics*) **9**: 651–661.

789 **Stutz E, Défago G, Kern H. 1986.** Naturally occurring fluorescent pseudomonads involved in  
790 suppression of black root rot of tobacco. *Phytopathology* **76**: 181–185.

791 **Su S, Stephens BB, Alexandre G, Farrand SK. 2006.** Lon protease of the alpha-  
792 proteobacterium *Agrobacterium tumefaciens* is required for normal growth, cellular  
793 morphology and full virulence. *Microbiology* **152**: 1197–1207.

794 **Taga ME, Walker GC. 2010.** *Sinorhizobium meliloti* requires a cobalamin-dependent  
795 ribonucleotide reductase for symbiosis with its plant host. *Molecular Plant-Microbe*  
796 *Interactions* **23**: 1643–1654.

797 **Thompson MA, Onyeziri MC, Fuqua C. 2018.** Function and regulation of *Agrobacterium*  
798 *tumefaciens* cell surface structures that promote attachment. In: Gelvin S, ed.  
799 *Agrobacterium Biology. Current Topics in Microbiology and Immunology*. Cham,  
800 Switzerland: Springer, 143–184.

801 **Van Opijnen T, Bodi KL, Camilli A. 2009.** Tn-seq: high-throughput parallel sequencing for  
802 fitness and genetic interaction studies in microorganisms. *Nature Methods* **6**: 767–772.

- Van Opijnen T, Camilli A. 2013.** Transposon insertion sequencing: a new tool for systems-level analysis of microorganisms. *Nature Reviews Microbiology* **11**: 435–442.
- Vattanaviboon P, Tanboon W, Mongkolsuk S. 2007.** Physiological and expression analyses of *Agrobacterium tumefaciens trxA*, encoding thioredoxin. *Journal of Bacteriology* **189**: 6477–6481.
- Vences-Guzmán MÁ, Geiger O, Sohlenkamp C. 2012.** Ornithine lipids and their structural modifications: from A to E and beyond. *FEMS Microbiology Letters* **335**: 1–10.
- Vogel J, Normand P, Thioulouse J, Nesme X, Grundmann GL. 2003.** Relationship between spatial and genetic distance in *Agrobacterium* spp. in 1 cubic centimeter of soil. *Applied and Environmental Microbiology* **69**: 1482–1487.
- Weissenmayer B, Gao JL, López-Lara IM, Geiger O. 2002.** Identification of a gene required for the biosynthesis of ornithine-derived lipids. *Molecular Microbiology* **45**: 721–733.
- Wheatley RM, Ford BL, Li L, Aroney STN, Knights HE. 2020.** Lifestyle adaptations of *Rhizobium* from rhizosphere to symbiosis. **117**: 23823–23834.
- Wood D, Setubal J, Kaul R, Monks D, Kitajima J, Okura V, Zhou Y, Chen L, Wood G, Almeida NJ, et al. 2001.** The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. *Science* **294**: 2317–2323.
- Xu XQ, Pan SQ. 2000.** An *Agrobacterium* catalase is a virulence factor involved in tumorigenesis. *Molecular Microbiology* **35**: 407–414.

## Supporting information

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

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

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

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

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

Notes S2. Mutant construction by reverse genetics.

Notes S3. Root colonization and tumorigenesis assays.

Notes S4. Validation of the fitness genes by reverse genetics

Notes S5. Proline, nopaline and spermidine pathways.

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

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

## Figure legends

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

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

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

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



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

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

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

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

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

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

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

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

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

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

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

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

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

1. Carbon and nitrogen metabolism
-----------------------------------

<b>Carbon metabolism</b>
--------------------------

- |  |
|--|
| <ul style="list-style-type: none"><li>• Sugar metabolism (<i>scrK</i>=<i>atu0401</i>, <i>pgi</i>=<i>atu0404</i>, <i>pgl</i>=<i>atu0599</i>, <i>zwf</i>=<i>atu0600</i>, <i>tal</i>=<i>atu4464</i>)</li><li>• Gluconeogenesis (<i>gapA</i>=<i>atu3737</i>, <i>atu3740</i>)</li></ul> |
|--|

---

## 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- $\beta$ -(1,2)-glucan (*chvB*=*atu2730*)