Full Title:

Genome wide identification of genes required for bacterial plant infection by Tn-seq

Short title: *Dickeya dadantii* virulence genes in chicory

Kevin Royet[1], Nicolas Parisot[2], Agnès Rodrigue[1], Erwan Gueguen[1]* and Guy Condemine[1]

1 Univ Lyon, Université Lyon 1, INSA de Lyon, CNRS UMR 5240 Microbiologie Adaptation et Pathogénie, F-69622 Villeurbanne, France.

2 Univ Lyon, INSA-Lyon, INRA, BF2I, UMR0203, F-69621, Villeurbanne, France

*Corresponding author. erwan.gueguen@univ-lyon1.fr

Abstract

Soft rot enterobacteria (*Dickeya* and *Pectobacterium*) are major pathogens that provoke diseases on plants of agricultural importance such as potato and ornamentals. Long term studies to identify virulence factors of these bacteria focused mostly on plant cell wall degrading enzymes secreted by the type II Out secretion system and the regulation of their expression. To identify new virulence factors we performed a Tn-seq genome-wide screen of a transposon mutant library during chicory infection followed by high-throughput sequencing. This allowed the detection of mutants with reduced but also increased fitness in the plant. Virulence factors identified differed from those previously known since diffusible ones (secreted enzymes, siderophores or metabolites) were not detected by this screen. In addition to genes encoding proteins of unknown function that could be new virulence factors, others could be assigned to known biological functions. The central role of the FlhDC regulatory cascade in the control of virulence was highlighted with the identification of new members of this pathway. Scarcity of the plant in certain amino acids and nucleic acids required presence of the corresponding biosynthetic genes in the bacteria. Their products could be targets for the development of antibacterial compounds. Among the genes required for full development in chicory we also identified six genes involved in the glycosylation of the flagellin FliC. We showed that absence of this modification reduces virulence of *D. dadantii* on celery. Since homologues of these genes are present in other *Dickeya* and *Pectobacterium* strains, this modification could be an important factor for soft rot enterobacteriale virulence.

Author summary

Identification of virulence factors of plant pathogenic bacteria has relied on the test of individual mutants on plants, a time-consuming method. New methods like transcriptomic or

proteomic can now be used but they only allow the identification of genes induced during the infection process and non-induced genes may be missed. Tn-seq is a very powerful method to identify genes required for bacterial growth in their host. We used for the first time this method in a plant pathogenic bacteria to identify genes required for the multiplication of *Dickeya dadantii* in chicory. We identified about 100 genes with decreased or increased fitness in the plant. Most of them had no previously described role in bacterial virulence. We unveiled important metabolic genes and regulators of motility and virulence. We showed that *D. dadantii* flagellin is glycosylated and that this modification confers fitness to the bacteria during plant infection. Our work opens the way to the use of Tn-seq with bacterial phytopathogens. Test by this method of large collections of environmental pathogenic strains now available will allow an easy and rapid identification of new virulence factors.

Introduction

Dickeya are broad-host range phytopathogenic bacteria belonging to the Pectobacteriaceae family [1] that provoke the soft rot disease on many plant species. They are the cause of significant losses on economically important crops such as potato, chicory and ornamentals. Identification and studies on the virulence factors of these bacteria have been performed mostly on the model strain *D. dadantii* 3937 and focused mainly on three domains/aspects, known to be important for disease development: plant cell wall degrading enzymes, the type III secretion system and iron metabolism [2]. Secretion of plant cell wall degrading enzymes has long ago been identified as the bacteria main virulence factor. Many studies focused on the identification and characterization of these secreted enzymes, mostly

65 pectinases [3], of the regulators controlling their production (*kdgR*, *pecS*, *pecT*, *hns*, *gacA*) [4-
66 8], of the genes whose expression is coregulated with that of these secreted enzyme genes [9,
67 10], and of the mechanism of their secretion by the Out type II secretion system [11].
68 Although of a lesser importance for *Dickeya* virulence, the same type of approach has been
69 used to identify Hrp type III secretion system regulators and effectors [12-14]. Moreover,
70 competition for iron within the plant is strong. *D. dadantii* acquires this metal through
71 production of two siderophores, chrysobactin and achromobactin [15-17]. More recently,
72 omics approaches have also been used to identify genes induced during plant infection [18]
73 [19] [20]. These studies now provide a clearer picture on a complex network of factors
74 required for *D. dadantii* virulence [2, 21]. However, these approaches may have missed some
75 important factors not targeted by these analyses. Subsequently, more global screens need to
76 be performed to identify these factors. Libraries of transposon-induced mutants were tested
77 on plants to find mutants showing reduced virulence with *Pectobacterium carotovorum* and
78 *P. atrosepticum*, two other soft rot enterobacteria [22, 23]. These studies identified
79 auxotrophs, mutants defective in production or secretion of exoenzymes and in motility.
80 Other mutants with a more complex phenotype were not characterized at this time. Moreover,
81 the number of tested mutants was limited by the necessity to test individually each mutant on
82 plant. This type of work has never been performed on *Dickeya* strains. To have a more
83 complete view of the genes required for the virulence of *Dickeya*, we used a high-throughput
84 sequencing of a saturated transposon library (Tn-seq) to screen tens of thousands random
85 insertion mutants of *D. dadantii* in laboratory medium and during infection of chicory. Tn-
86 seq has been extensively used to uncover essential genes required for mouse colonization by
87 human pathogens *Vibrio cholerae* [24], *Pseudomonas aeruginosa* [25] and *Streptococcus*
88 *pneumoniae* [26]. Thus, Tn-seq is a very powerful method to identify genes required for
89 bacterial growth in their host. However, this technology has not yet been employed to unveil

genes required for the multiplication of a phytopathogen in a plant host. By applying this technique to screen a *D. dadantii* mutant library in chicory, we identified metabolic pathways and bacterial genes required for growth *in planta*. Among them, we found a cluster of genes required for flagellin glycosylation, a modification known to be important for several plant pathogenic bacteria virulence.

Results and discussion

Characterization of *D. dadantii* 3937 *Himar1* transposon library

Many tools are available to perform Tn-seq [27]. In order to perform a Tn-seq experiment with *D. dadantii* 3937, we used a *Himar9* mariner transposon derivative carrying *MmeI* restriction sites in the inverted repeats (IR) and a kanamycin resistance cassette between the IRs [28]. We realized a biparental mating between *E. coli* and *D. dadantii* on M63 agar medium without carbon source and amino acids. We obtained approximately 300 000 colonies that were pooled. Subsequent DNA sequencing (see below) showed the presence of transposon insertions in amino acid biosynthesis pathways, demonstrating that mating on M63 minimal medium does not prevent the acquisition of auxotroph mutants. To identify essential genes, mutants were grown in LB medium during several generations. Two DNA libraries were prepared from two cultures and subjected to high-throughput sequencing. The mariner transposon inserts into TA dinucleotides. The TPP software [29] was used to determine the number of reads at each TA site for each biological replicate. *D. dadantii* genome has 171,791 TA sites that can be targeted by the *Himar9* transposase. Pairs of biological replicates were compared. 37,794 and 48,101 unique insertions in TAs were detected in each sample, which corresponds to 22 and 28% density of insertion, respectively

115 (Table 1). The average number of reads per TA is 88 and 75, respectively. The results were
116 reproducible with a Pearson correlation coefficient of 72% (Fig. S1) The location of the
117 unique insertions showed an even distribution around the chromosome (Fig. 1A). For each
118 gene, we calculated a $\log_2$FC corresponding to a ratio between the measured number of reads
119 and the expected number of reads. The density plot (Fig. 1B) indicates that essential and non-
120 essential genes are easily distinguishable, confirming the good quality of our Tn-seq libraries.
121 Indeed non essential genes are expected to have a positive FC, whereas essential genes are
122 expected to have none or few insertions.
123 Then, gene essentiality of the Tn-seq input libraries was determined by using the TRANSIT
124 software [29]. We decided to use the Hidden Markov Model (HMM) method which predicts
125 essentiality and non-essentiality for individual insertion sites since it has been shown to give
126 good prediction in datasets with density as low as 20% [29]. The HMM analysis led to the
127 identification of 665 genes essential for growth in LB (ES), representing 14% of the genes of
128 *D. dadantii* 3937, a number in the range of those found for this type of analysis with bacteria.
129 552 genes were categorized as Growth Defect genes (GD, i.e. mutations in these genes lead
130 to loss of fitness), 125 as growth advantage genes (GA, i.e mutations in these genes lead to
131 gain of fitness) and 3320 as non-essential genes (NE) (Table S2 and Fig. 1B)).

132 **TABLE 1** Tn-Seq analysis of *Dickeya dadantii* 3937

Mutant pool	Total no. of reads	No. of reads containing Tn end	No. of reads normalized[a]	No. of mapped reads to unique TA sites	No. of mapped reads to unique TA sites after LOESS correction	Density (%)[b]	Mean read count per TA[c]
LB #1	23,152,186	22,647,343	18,748,028	13,166,770 (70 %)	12,904,900 (69 %)	28 %	75
LB #2	30,105,412	27,963,154	18,748,028	15,535,291 (83 %)	15,195,582 (81 %)	22 %	88
Chicory #1	18,925,029	18,748,028	18,748,028	17,535,146 (94 %)	14,906,888 (79 %)	24 %	87
Chicory #2	27,607,717	26,555,297	18,748,028	17,477,706 (93 %)	16,955,724 (90 %)	23 %	99

133 [a] The number of reads were normalized for each sample according to the Chicory #1 sample.
134 [b] *Dickeya dadantii* 3937 genome has 171,791 TA sites. The density is the % of TAs for which mapped
135 reads has been assigned by the TPP software.
136 [c] The mean value of mapped reads per TA.
137

138 **Genes necessary for chicory leaf maceration.** We used chicory leaf infection as a model to
139 identify *D. dadantii* genes required for growth in plant tissues. Biological duplicates were
140 performed to insure the reproducibility of the results. Each chicory was inoculated with 10^7
141 bacteria from the mutant pool and after 2 days more than 10^{10} bacteria were collected from
142 the rotten tissue. Sequencing transposon insertion sites in these bacteria followed by the TPP
143 analysis indicated a density of unique insertion in TAs comparable to that of the input
144 datasets (23-24%). Surprisingly, the results were more highly reproducible than in LB with a
145 very high Pearson correlation coefficient of 98% (Fig. S1).
146 In order to test the statistical significance of the identified genes conferring to *D. dadantii* a
147 loss or a gain of fitness *in planta*, we performed the RESAMPLING (permutation test)
148 analysis of the TRANSIT software. Applied to our Tn-seq datasets and selecting only genes
149 achieving a FDR adjusted p-value (q-value) ≤ 0.05, we identified 122 genes out of 4666
150 required for fitness *in planta*, as shown with the volcano plot of RESAMPLING results
151 comparing replicates grown in LB versus *in planta* (Fig. S2). From these 122 genes, we
152 applied an additional cutoff by removing 20 genes with a mean read count in LB <5 (less
153 than 5 reads in average per TA). These genes were categorized as ES or GD in LB. We also
154 removed from the analysis 6 genes with a $\log_2$FC comprised between -2 and 2. By applying
155 all these criteria, we retained only 96 genes for a further analysis (Fig. 2). 92 of them were
156 identified as GD genes in the chicory ($\log_2$FC $\leq$2), the 4 left as GA genes in the chicory
157 ($\log_2$FC $\geq$2). Some of these genes, in bold in Fig. 2, were already known to play a role in *D.*
158 *dadantii* virulence, confirming the validity of the Tn-seq approach. Using the Kyoto
159 Encyclopedia of Genes and Genomes (KEGG) [30], we discovered that certain metabolic
160 pathways and biological functions are very important for growth in chicory (Table S3). We
161 highlight some of them in the next sections of the article.
162

163 **Analysis of the genes of *D. dadantii* required for plant colonization.**

164 **(i) Metabolism.** Chicory appears as an environment in which amino acids, nucleic acids and
165 some vitamins (pyridoxal) are scarce. Of the 92 genes identified as GD genes *in planta*, 8 are
166 involved in purine and 7 in pyrimidine metabolisms (Table S3). In the purine metabolism
167 pathway, the inosine monophosphate (IMP) biosynthesis pathway that produces IMP from L-
168 glutamine and 5-phosphoribosyl diphosphate is particularly important for *D. dadantii in*
169 *planta* since 5 out of the 10 genes of this pathway were significantly GD genes *in planta* (Fig.
170 3). IMP is the precursor of adenine and guanine. Next, IMP can be converted in xanthosine
171 5'-phosphate (XMP) by the IMP dehydrogenase GuaB. *guaB* gene was also a GD gene *in*
172 *planta*, with a strong $\log_2$FC of -10.06 (Fig. 3). In the pyrimidine synthesis, the uridine
173 monophosphate (UMP) biosynthesis pathway that converts L-glutamine to UMP, a precursor
174 of uracyl, is very important *in planta* since *carAB*, *pyrB*, *pyrC* and *pyrE*, involved in this
175 enzymatic pathway, were all required for growth *in planta* (Fig. 3). This pyrimidine
176 biosynthesis pathway is specific to bacteria. It is noteworthy that in the human pathogen *S.*
177 *pneumoniae*, mutants of this pathway have a fitness defect in the nasopharynx of infected
178 mice [26]. Hence, it looks that the pyrimidine biosynthesis pathway is particularly important
179 for multiplication of some bacterial species in the host.

180 Mutants in genes involved in the synthesis of sulfur-containing amino acids (*cysIJQ, metB),*
181 lysine (*lysA*) and leucine (*leuABC)* are disadvantaged in chicory (Fig. 2 and Fig. 4A). These
182 amino acids are known to be present in low concentration in plant tissues. Other amino acids
183 seem to be present in quantity sufficient for growth of *D. dadantii* auxotrophs. Low level of
184 certain amino acids probably induced the stringent response in the bacteria. Reduced growth
185 in the plant of the *relA* mutant, unable to synthesize the alarmone ppGpp, supports this
186 hypothesis. Glucose is the main sugar present in plant tissue, present as a circulating sugar or
187 a cellulose degradation product. Mutants in the PTS glucose transport system genes *ptsI* and

188 *ptsG* had a reduced growth in bacteria (Fig. 2) showing its importance as a carbon source *in*
189 *planta*.
190 Degradation of cell wall pectin by a battery of extracellular enzymes is the main determinant
191 of *Dickeya* pathogenicity. Mutants unable to produce or to secrete these enzymes by the type
192 II Out secretion system were not disfavored in chicory since these mutants could use for their
193 growth the pectin degradation compounds produced by enzymes secreted by other bacteria.
194 The redundancy of oligogalacturonate specific porins (KdgM and KdgN) and inner
195 membrane transporters (TogT and TogMNABC) allow entry of these compounds into the
196 bacteria even in a mutant in one of these transport systems. However, *kduI* mutants, blocked
197 in the intracellular part of the pectin degradation pathway, had a limited growth *in planta*,
198 confirming the importance of the pectin degradation pathway in the disease progression.
199 **(ii) Stress resistance**. Plant is an hostile environment for the bacteria that has to cope with
200 antimicrobial peptides, ROS, toxic compounds and acidic pH [31]. We observed that the
201 pump AcrABTolC, that can efflux a wide range of compounds [32], was important for
202 survival in chicory (Fig. S3). Stress can lead to the accumulation of phospholipids in the
203 outer membrane. This accumulation makes the bacteria more sensitive to small toxic
204 molecules [33]. Such a phospholipid accumulation probably occured when the bacteria
205 infected chicory since *mlaC* and *mlaF* mutants, which are unable to prevent phospholipid
206 accumulation in the outer membrane, had a reduced growth in plant. Production of
207 exopolysaccharides (EPS) was shown to protect the bacteria during the first steps of infection
208 [9]. We observed that *rffG* and *wzx* mutants unable to synthesize EPS had a growth defect in
209 chicory. A set of genes required to repair or degrade altered proteins (*clpA, degQ, trxB*) were
210 also important for survival *in planta*. No gene directly involved in detoxification of ROS was
211 detected in our analysis. However, ROS can create DNA damage. The two helicases involved
212 in DNA repair, UvrD and HelD, gave growth advantage in plant. Osmoregulated periplasmic

213 glycans (OPG) are polymers of glucose found in the periplasm of α, β and γ proteobacteria.
214 Their exact role is unknown but their absence leads to avirulence in certain bacteria such as
215 *D. dadantii* [34]. This absence induces a membrane stress that is sensed and transduced by
216 the Rcs envelope stress response system. This system controls the expression of many genes,
217 including those involved in motility, and those encoding plant cell wall degrading enzymes
218 through the RsmA-RsmB system [35-37]. Thus, mutants defective in OPG synthesis are
219 expected to have a reduced virulence. Indeed, in our experiment, mutants in the two genes
220 involved in OPG synthesis, *opgG* and *opgH* were non competitive in chicory (Fig. 2).
221 **(iii) Iron uptake**. *D. dadantii* produces two types of siderophores, achromobactin and
222 chrysobactin, that are required for the development of maceration symptoms in the iron
223 limited environment of plant hosts [38]. Once iron loaded, the siderophores are imported into
224 the bacteria. Import through the outer membrane requires a specific outer membrane channel
225 and the energy transducing complex formed by TonB ExbB and ExbD. While the absence of
226 synthesis of one of the siderophores can be compensated by the presence of siderophore
227 secreted by other bacteria in the growth medium, mutants of the TonB complex are totally
228 unable to acquire iron and thus are unable to grow in the plant. In accordance, *tonB* was
229 essential in chicory while the genes coding for siderophore synthesis or secretion were not.
230 Similarly a mutant devoid of the iron-loaded chrysobactin transport gene (*fct)* was non-
231 competitive.
232 **(iv) Regulation.** Mutants in several genes controlling virulence factor production have a
233 growth defect in the plant. The master regulator FlhDC acts as a regulator of both flagella and
234 virulence factor synthesis in many bacteria such as *Yersinia ruckeri*, *Edwardsiella tarda* and
235 *Ralstonia solanacearum* [39-41]. In *D. dadantii* FlhDC has recently been shown to control, in
236 addition to flagellar motility, type III secretion system and virulence factor synthesis through
237 several pathways [42]. We observed that *flhC* gave a growth advantage in chicory. In

238 addition, we uncovered that some genes regulating *flhDC* in other bacteria regulated *D.*
239 *dadantii* virulence, probably by controlling *flhDC* expression. *rsmC* is a poorly characterized
240 gene in *D. dadantii* but that has been studied in *P. carotovorum*. It negatively controls
241 motility and extracellular enzyme production through modulating transcriptional activity of
242 FlhCD [43]. HdfR is a poorly characterized LysR family regulator that controls the *std*
243 fimbrial operon in *S. enterica* and FlhDC expression in *E. coli* [44]. *rsmC* mutants were
244 overrepresented in the chicory (Fig. 4B), indicating a gain of virulence for these mutants.
245 *hdfR* conferred fitness benefits during growth in chicory and could also act in *D. dadantii* as
246 activator of *flhDC* expression.
247 The GGDEF proteins are c-di-GMP synthase. Their genes are often located next to their
248 cognate EAL diguanylate phosphodiesterase gene. *ecpC (yhjH)* encodes an EAL protein that
249 was shown to activate virulence factor production in *D. dadantii* [45]. *gcpA*, which is located
250 next to *ecpC* encodes a GGDEF protein. However, a *gcpA* mutant could not be constructed
251 and analysed in this previous study. We observed that *gcpA* mutants (Dda_03858) were
252 overrepresented in chicory (Fig. 2). This increased virulence is a phenotype opposite to that
253 described for the *ecpC* mutants, indicating that overproduction of c-di-GMP could reduce *D.*
254 *dadantii* virulence.
255 Of the eighteen regulators of the LacI family present in *D. dadantii*, four were found to be
256 involved in plant infection [46]. One of those, LfcR, which has been found important for
257 infection of chicory, Saintpaulia and Arabidopsis, was identified as important for chicory
258 infection in our experiment. LfcR is a repressor of adjacent genes [46]. Surprisingly none of
259 these genes appeared to play a role for chicory infection suggesting that other targets of LfcR
260 probably remain to be discovered.
261 Finally, it is noteworthy to mention that the *ackA* and *pta* genes were GD *in planta*. These
262 genes constitute the reversible Pta-AckA pathway. The steady-state concentration of acetyl-

263 phosphate (acetyl-P), a signaling molecule in bacteria, depends upon the rate of its formation
264 catalyzed by Pta and of its degradation catalyzed by AckA [47]. The GD phenotype of *D.*
265 *dadantii ackA* and *pta* mutants during infection suggests that acetyl-P might play a crucial
266 signaling role in the adaptation of *D. dadantii* to the plant tissue.
267 **(v) Motility**. Motility is an essential virulence factor of *D. dadantii* required to move on the
268 surface of the leaf, enter the wounds and propagate into the plant tissue [48-50]. Accordingly,
269 all the genes required for flagella synthesis, the flagella motor and genes regulating their
270 synthesis (*flhC*, *flhD*, *fliA*) (see above) were necessary for fitness during chicory infection
271 (Fig. 4C and 5A). All the genes responsible for the transduction of the chemotaxis signal
272 (*cheA*, *B*, *R*, *W*, *X*, *Y* and *Z*) also confered a benefit *in planta* (Fig. 2). No methyl-accepting
273 chemoreceptor gene mutant was found. Like other environmental bacteria, *D. dadantii*
274 encodes many such proteins (47). They probably present some redundancy in the recognized
275 signal which prevented their detection by our screen.
276
277 ***D. dadantii* flagellin is modified by glycosylation**
278 A group of six genes located between *fliA* and *fliC* retained our interest since insertions in
279 these genes led to a growth defect in chicory (Fig. 5A). Dda3937_03424 encodes an O-linked
280 N-acetylglucosamine transferase and Dda3937_03419 encodes a protein with a nucleotide
281 diphospho sugar transferase predicted activity. The other ones could be involved in the
282 modification of sugars (predicted function of Dda3937_03423: nucleotide sugar
283 transaminase, Dda3937_03422: carbamoyl phosphate synthase, Dda3937_03421:
284 oxidoreductase; Dda3937_03420: methyltransferase). Their location let suppose that this
285 group of genes could be involved in flagellin glycosylation. Analysis by SDS-PAGE of FliC
286 produced by the wild type, and the A4277 and A3422 strains (Dda3937_03424 and
287 Dda3937_03419 mutants, respectively) showed that in the two latter strains the molecular

288 weight of the protein diminished (Fig. 5B). The molecular weight determined by mass
289 spectroscopy was 28,911 Da for $FliC_{A4277}$, 31,034 Da for $FliC_{A3422}$ and 32,258 Da for the WT
290 FliC. Thus, in the wild type strain FliC is modified by the products of the genes Dda_03424
291 to Dda_03419, probably by multiple glycosylation with a disaccharide. Absence of
292 modification did not alter *D. dadantii* motility (data not shown). Glycosylation of Gram
293 negative bacteria flagellin is limited to a few bacterial species. However, this modification
294 has been described in the plant pathogens *Pseudomonas syringae* pv *tabaci* and *Burkholderia*
295 *cenocepacia*. Absence of this modification lowered the ability of these bacteria to cause
296 disease on tobacco and *Arabidopsis*, respectively [51, 52]. Virulence of the glycosylation
297 mutant A4277 was tested on several plants. While no difference could be observed when
298 infecting chicory leaves (data not shown), length of rotten tissue was significantly reduced on
299 celery leaves compared to the wild type strain (Fig. 5C). Glycosylation prevents flagellin
300 epitope recognition and reduces induction of plant defense reactions. Thus, glycosylation
301 mutants are more efficiently killed by the plant. Glycosylation genes can be found in the
302 flagellar gene clusters of other *Dickeya* strains but also in *Pectobacterium* strains. This type
303 of modification that confers an increased virulence to the bacteria could be common feature
304 of flagellin in soft rot enterobacteriales.
305
306 **Additional genes could be involved in virulence**
307 Several genes have a $\log_2FC$ >4 or <-4 but do not satisfy the statistical permutation test
308 (Table S4). However, most of them belong to the categories described above and could be
309 required for growth *in planta*. Among those with a $\log_2FC$< -4 can be found genes involved
310 in amino acid and nucleic acid synthesis (*cysH*, *ilvC*, *pyrF*, *pyrD*, *purC*, *thrC*, *metA*, *cysK*,
311 *lysC*), flagella and motility *(flgJ*, *fliO*, *flgC*, *fliS*, *flgG*, *flgA*, *flgL*, *cheW*, *fliN*, *fliP*, *fliK*, *fliG*,

312 *fliL*), pectin and glucose metabolism (*kduD*, *pgi*), EPS synthesis (*gmd*), flagella glycosylation
313 (*vioA*) and regulation (*zur*, *ecpC* and the general RNA chaperone *hfq*).
314 Among the genes with a $\log_2$FC > 4, three regulators can be noticed: *pecS*, *pecT* and
315 *Dda3937_00840*. *pecS* and *pecT* are known regulators of *D. dadantii* controlling the
316 expression of many factors involved in virulence [5, 6]. Thus, their mutation could confer an
317 increased fitness of the bacteria in chicory. *D. dadantii* possesses a functional *expI-expR*
318 quorum sensing system which does not seem to control plant virulence factor production [53].
319 However, several LuxR family regulator genes which are not associated with a *luxI* gene are
320 present in the genome of the bacteria. Mutants of one of them (*Dda3937_00840*) were
321 overrepresented in the chicory. Its product is probably a repressor of genes conferring an
322 increased fitness i*n planta*.
323
324
325 **Validation of the Tn-seq results**.
326 To validate the Tn-seq results, we performed coinoculation experiments in chicory leaves
327 with the wild type strain and various mutants in genes conferring a growth advantage, a
328 growth defect or no fitness benefit in a 1/1 ratio. We calculated a competitivity index (CI) by
329 counting the number of each type of bacteria in the rotten tissue after 24 h. We found a
330 correlation between the competitivity index of mutants with the $\log_2$FC of the corresponding
331 genes with a Pearson coefficient of 0.50 (Fig. 6), indicating that Tn-seq is a reliable technique
332 to identify genes involved in plant colonization and virulence. Additional validations were
333 performed by inoculation of uncharacterized leucine and cytosine auxotroph mutants in
334 chicory leaves which confirmed that these mutants are unable to grow in plant and are thus
335 avirulent (Fig S4).
336

337

338 **Conclusion**

339 This Tn-seq experiment highlights new factors required for *D. dadantii* successful rotting of
340 chicory. Many genes known to be important for pathogenesis were not found in this screen
341 because their products are secreted and can be shared with other strains in the community.
342 This includes all the proteins secreted by the type II secretion system and small molecules
343 such as siderophores and butanediol. Other categories of genes were not found: for example,
344 no genes involved in response to acidic or oxidative stresses were identified. However,
345 chicory has been described as an inadequate model to study the response of *D. dadantii* to
346 oxidative stress [54]. Similarly, the type III *hrp* genes were not identified in our study. The
347 Hrp system is not necessary for *D. dadantii* virulence and in our experimental conditions
348 (high inoculum on isolated chicory leaves) the necrotrophic capacities of *D. dadantii*
349 (production of plant cell wall degrading enzymes) is probably sufficient to provoke the
350 disease. Studies on other host plants will allow to determine all the full virulence repertoire of
351 the bacteria.

352 Our results give also some information on the metabolic status of the plant. The amount of
353 nucleic acids and of the cysteine, leucine, methionine, threonine and isoleucine amino acids is
354 too low in chicory to allow the multiplication of mutant bacteria. Some enzymatic steps
355 involved in their synthesis are specific to bacteria and fungi. Thus, they could be good target
356 for the development of specific inhibitors [55] to fight *D. dadantii.*

357 While Tn-seq has been used to study genes required for the infection of animals, there has
358 been no genome-wide study of factors necessary for a plant pathogen to develop and provoke
359 disease on a plant. Besides the genes of known function described in the Results section, this
360 study allowed the identification of several genes of unknown function required for chicory

361 rotting. Repetition of this experiment with other strains or on other plants will tell if these
362 genes encode strain or host specific virulence factors.
363
364 **Methods**

365 **Bacterial strains and growth conditions.** Bacterial strains, phages, plasmids and
366 oligonucleotides used in this study are described in Table S1. *D. dadantii* and *E. coli* cells
367 were grown at 30 and 37°C respectively in LB medium or M63 minimal medium
368 supplemented with a carbon source (2 g/L). When required antibiotics were added at the
369 following concentration: ampicillin, 100 µg/L, kanamycin and chloramphenicol, 25 µg/L.
370 Media were solidified with 1.5 g/L agar. Transduction with phage PhiEC2 was performed
371 according to [56]. The motility of each mutant was compared with that of the wild-type strain
372 on semisolid (0.4%) LB agar plates as previously described [57].

373 **Construction of the transposon library**

374 5 mL of an overnight culture of *D. dadantii* strain A350 and of *E. coli* MFDpir/pSamEC were
375 mixed and centrifuged 2 min at 6000 g. The bacteria were resuspended in 1 mL of M63
376 medium and spread onto a 0.45 µm cellulose acetate filter placed on a M63 medium agar
377 plate. After 8h, bacteria were resuspended in 1 mL M63 medium. An aliquot was diluted and
378 spread onto LB agar + kanamycin plates to estimate the efficiency of mutagenesis. The other
379 part was inoculated in 100 mL of LB medium + kanamycin and grown for 24 h at 30°C. To
380 confirm that the bacteria that grew were *D. dadantii* strains with a transposon but without
381 plasmid pSamEC, we checked that all the grown bacteria were kan^R, amp^S and
382 diaminopimelate (DAP) prototrophs (MFDpir is DAP^-). The bacteria were frozen in 40%
383 glycerol at -80°C and represent a library of about 300 000 mutants.

384 **DNA preparation for high-throughput sequencing**

385 An aliquot of the mutant library was grown overnight in LB medium + kanamycin. To
386 identify essential genes in LB, the culture was diluted 100-fold in LB and grown for 6 h. To
387 infect chicory, the overnight culture was centrifuged and resuspended at OD_{600} = 1 in M63
388 medium. Chicories cut in half were inoculated with 10 µL of this bacterial suspension and
389 incubated at 30°C with maximum moist. After 60 h, the rotten tissue was collected and
390 filtered through a cheesecloth. The bacteria were collected by centrifugation and washed
391 twice in M63 medium. DNA was extracted from 1.5 mL aliquots of bacterial suspension
392 adjusted to OD_{600}=1.5 with the Promega Wizard Genomic DNA purification kit. Next steps
393 of the DNA preparation methods were adapted from [25]. All DNA gel-extraction were
394 performed onto a blue-light transilluminator of DNA stained with gel-green (Biotium) to
395 avoid DNA mutation and double-stranded breaks. 50 µg of DNA samples were digested with
396 50 U *MmeI* in a total volume of 1.2 mL for one hour at 37°C according to manufacturer's
397 instructions, then heat-inactivated for 20 minutes at 80°C, purified (QIAquick PCR
398 purification kit, Qiagen) and concentrated using a vacuum concentrator to a final volume of
399 25 µL. Digested DNA samples were run on a 1% agarose gel, the 1.0–1.5 kb band containing
400 the transposon and adjacent DNA was cut out and DNA was extracted from the gel according
401 to manufacturer's instructions (QIAquick Gel Extraction Kit, Qiagen). This allowed recovery
402 of all the fragments containing genomic DNA adjacent to transposons (1201 bp of
403 transposable element with 32-34 bp of genomic DNA). A pair of single-stranded
404 complementary oligonucleotides containing an unique 5-nt barcode sequence (LIB_AdaptT
405 and LIB_AdaptB) was mixed and heated to 100°C, then slowly cooled down in a water bath
406 to obtain double-stranded adaptors with two-nucleotide overhangs. 1 µg DNA of each sample
407 was ligated to the barcoded adaptors (0.44 mM) with 2000 U T4 DNA ligase in a final
408 volume of 50 µL at 16°C overnight. Five identical PCR reactions from the ligation product
409 were performed to amplify the transposon adjacent DNA. One reaction contained 100 ng of

410 DNA, 1 unit of Q5 DNA polymerase (Biolabs), 1X Q5 Buffer, 0.2 mM dNTPs, 0.4 µM of the
411 forward primer (LIB_PCR_5, which anneals to the P7 Illumina sequence of the transposon)
412 and the reverse primer (LIB_PCR_3, which anneals to the P5 adaptor). Only 18 cycles were
413 performed to keep a proportional amplification of the DNA. Samples were concentrated
414 using a vacuum concentrator to a final volume of 25 µL. Amplified DNA was run on a 1.8%
415 agarose gel and the 125 bp band was cut-out and gel extracted (QIAquick PCR purification
416 kit, Qiagen). DNA was finally dialysed (MF-Millipore™ Membrane Filters) for 4 hours.
417 Quality control of the Tn-seq DNA libraries (size of the fragments and concentration) and
418 High-throughput sequencing on Illumina HiSeq 2500 was performed by MGX (CNRS
419 sequencing service, Montpellier). 6 DNA libraries were multiplexed on one flow-cell. After
420 demultiplexing, the total number of reads was comprised between 18 and 31 millions (Table
421 1).
422
423 **Bioinformatics analysis:**
424 Raw reads from the fastQ files were first filtered using cutadapt v1.11 [58] and only reads
425 containing the *mariner* inverted left repeat (ACAGGTTGGATGATAAGTCCCCGGTCTT)
426 were trimmed and considered *bona fide* transposon-disrupted genes. Trimmed reads were
427 then analyzed using a modified version of the TPP script available from the TRANSIT
428 software v2.0.2 [29]. The mapping step was modified to select only reads mapping uniquely
429 and without mismatch in the *D. dadantii* 3937 genome (Genbank CP002038.1). Then, the
430 counting step was modified to accurately count the reads mapping to each TA site in the
431 reference genome according to the Tn-seq protocol used in this study. Read counts per
432 insertion were normalized using the LOESS method as described in [59]. We next used the
433 TRANSIT software (version 2.0) to compare the Tn-seq datasets.
434

435 **Strain construction.** To construct the A4277 strain, gene Dda3937_03424 was
436 amplified with the oligonucleotides 19732+ and 19732-. The resulting fragment was
437 inserted into the pGEM-T plasmid (Promega). A *uidA*-kan^R cassette [60] was inserted
438 into the unique AgeI site of the fragment. The construct was recombined into the *D.*
439 *dadantii* chromosome according to [61]. Recombination was checked by PCR.
440
441 **Protein techniques.** Flagella were prepared from overnight LB grown cells. Bacteria
442 were pelleted, resuspended in 1/10 volume of water and passed 20 fold through a needle
443 on a syringe. Cells and cells debris were removed by centrifugation 5 min at 20,000 g
444 [57]. Proteins were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).
445
446 **Coinoculation experiments**. To determine the competitivity index, the wild type strain and
447 the mutant to test, marked with an antibiotic resistance gene, were grown overnight in M63 +
448 glycerol medium. Bacteria were washed in M63 medium and the OD_{600} was adjusted to 1.0.
449 Bacteria were mixed to a 1:1 ratio and diluted 10-fold. 10 µL of the mixture were inoculated
450 into chicory leaves. The wound was covered with mineral oil and the leaves were incubated
451 at 30°C at high humidity. After 24 h the rotten tissue was collected, homogenized, diluted in
452 M63 and spread onto LB and LB + antibiotic plates. After 48 h at 30°C, colonies were
453 counted. The competitivity index is the ratio (number of mutant bacteria/number of WT
454 bacteria) in the rotten tissue / (number of mutant bacteria/number of WT bacteria) in the
455 inoculum.
456
457 **Plant inoculation experiments.** Bacteria to test were grown overnight in M63 + glycerol
458 medium. Bacteria were washed in M63 medium and the OD_{600} was adjusted to 1.0. Bacteria
459 were diluted 10-fold (for celery infection) or 100-fold (for chicory infection) in the same

460 medium. 10 µL of the bacterial suspension were inoculated into leaves in a hole made with a
461 pipet tip. The wound was covered with mineral oil and the leaves were incubated at 30°C at
462 high humidity for 2 days (celery) or 24 h (for chicory). Length or rotten tissue was measured.
463
464 **Nucleotide sequence accession numbers.** The transposon sequence reads we obtained have
465 been submitted to the ENA database under accession number PRJEB20574.
466

467 **Acknowledgment**
468 We thank Geraldine Effantin, Veronique Utzinger and Matthias Schulz for technical
469 assistance, the members of the MTSB team and Xavier Charpentier for discussion, Nicole
470 Cotte-Pattat for critical read of the manuscript and James Paslesvert for encouragements.
471

472 **Funding**
473 This work was supported by the CNRS, INSA and funded by a grant from the University
474 Lyon I to E.G. (BQR UCBL). K.R was supported by a PhD grant from the Ministère de
475 l'éducation nationale et de la recherche.

476 **Author contributions**
477 Conceived and designed the experiments: EG GC. Performed the experiments: KR NP GC.
478 Analyzed the data: KR NP EG GDC. Contributed reagents/materials/analysis tools: NP AR
479 EG GC. Wrote the paper: KR EG GC.
480

481 **Legend of figures**
482

483 **Fig. 1**. **Quality control of the Tn-seq *D. dadantii* 3937 libraries**.
484 (A) Frequency and distribution of transposon sequence reads across the entire *D. dadantii*

485 *3937* genome. The localization of transposon insertions shows no bias throughout the genome
486 of *D. dadantii 3937*. B) Density plot of log_2FC of measured read counts versus expected
487 reads count per gene.
488
489 **Fig 2. Genes identified by Tn-seq exhibiting a fitness change in chicory compared to LB.**
490 Data were obtained with the TRANSIT software**.** From left to right: (a) classification of the
491 genes with the HMM method performed in the LB dataset. (b) Number of TA sites left after
492 removing the first 10% bp of 5' and 3' extremities, in order to minimize Tn insertions that
493 would not result in complete inactivation of a given gene. (c) Mean read number per gene in
494 the LB or chicory dataset. (d) Ratio of read number between chicory and LB expressed in
495 log_2. (e) FDR adjusted p-values (q-value). Genes for which a role in *D. dadantii* virulence has
496 been described before are in bold.

497 **Fig 3. Scheme of the purine and pyrimidine biosynthesis pathways in *D. dadantii* that**
498 **produce XMP (purine metabolism) and UMP (pyrimidine metabolism) from L-**
499 **glutamine.**
500 In red are indicated the growth defect genes in chicory that pass the permutation test (q-value
501 ≤ 0.05). The log_2FC of read numbers between chicory and LB for each gene is indicated in
502 bracket. Some genes do not pass the permutation test (in black) but have a strong negative
503 log_2FC. PRPP: 5-phosphoribosyl-1-pyrophosphate ; GAR: 5'-phosphoribosyl-1-glycinamide
504 ; FGAM: 5'-phosphoribosyl-*N*-formylglycinamide ; AIR: 5'-phosphoribosyl-5-
505 aminoimidazole ; CAIR: 5'-phosphoribosyl-5-aminoimidazole carboxylic acid ; SAICAR: 5'-
506 phosphoribosyl-4-(*N*-succino-carboxamide)-5-aminoimidazole ; AICAR: 5-aminoimidazole-
507 4-carboxamide ribonucleotide ; IMP: inosine monophosphate ; XMP: xanthine
508 monophosphate ; UMP: uridine monophosphate.
509

510 **Fig 4. Examples of essential and important genes revealed by Tn-seq**.

511 Number of reads at each transposon location in the sample grown in LB or in chicory. Data
512 are averaged from biological replicates and normalized as described in the methods section.
513 Three regions of the genome representative of Tn-seq results are shown, with the predicted
514 genes represented at the bottom of each panel. Peaks represent read number at TA sites.
515 Black arrows represent genes that pass the permutation test (q-value ≤ 0.05). (A) Essentiality
516 of leucine biosynthetic genes in chicory. (B) Insertions in the 5' region of *rsmC* generate
517 growth advantage for the bacteria in chicory. (C) Importance of genes involved in motility for
518 growth in chicory.

519 **Fig 5. Modification of FliC revealed by Tn-seq analysis and SDS-PAGE. (**A) Importance
520 of 6 genes located between *fliA* and *fliC* for growth in chicory. Log_2FC are indicated in
521 bracket. Dda3937_03425 and Dda3937_03426 are duplicated transposase genes that have
522 been removed from the analysis. Black arrow: GD in chicory (q-value ≤ 0.05) ; white arrow:
523 genes that do not pass the permutation test (q-value > 0.05). (B) Analysis by SDS-PAGE of
524 FliC produced by the wild type (lane 2), the A3422 (lane 3) and A4277 (lane 4) strains. MM
525 (lane 1) is the Molecular Marker. (C) (C) Maceration of celery leaves by the Wild Type and
526 A4277 (glycosylation) mutant. Length of rotten tissue was measured 48 h post infection. Box
527 plot were generated by BoxPlotR from 9 data points. The calculated median value is 109 for
528 the WT strain, 40 for the A4277 strain.

529

530 **Fig 6. Correlation between the competitivity index (IC) of several mutant strains with**
531 **the log_2FC of the corresponding genes.** IC values were determined in chicory leaves as
532 described in Methods. The IC values for *dltB*, *anrB*, *phoS* and *phoH* are from [62]. Each

533 value is the mean of 5 experiments.

534

535 **Fig S1. Biological reproducibility of the Tn-seq results**.

536 Pairs of Tn-seq assay results are compared, with the total number of reads per gene plotted.

537 Analysis of DNA samples corresponding to two independent cultures of the mutant pool

538 grown (A) in LB medium (correlation coefficient R = 0.72) and (B) in chicory (correlation

539 coefficient R = 0.98). Values represent average numbers of reads per gene from the pairs of

540 biological replicates.

541

542 **Fig S2. Volcano plot of resampling results comparing replicates grown in chicory versus**

543 **in LB**. Significant hits have q-value < 0.05 or $-\log_{10}$ q-value > 1.3. Growth defect (GD) or

544 growth advantage (GA) genes are indicated by a red frame.

545 **Fig S3. *acrAB* are essential in chicory.** Number of Tn-seq reads at each insertion site in the

546 *acrA acrB* region in samples grown in LB or in chicory. Data are averaged from biological

547 replicates and normalized as described in Methods. *dnaX* which encodes both the tau and

548 gamma subunits of DNA polymerase is represented by a grey arrow. *dnaX* is essential gene in

549 LB. *acrAB* represented by black arrows are GD in chicory (q-value ≤ 0.05).

550 **Fig S4. Pathogenicity tests on chicory with leucine (A5969) and cytosine (A5968)**

551 **mutants of *D. dadantii*.**

552 Each leaf was inoculated with 10^6 bacteria. Length of rotten tissue was observed after 24h.

553 **References**

554

555 1. Adeolu M, Alnajar S, Naushad S, R SG. Genome-based phylogeny and taxonomy of

556 the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families

557 Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam.
558 nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. Int J Syst
559 Evol Microbiol. 2016;66(12):5575-99. Epub 2016/09/14. doi: 10.1099/ijsem.0.001485.
560 PubMed PMID: 27620848.
561 2. Charkowski A, Blanco C, Condemine G, Expert D, Franza T, Hayes C, et al. The
562 Role of Secretion Systems and Small Molecules in Soft-Rot Enterobacteriaceae
563 Pathogenicity. Annu Rev Phytopathol. 2012;50:425-49. PubMed PMID: 22702350.
564 3. Hugouvieux-Cotte-Pattat N, Condemine G, Nasser W, Reverchon S. Regulation of
565 pectinolysis in *Erwinia chrysanthemi.* Annu Rev Microbiol. 1996;50:213-57. PubMed PMID:
566 8905080.
567 4. Condemine G, Robert-Baudouy J. Analysis of an *Erwinia chrysanthemi* gene cluster
568 involved in pectin degradation. Mol Microbiol. 1991;5(9):2191-202. PubMed PMID:
569 1766386.
570 5. Reverchon S, Nasser W, Robert-Baudouy J. pecS: a locus controlling pectinase,
571 cellulase and blue pigment production in Erwinia chrysanthemi. Mol Microbiol.
572 1994;11(6):1127-39. Epub 1994/03/01. PubMed PMID: 8022282.
573 6. Surgey N, Robert-Baudouy J, Condemine G. The Erwinia chrysanthemi pecT gene
574 regulates pectinase gene expression. J Bacteriol. 1996;178(6):1593-9. PubMed PMID:
575 8626286.
576 7. Nasser W, Faelen M, Hugouvieux-Cotte-Pattat N, Reverchon S. Role of the nucleoid-
577 associated protein H-NS in the synthesis of virulence factors in the phytopathogenic
578 bacterium Erwinia chrysanthemi. Mol Plant Microbe Interact. 2001;14(1):10-20. Epub
579 2001/02/24. doi: 10.1094/mpmi.2001.14.1.10. PubMed PMID: 11194867.
580 8. Lebeau A, Reverchon S, Gaubert S, Kraepiel Y, Simond-Cote E, Nasser W, et al. The
581 GacA global regulator is required for the appropriate expression of Erwinia chrysanthemi

582 3937 pathogenicity genes during plant infection. Environ Microbiol. 2008;10(3):545-59.
583 PubMed PMID: 18177376.
584 9. Condemine G, Castillo A, Passeri F, Enard C. The PecT repressor coregulates
585 synthesis of exopolysaccharides and virulence factors in Erwinia chrysanthemi. Mol Plant
586 Microbe Interact. 1999;12(1):45-52. PubMed PMID: 9885192.
587 10. Reverchon S, Rouanet C, Expert D, Nasser W. Characterization of indigoidine
588 biosynthetic genes in Erwinia chrysanthemi and role of this blue pigment in pathogenicity. J
589 Bacteriol. 2002;184(3):654-65. Epub 2002/01/16. PubMed PMID: 11790734; PubMed
590 Central PMCID: PMCPMC139515.
591 11. Condemine G, Dorel C, Hugouvieux-Cotte-Pattat N, Robert-Baudouy J. Some of the
592 out genes involved in the secretion of pectate lyases in Erwinia chrysanthemi are regulated by
593 kdgR. Mol Microbiol. 1992;6(21):3199-211. PubMed PMID: 1453958.
594 12. Yang CH, Gavilanes-Ruiz M, Okinaka Y, Vedel R, Berthuy I, Boccara M, et al. hrp
595 genes of Erwinia chrysanthemi 3937 are important virulence factors. Mol Plant Microbe
596 Interact. 2002;15(5):472-80. Epub 2002/05/31. doi: 10.1094/mpmi.2002.15.5.472. PubMed
597 PMID: 12036278.
598 13. Li Y, Hutchins W, Wu X, Liang C, Zhang C, Yuan X, et al. Derivative of plant
599 phenolic compound inhibits the type III secretion system of Dickeya dadantii via HrpX/HrpY
600 two-component signal transduction and Rsm systems. Molecular plant pathology.
601 2015;16(2):150-63. Epub 2014/07/06. doi: 10.1111/mpp.12168. PubMed PMID: 24986378.
602 14. Yang S, Peng Q, Zhang Q, Zou L, Li Y, Robert C, et al. Genome-wide identification
603 of HrpL-regulated genes in the necrotrophic phytopathogen Dickeya dadantii 3937. PLoS
604 One. 2010;5(10):e13472. Epub 2010/10/27. doi: 10.1371/journal.pone.0013472. PubMed
605 PMID: 20976052; PubMed Central PMCID: PMCPMC2957411.

606 15. Franza T, Mahe B, Expert D. Erwinia chrysanthemi requires a second iron transport
607 route dependent of the siderophore achromobactin for extracellular growth and plant
608 infection. Mol Microbiol. 2005;55(1):261-75. Epub 2004/12/23. doi: 10.1111/j.1365-
609 2958.2004.04383.x. PubMed PMID: 15612933.
610 16. Franza T, Sauvage C, Expert D. Iron regulation and pathogenicity in Erwinia
611 chrysanthemi 3937: role of the Fur repressor protein. Mol Plant Microbe Interact.
612 1999;12(2):119-28. Epub 1999/02/02. doi: 10.1094/mpmi.1999.12.2.119. PubMed PMID:
613 9926414.
614 17. Franza T, Expert D. The virulence-associated chrysobactin iron uptake system of
615 Erwinia chrysanthemi 3937 involves an operon encoding transport and biosynthetic
616 functions. J Bacteriol. 1991;173(21):6874-81. Epub 1991/11/01. PubMed PMID: 1657869;
617 PubMed Central PMCID: PMCPMC209040.
618 18. Okinaka Y, Yang CH, Perna NT, Keen NT. Microarray profiling of Erwinia
619 chrysanthemi 3937 genes that are regulated during plant infection. Mol Plant Microbe
620 Interact. 2002;15(7):619-29. Epub 2002/07/18. doi: 10.1094/mpmi.2002.15.7.619. PubMed
621 PMID: 12118877.
622 19. Yang S, Perna NT, Cooksey DA, Okinaka Y, Lindow SE, Ibekwe AM, et al.
623 Genome-wide identification of plant-upregulated genes of Erwinia chrysanthemi 3937 using
624 a GFP-based IVET leaf array. Mol Plant Microbe Interact. 2004;17(9):999-1008. Epub
625 2004/09/24. doi: 10.1094/mpmi.2004.17.9.999. PubMed PMID: 15384490.
626 20. Chapelle E, Alunni B, Malfatti P, Solier L, Pedron J, Kraepiel Y, et al. A
627 straightforward and reliable method for bacterial in planta transcriptomics: application to the
628 Dickeya dadantii/Arabidopsis thaliana pathosystem. Plant J. 2015;82(2):352-62. Epub
629 2015/03/06. doi: 10.1111/tpj.12812. PubMed PMID: 25740271.

630 21. Reverchon S, Muskhelisvili G, Nasser W. Virulence Program of a Bacterial Plant
631 Pathogen: The Dickeya Model. Progress in molecular biology and translational science.
632 2016;142:51-92. Epub 2016/08/31. doi: 10.1016/bs.pmbts.2016.05.005. PubMed PMID:
633 27571692.
634 22. Hinton JC, Sidebotham JM, Hyman LJ, Perombelon MC, Salmond GP. Isolation and
635 characterisation of transposon-induced mutants of Erwinia carotovora subsp. atroseptica
636 exhibiting reduced virulence. Mol Gen Genet. 1989;217(1):141-8. Epub 1989/05/01. PubMed
637 PMID: 2549365.
638 23. Pirhonen M, Saarilahti H, Karlsson M-B, Palva ET. Identification of pathogenicity
639 determinants of Erwinia carotovora subsp. carotovora by transposon mutagenesis. Molecular
640 Plant-Microbe Interaction. 1991;4:276-83.
641 24. Fu Y, Waldor MK, Mekalanos JJ. Tn-Seq analysis of Vibrio cholerae intestinal
642 colonization reveals a role for T6SS-mediated antibacterial activity in the host. Cell host &
643 microbe. 2013;14(6):652-63. Epub 2013/12/18. doi: 10.1016/j.chom.2013.11.001. PubMed
644 PMID: 24331463; PubMed Central PMCID: PMCPMC3951154.
645 25. Skurnik D, Roux D, Aschard H, Cattoir V, Yoder-Himes D, Lory S, et al. A
646 comprehensive analysis of in vitro and in vivo genetic fitness of Pseudomonas aeruginosa
647 using high-throughput sequencing of transposon libraries. PLoS Pathog. 2013;9(9):e1003582.
648 Epub 2013/09/17. doi: 10.1371/journal.ppat.1003582. PubMed PMID: 24039572; PubMed
649 Central PMCID: PMCPMC3764216.
650 26. van Opijnen T, Camilli A. A fine scale phenotype-genotype virulence map of a
651 bacterial pathogen. Genome research. 2012;22(12):2541-51. Epub 2012/07/25. doi:
652 10.1101/gr.137430.112. PubMed PMID: 22826510; PubMed Central PMCID:
653 PMCPMC3514683.

654 27. van Opijnen T, Camilli A. Transposon insertion sequencing: a new tool for systems-
655 level analysis of microorganisms. Nat Rev Microbiol. 2013;11(7):435-42. Epub 2013/05/29.
656 doi: 10.1038/nrmicro3033. PubMed PMID: 23712350; PubMed Central PMCID:
657 PMCPMC3842022.
658 28. Wiles TJ, Norton JP, Russell CW, Dalley BK, Fischer KF, Mulvey MA. Combining
659 quantitative genetic footprinting and trait enrichment analysis to identify fitness determinants
660 of a bacterial pathogen. PLoS Genet. 2013;9(8):e1003716. Epub 2013/08/31. doi:
661 10.1371/journal.pgen.1003716. PubMed PMID: 23990803; PubMed Central PMCID:
662 PMCPMC3749937.
663 29. DeJesus MA, Ambadipudi C, Baker R, Sassetti C, Ioerger TR. TRANSIT--A
664 Software Tool for Himar1 TnSeq Analysis. PLoS computational biology.
665 2015;11(10):e1004401. Epub 2015/10/09. doi: 10.1371/journal.pcbi.1004401. PubMed
666 PMID: 26447887; PubMed Central PMCID: PMCPMC4598096.
667 30. Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M. KEGG: Kyoto
668 Encyclopedia of Genes and Genomes. Nucleic Acids Res. 1999;27(1):29-34. Epub
669 1998/12/10. PubMed PMID: 9847135; PubMed Central PMCID: PMCPMC148090.
670 31. Reverchon S, Nasser W. Dickeya ecology, environment sensing and regulation of
671 virulence programme. Environ Microbiol Rep. 2013;5(5):622-36. PubMed PMID: 24115612.
672 32. Ravirala RS, Barabote RD, Wheeler DM, Reverchon S, Tatum O, Malouf J, et al.
673 Efflux pump gene expression in Erwinia chrysanthemi is induced by exposure to phenolic
674 acids. Mol Plant Microbe Interact. 2007;20(3):313-20. Epub 2007/03/24. doi: 10.1094/mpmi-
675 20-3-0313. PubMed PMID: 17378434.
676 33. Malinverni JC, Silhavy TJ. An ABC transport system that maintains lipid asymmetry
677 in the gram-negative outer membrane. Proc Natl Acad Sci U S A. 2009;106(19):8009-14.

678 Epub 2009/04/23. doi: 10.1073/pnas.0903229106. PubMed PMID: 19383799; PubMed
679 Central PMCID: PMCPMC2683108.
680 34. Page F, Altabe S, Hugouvieux-Cotte-Pattat N, Lacroix JM, Robert-Baudouy J, Bohin
681 JP. Osmoregulated periplasmic glucan synthesis is required for Erwinia chrysanthemi
682 pathogenicity. J Bacteriol. 2001;183(10):3134-41. PubMed PMID: 11325942.
683 35. Bouchart F, Boussemart G, Prouvost AF, Cogez V, Madec E, Vidal O, et al. The
684 virulence of a Dickeya dadantii 3937 mutant devoid of osmoregulated periplasmic glucans is
685 restored by inactivation of the RcsCD-RcsB phosphorelay. J Bacteriol. 2010;192(13):3484-
686 90. Epub 2010/04/27. doi: 10.1128/jb.00143-10. PubMed PMID: 20418397; PubMed Central
687 PMCID: PMCPMC2897653.
688 36. Madec E, Bontemps-Gallo S, Lacroix JM. Increased phosphorylation of the RcsB
689 regulator of the RcsCDB phosphorelay in strains of Dickeya dadantii devoid of
690 osmoregulated periplasmic glucans revealed by Phos-tag gel analysis. Microbiology.
691 2014;160(Pt 12):2763-70. Epub 2014/10/17. doi: 10.1099/mic.0.081273-0. PubMed PMID:
692 25320363.
693 37. Wu X, Zeng Q, Koestler BJ, Waters CM, Sundin GW, Hutchins W, et al. Deciphering
694 the components that coordinately regulate virulence factors of the soft rot pathogen Dickeya
695 dadantii. Mol Plant Microbe Interact. 2014;27(10):1119-31. Epub 2014/09/03. doi:
696 10.1094/mpmi-01-14-0026-r. PubMed PMID: 25180688.
697 38. Franza T, Expert D. Role of iron homeostasis in the virulence of phytopathogenic
698 bacteria: an 'a la carte' menu. Molecular plant pathology. 2013;14(4):429-38. Epub
699 2012/11/23. doi: 10.1111/mpp.12007. PubMed PMID: 23171271.
700 39. Tans-Kersten J, Brown D, Allen C. Swimming motility, a virulence trait of Ralstonia
701 solanacearum, is regulated by FlhDC and the plant host environment. Mol Plant Microbe

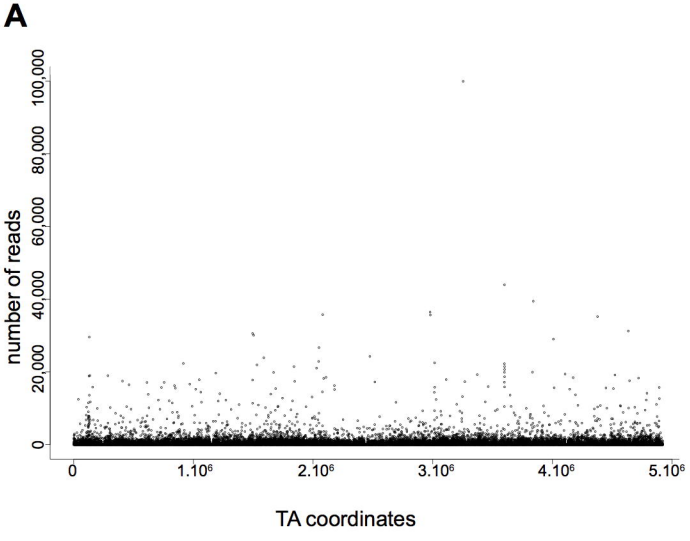
702 Interact. 2004;17(6):686-95. Epub 2004/06/16. doi: 10.1094/mpmi.2004.17.6.686. PubMed
703 PMID: 15195951.
704 40. Jozwick AK, Graf J, Welch TJ. The flagellar master operon flhDC is a pleiotropic
705 regulator involved in motility and virulence of the fish pathogen Yersinia ruckeri. J Appl
706 Microbiol. 2016. Epub 2016/12/17. doi: 10.1111/jam.13374. PubMed PMID: 27981729.
707 41. Xu T, Su Y, Xu Y, He Y, Wang B, Dong X, et al. Mutations of flagellar genes fliC12,
708 fliA and flhDC of Edwardsiella tarda attenuated bacterial motility, biofilm formation and
709 virulence to fish. J Appl Microbiol. 2014;116(2):236-44. Epub 2013/10/15. doi:
710 10.1111/jam.12357. PubMed PMID: 24118854.
711 42. Yuan X, Khokhani D, Wu X, Yang F, Biener G, Koestler BJ, et al. Cross-talk
712 between a regulatory small RNA, cyclic-di-GMP signalling and flagellar regulator FlhDC for
713 virulence and bacterial behaviours. Environ Microbiol. 2015;17(11):4745-63. Epub
714 2015/10/16. doi: 10.1111/1462-2920.13029. PubMed PMID: 26462993.
715 43. Chatterjee A, Cui Y, Chatterjee AK. RsmC of Erwinia carotovora subsp. carotovora
716 negatively controls motility, extracellular protein production, and virulence by binding FlhD
717 and modulating transcriptional activity of the master regulator, FlhDC. J Bacteriol.
718 2009;191(14):4582-93. Epub 2009/05/19. doi: 10.1128/jb.00154-09. PubMed PMID:
719 19447906; PubMed Central PMCID: PMCPMC2704716.
720 44. Ko M, Park C. H-NS-Dependent regulation of flagellar synthesis is mediated by a
721 LysR family protein. J Bacteriol. 2000;182(16):4670-2. Epub 2000/07/27. PubMed PMID:
722 10913108; PubMed Central PMCID: PMCPMC94646.
723 45. Yi X, Yamazaki A, Biddle E, Zeng Q, Yang CH. Genetic analysis of two
724 phosphodiesterases reveals cyclic diguanylate regulation of virulence factors in Dickeya
725 dadantii. Mol Microbiol. 2010;77(3):787-800. Epub 2010/06/30. doi: 10.1111/j.1365-
726 2958.2010.07246.x. PubMed PMID: 20584146.

727 46. Van Gijsegem F, Wlodarczyk A, Cornu A, Reverchon S, Hugouvieux-Cotte-Pattat N.
728 Analysis of the LacI family regulators of Erwinia chrysanthemi 3937, involvement in the
729 bacterial phytopathogenicity. Mol Plant Microbe Interact. 2008;21(11):1471-81. PubMed
730 PMID: 18842096.
731 47. Wolfe AJ. The acetate switch. Microbiol Mol Biol Rev. 2005;69(1):12-50. Epub
732 2005/03/10. doi: 10.1128/mmbr.69.1.12-50.2005. PubMed PMID: 15755952; PubMed
733 Central PMCID: PMCPMC1082793.
734 48. Antunez-Lamas M, Cabrera-Ordonez E, Lopez-Solanilla E, Raposo R, Trelles-Salazar
735 O, Rodriguez-Moreno A, et al. Role of motility and chemotaxis in the pathogenesis of
736 Dickeya dadantii 3937 (ex Erwinia chrysanthemi 3937). Microbiology. 2009;155(Pt 2):434-
737 42. Epub 2009/02/10. doi: 10.1099/mic.0.022244-0. PubMed PMID: 19202091.
738 49. Rio-Alvarez I, Munoz-Gomez C, Navas-Vasquez M, Martinez-Garcia PM, Antunez-
739 Lamas M, Rodriguez-Palenzuela P, et al. Role of Dickeya dadantii 3937 chemoreceptors in
740 the entry to Arabidopsis leaves through wounds. Molecular plant pathology. 2015;16(7):685-
741 98. Epub 2014/12/10. doi: 10.1111/mpp.12227. PubMed PMID: 25487519.
742 50. Jahn CE, Willis DK, Charkowski AO. The flagellar sigma factor fliA is required for
743 Dickeya dadantii virulence. Mol Plant Microbe Interact. 2008;21(11):1431-42. Epub
744 2008/10/10. doi: 10.1094/mpmi-21-11-1431. PubMed PMID: 18842093.
745 51. Taguchi F, Yamamoto M, Ohnishi-Kameyama M, Iwaki M, Yoshida M, Ishii T, et al.
746 Defects in flagellin glycosylation affect the virulence of Pseudomonas syringae pv. tabaci
747 6605. Microbiology. 2010;156(Pt 1):72-80. Epub 2009/10/10. doi: 10.1099/mic.0.030700-0.
748 PubMed PMID: 19815579.
749 52. Khodai-Kalaki M, Andrade A, Fathy Mohamed Y, Valvano MA. Burkholderia
750 cenocepacia Lipopolysaccharide Modification and Flagellin Glycosylation Affect Virulence
751 but Not Innate Immune Recognition in Plants. MBio. 2015;6(3):e00679. Epub 2015/06/06.

752 doi: 10.1128/mBio.00679-15. PubMed PMID: 26045541; PubMed Central PMCID:
753 PMCPMC4462625.
754 53. Nasser W, Bouillant ML, Salmond G, Reverchon S. Characterization of the Erwinia
755 chrysanthemi expI-expR locus directing the synthesis of two N-acyl-homoserine lactone
756 signal molecules. Mol Microbiol. 1998;29(6):1391-405. Epub 1998/10/22. PubMed PMID:
757 9781877.
758 54. Santos R, Franza T, Laporte ML, Sauvage C, Touati D, Expert D. Essential role of
759 superoxide dismutase on the pathogenicity of Erwinia chrysanthemi strain 3937. Mol Plant
760 Microbe Interact. 2001;14(6):758-67. Epub 2001/06/02. doi: 10.1094/mpmi.2001.14.6.758.
761 PubMed PMID: 11386371.
762 55. Thangavelu B, Bhansali P, Viola RE. Elaboration of a fragment library hit produces
763 potent and selective aspartate semialdehyde dehydrogenase inhibitors. Bioorg Med Chem.
764 2015;23(20):6622-31. Epub 2015/09/26. doi: 10.1016/j.bmc.2015.09.017. PubMed PMID:
765 26404410; PubMed Central PMCID: PMCPMC4601562.
766 56. Résibois A, Colet M, Faelen M, Schoonejans E, Toussaint A. phiEC2, a new
767 generalized transducing phage of *Erwinia chrysanthemi*. Virology. 1984;137(1):102-12.
768 PubMed PMID: 18639822.
769 57. Shevchik VE, Condemine G, Robert-Baudouy J. Characterization of DsbC, a
770 periplasmic protein of Erwinia chrysanthemi and Escherichia coli with disulfide isomerase
771 activity. Embo J. 1994;13(8):2007-12. PubMed PMID: 8168497.
772 58. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing
773 reads. EMBnetjournal. 2011;17:10-2.
774 59. Zomer A, Burghout P, Bootsma HJ, Hermans PW, van Hijum SA. ESSENTIALS:
775 software for rapid analysis of high throughput transposon insertion sequencing data. PLoS

776 One. 2012;7(8):e43012. Epub 2012/08/18. doi: 10.1371/journal.pone.0043012. PubMed
777 PMID: 22900082; PubMed Central PMCID: PMCPMC3416827.
778 60. Bardonnet N, Blanco C. Improved vectors for transcriptional signal screening in
779 corynebacteria. FEMS Microbiol Lett. 1991;68(1):97-102. PubMed PMID: 1722768.
780 61. Roeder DL, Collmer A. Marker-exchange mutagenesis of a pectate lyase isozyme
781 gene in Erwinia chrysanthemi. J Bacteriol. 1985;164(1):51-6. PubMed PMID: 2995324.
782 62. Pandin C, Caroff M, Condemine G. Antimicrobial Peptide Resistance Genes in the
783 Plant Pathogen Dickeya dadantii. Appl Environ Microbiol. 2016;82(21):6423-30. Epub
784 2016/08/28. doi: 10.1128/aem.01757-16. PubMed PMID: 27565623; PubMed Central
785 PMCID: PMCPMC5066359.
786

Figure 1


A
number of reads
0
20,000
40,000
60,000
80,000
100,000
0
1.10⁶
2.10⁶
3.10⁶
4.10⁶
5.10⁶
TA coordinates

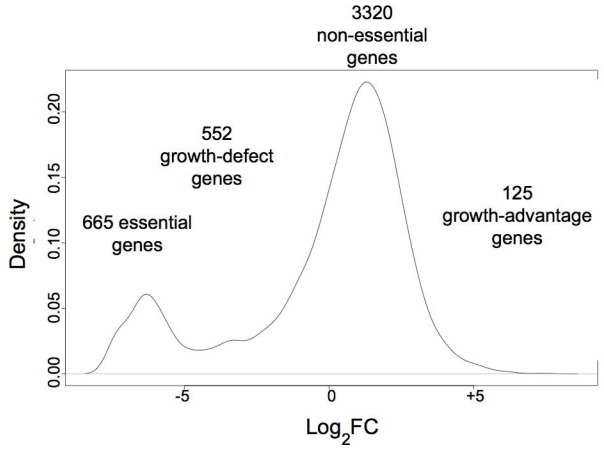

B
3320
non-essential
genes
552
growth-defect
genes
665 essential
genes
125
growth-advantage
genes
Density
0.00
0.05
0.10
0.15
0.20
-5
0
+5
Log2FC

Figure 2

Locus	Gene	Function	HMM State in LB[a]	RESAMPLING No. of TAs[b]	Mean reads[c] LB	Chicory	log_2FC[d]	q-value[e]
Dda3937_00335	*glpD*	glycerol-3-phosphate dehydrogenase	GD	33	650	0	-12.56	0.00
Dda3937_03379	*purL*	phosphoribosylformyl-glycineamide synthetase	NE	73	378	0	-11.91	0.00
Dda3937_03564	***opgG***	**Glucans biosynthesis protein G precursor**	**GA**	**40**	**1976**	**1**	**-11.41**	**0.00**
Dda3937_00244	*purH*	phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase	NE	37	145	0	-11.25	0.00
Dda3937_00432	*hflK*	FtsH protease regulator	GD	28	339	0	-11.12	0.03
Dda3937_02515	*purM*	phosphoribosylaminoimidazole synthetase	NE	21	344	0	-10.57	0.00
Dda3937_02627		4-hydroxythreonine-4-phosphate dehydrogenase	NE	26	129	0	-10.06	0.00
Dda3937_00004	*guaB*	IMP dehydrogenase	NE	33	151	0	-9.97	0.00
Dda3937_03563	***opgH***	**Glucans biosynthesis glucosyltransferase H**	**GA**	**62**	**1409**	**2**	**-9.79**	**0.00**
Dda3937_01284	*pyrB*	aspartate carbamoyltransferase	NE	17	159	0	-9.68	0.00
Dda3937_03924	***rffG***	**dTDP-glucose 4,6-dehydratase**	**NE**	**23**	**317**	**1**	**-9.38**	**0.02**
Dda3937_01389	*carB*	carbamoyl-phosphate synthase large subunit	NE	48	249	0	-9.23	0.00
Dda3937_03299	***acrA***	**MexE family multidrug efflux RND transporter periplasmic adaptor subunit**	**NE**	**34**	**196**	**0**	**-9.03**	**0.00**
Dda3937_03300	***acrB***	**multidrug efflux system protein**	**NE**	**89**	**422**	**1**	**-8.90**	**0.00**
Dda3937_03258	*pyrE*	orotate phosphoribosyltransferase	NE	14	175	0	-8.81	0.00
Dda3937_02336	*nlpI*	lipoprotein	GD	33	27	0	-8.69	0.00
Dda3937_02506	*nlpB (bamC)*	outer membrane protein assembly factor BamC	NE	20	47	0	-8.69	0.00
Dda3937_04018	*pta*	phosphate acetyltransferase	GD	36	579	2	-8.59	0.02
Dda3937_03554	*pyrC*	dihydro-orotase	NE	25	343	1	-8.44	0.00
Dda3937_04573	*lpxM*	acyl (myristate) transferase	NE	33	63	0	-8.31	0.00
Dda3937_01116	*glnG*	Nitrogen regulation protein NR(I), Two-component system	NE	26	39	0	-8.22	0.00
Dda3937_02099	*purF*	amidophosphoribosyltransferase	NE	32	107	0	-8.19	0.00
Dda3937_04019	*ackA*	acetate kinase A and propionate kinase 2	NE	29	45	0	-8.16	0.00
Dda3937_02189	*yejM*	Membrane-anchored periplasmic protein, alkaline phosphatase superfamily	GA	34	4160	15	-8.08	0.00
Dda3937_01390	*carA*	carbamoyl-phosphate synthase small subunit	NE	21	69	0	-8.05	0.00
Dda3937_01426	*ptsI*	Phosphoenolpyruvate-protein phosphotransferase of PTS system	NE	33	45	0	-7.85	0.00
Dda3937_00161	*cysQ*	3'(2'),5'-bisphosphate nucleotidase	NE	16	44	0	-7.81	0.02
Dda3937_00210	*cysI*	sulfite reductase beta subunit	NE	40	252	1	-7.65	0.00
Dda3937_04075	*lysR*	LysR family transcriptional regulator	NE	13	2385	13	-7.51	0.00
Dda3937_02526		conserved protein	NE	18	50	0	-7.50	0.00
Dda3937_03888	*metB*	Cystathionine gamma-synthase	NE	21	118	1	-7.34	0.01
Dda3937_00195	*relA*	(p)ppGpp synthetase I/GTP pyrophosphokinase	NE	55	256	2	-7.12	0.00
Dda3937_02532	***lfcR***	**Fructose repressor FruR, LacI family**	**NE**	**15**	**399**	**3**	**-7.04**	**0.00**
Dda3937_02226	*fliF*	Flagellar M-ring protein fliF	NE	46	476	4	-7.02	0.00
Dda3937_02206	*flgE*	Flagellar hook protein flgE	NE	50	597	5	-7.00	0.00
Dda3937_04507	*gnd*	phosphogluconate dehydrogenase (NADP(+)-dependent, decarboxylating)	GD	36	7	0	-6.91	0.00
Dda3937_00697	*degQ*	Protease	NE	28	80	1	-6.87	0.01
Dda3937_03631	*trxB*	thioredoxin-disulfide reductase	GD	25	16	0	-6.85	0.03
Dda3937_00361	*yrfF (igaA)*	intracellular growth attenuator protein	GD	38	22	0	-6.78	0.03
Dda3937_00588	*cysB*	Transcriptional dual regulator, O-acetyl-L-serine-binding protein	NE	29	90	1	-6.75	0.00
Dda3937_03783	*prc*	carboxy-terminal protease for penicillin-binding protein 3	NE	46	243	2	-6.71	0.00
Dda3937_00433	*hflX*	predicted GTPase	GD	27	16	0	-6.69	0.04
Dda3937_03427	*fliC*	flagellar filament structural protein (flagellin)	NE	33	96	1	-6.61	0.03
Dda3937_02223	*fliI*	Flagellum-specific ATP synthase fliI	NE	42	236	3	-6.56	0.00
Dda3937_04419	*hdfR*	DNA-binding transcriptional regulator	NE	29	117	1	-6.34	0.00
Dda3937_00209	*cysJ*	sulfite reductase alpha subunit	NE	41	180	2	-6.25	0.00
Dda3937_02209	*flgH*	Flagellar L-ring protein flgH	NE	23	586	8	-6.22	0.01
Dda3937_02246	*fabF*	beta-ketoacyl-[acyl-carrier-protein] synthase II	GD	41	10	0	-6.15	0.00
Dda3937_00301	*uvrD*	ATP-dependent DNA helicase UvrD/PcrA	NE	42	29	0	-6.11	0.00
Dda3937_02212	*flgK*	Flagellar hook-associated protein flgK	NE	63	116	2	-6.07	0.00
Dda3937_04046	*purU*	Formyltetrahydrofolate deformylase	NE	28	51	1	-5.84	0.00
Dda3937_03965	*flhA*	predicted flagellar export pore protein	NE	49	106	2	-5.80	0.00
Dda3937_02205	*flgD*	Flagellar basal-body rod modification protein flgD	NE	22	227	4	-5.73	0.01
Dda3937_01352	*leuC*	3-isopropylmalate dehydratase large subunit	NE	21	139	3	-5.73	0.01
Dda3937_02784	*flhC*	Flagellar transcriptional activator flhC	NE	20	477	9	-5.66	0.01
Dda3937_02782	*motB*	Flagellar motor rotation protein motB	NE	40	109	2	-5.55	0.01
Dda3937_02210	*flgI*	Flagellar P-ring protein flgI	NE	26	163	4	-5.49	0.00
Dda3937_02222	*fliJ*	Flagellar protein fliJ	NE	14	182	4	-5.44	0.03
Dda3937_02219	*fliM*	Flagellar motor switch protein fliM	NE	27	143	3	-5.40	0.00
Dda3937_02774	*flhB*	Flagellar biosynthesis protein flhB	NE	32	186	5	-5.31	0.00
Dda3937_02777	*cheB*	Chemotaxis response regulator protein-glutamate methylesterase CheB	NE	31	282	8	-5.14	0.00
Dda3937_02783	*motA*	Flagellar motor rotation protein motA	NE	24	39	1	-5.06	0.00
Dda3937_00565	***tonB***	**TonB protein**	**NE**	**14**	**106**	**3**	**-5.00**	**0.05**
Dda3937_00427	*fbp*	fructose-bisphosphatase	GA	33	805	27	-4.92	0.01
Dda3937_02781	*cheA*	Chemotaxis protein CheA	NE	50	151	5	-4.89	0.00
Dda3937_03422		Carbamoyl-phosphate synthase small subunit	NE	43	379	13	-4.85	0.02
Dda3937_02577	*lysA*	diaminopimelate decarboxylase	NE	23	332	0	-4.79	0.00
Dda3937_02207	*flgF*	Flagellar basal-body rod protein flgF	NE	21	35	1	-4.76	0.00
Dda3937_02230	*fliD*	Flagellar hook-associated protein fliD	NE	47	93	3	-4.75	0.00
Dda3937_04301	*leuA*	2-isopropylmalate synthase	NE	36	35	1	-4.69	0.02
Dda3937_02778	*cheR*	Chemotaxis protein methyltransferase CheR	NE	30	462	18	-4.67	0.05
Dda3937_02228	*fliT*	Flagellar biosynthesis protein fliT	GD	16	8	0	-4.63	0.05
Dda3937_04404	*leuB*	3-isopropylmalate dehydrogenase	NE	16	285	12	-4.63	0.05
Dda3937_02214	*fliR*	Flagellar biosynthesis protein fliR	NE	33	268	11	-4.56	0.00
Dda3937_03727	***kduI***	**4-deoxy-L-threo-5-hexosulose-uronate ketol-isomerase**	**NE**	**26**	**70**	**3**	**-4.54**	**0.03**
Dda3937_03267		O-antigen, teichoic acid lipoteichoic acids export membrane protein	ES	107	89	4	-4.33	0.05
Dda3937_00415	*epd*	D-erythrose 4-phosphate dehydrogenase	NE	26	316	16	-4.27	0.02
Dda3937_02337	*pnp*	polynucleotide phosphorylase/polyadenylase	GD	50	5	0	-3.97	0.00
Dda3937_01683	*purK*	N5-carboxyaminoimidazole ribonucleotide synthase	NE	16	90	0	-3.49	0.01
Dda3937_00689	*yrbF (mlaF)*	predicted toluene transporter subunit	GA	9	1254	114	-3.47	0.01
Dda3937_02829	*helD*	DNA helicase IV	NE	26	99	9	-3.46	0.01
Dda3937_02252	*ptsG*	PTS system glucose-specific IICB component	NE	37	81	8	-3.38	0.03
Dda3937_00726	***tolC***	**transport channel**	**NE**	**34**	**184**	**0**	**-3.35**	**0.00**
Dda3937_02363	*clpA*	ATP-dependent Clp protease ATP-binding subunit	NE	44	64	8	-3.02	0.03
Dda3937_02470	*corC*	magnesium and cobalt ions transport	NE	13	159	21	-2.90	0.02
Dda3937_00692	*yrbC (mlaC)*	predicted ABC-type organic solvent transporter	GA	23	740	106	-2.81	0.01
Dda3937_02045	*envC*	murein hydrolase activator	NE	17	71	12	-2.59	0.00
Dda3937_01807	*nuoM*	NADH-quinone oxidoreductase subunit M	NE	29	57	10	-2.47	0.03
Dda3937_03668	*sufB*	Fe-S cluster assembly protein	NE	32	116	21	-2.44	0.00
Dda3937_02080	*trkG*	Potassium uptake protein	NE	36	65	13	-2.33	0.05
Dda3937_03042	***fct***	**ferrichrysobactin outer membrane receptor**	**NE**	**80**	**244**	**51**	**-2.25**	**0.01**
Dda3937_01287	*argI*	Ornithine carbamoyltransferase	NE	24	279	59	-2.23	0.03
Dda3937_02456	*rsmC*	global regulatory protein RsmC	NE	10	116	221,705	10.90	0.028
Dda3937_03858	*gcpA*	hypothetical protein	GA	55	3728	140,136	5.23	0.00
Dda3937_03971	*mltD*	outer membrane-bound lytic murein transglycosylase D	NE	46	276	10,885	5.30	0.00
Dda3937_00363	*mrcA*	penicillin-binding protein 1A (PBP1A)	NE	53	85	468	2.47	0.021

Figure 3

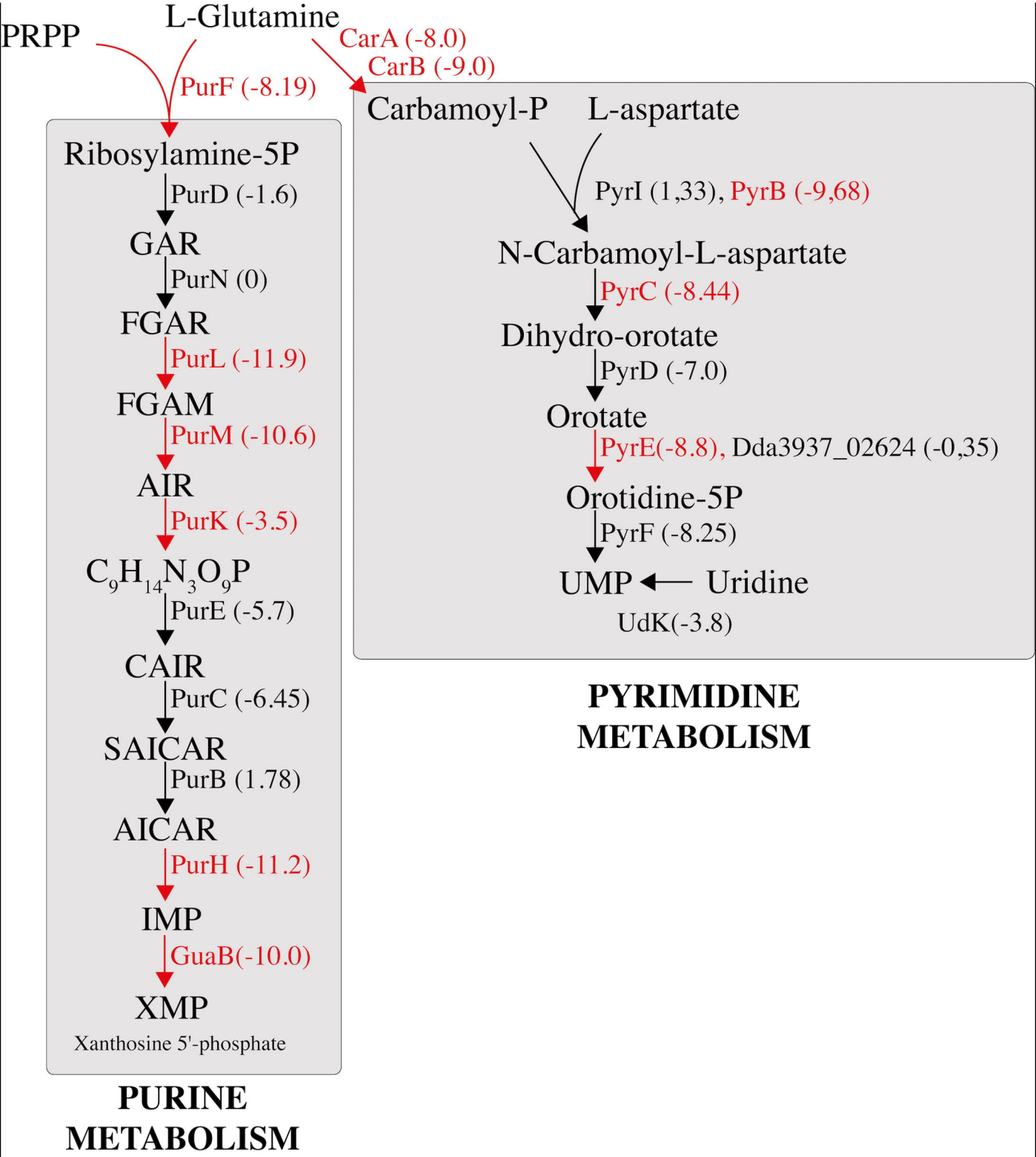

PRPP
L-Glutamine
PurF (-8.19)
CarA (-8.0)
CarB (-9.0)
Ribosylamine-5P
PurD (-1.6)
GAR
PurN (0)
FGAR
PurL (-11.9)
FGAM
PurM (-10.6)
AIR
PurK (-3.5)
$C_9H_{14}N_3O_9P$
PurE (-5.7)
CAIR
PurC (-6.45)
SAICAR
PurB (1.78)
AICAR
PurH (-11.2)
IMP
GuaB(-10.0)
XMP
Xanthosine 5'-phosphate
PURINE METABOLISM
Carbamoyl-P
L-aspartate
PyrI (1,33), PyrB (-9,68)
N-Carbamoyl-L-aspartate
PyrC (-8.44)
Dihydro-orotate
PyrD (-7.0)
Orotate
PyrE(-8.8), Dda3937_02624 (-0,35)
Orotidine-5P
PyrF (-8.25)
UMP
Uridine
UdK(-3.8)
PYRIMIDINE METABOLISM

Figure 4

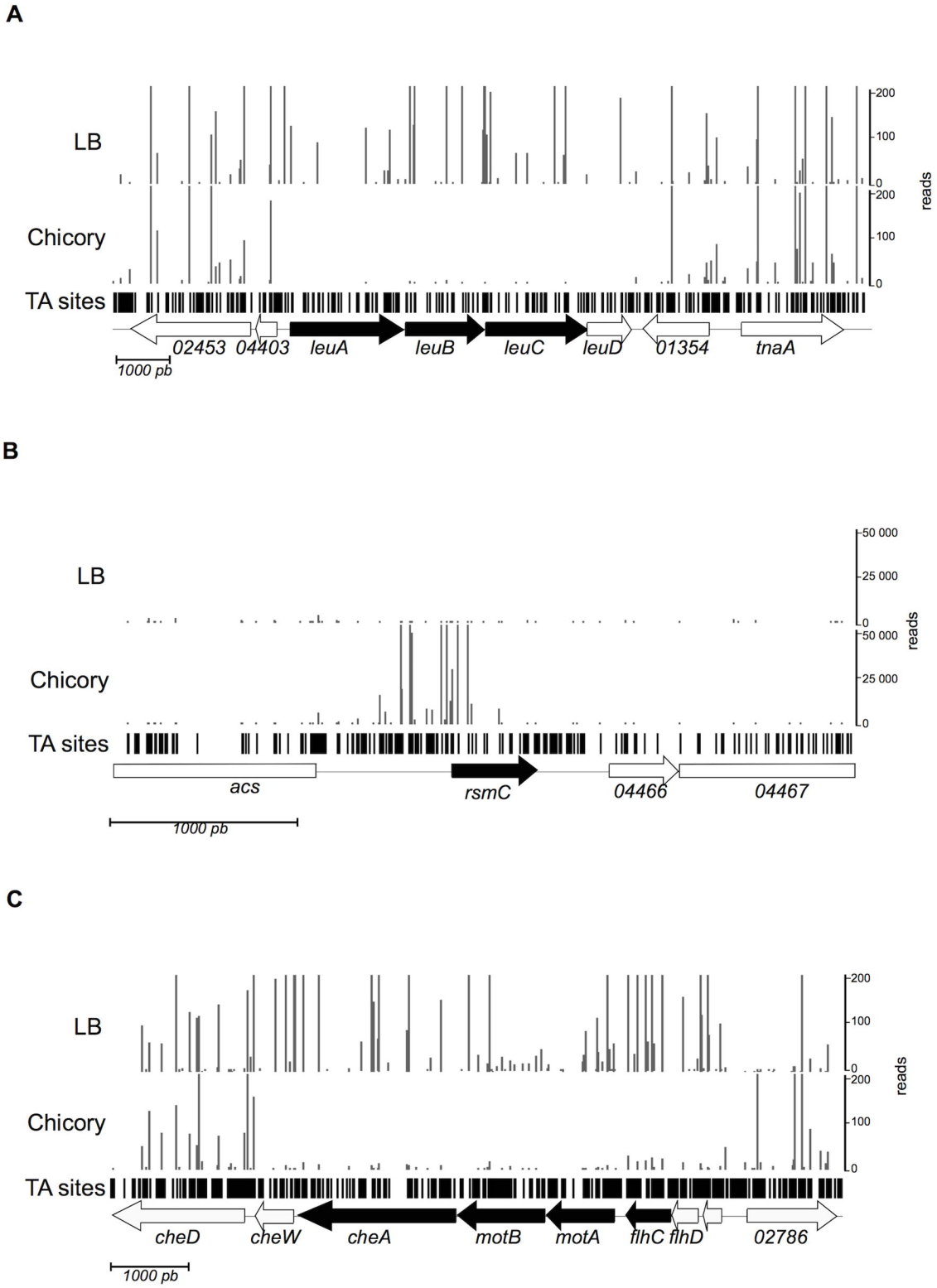
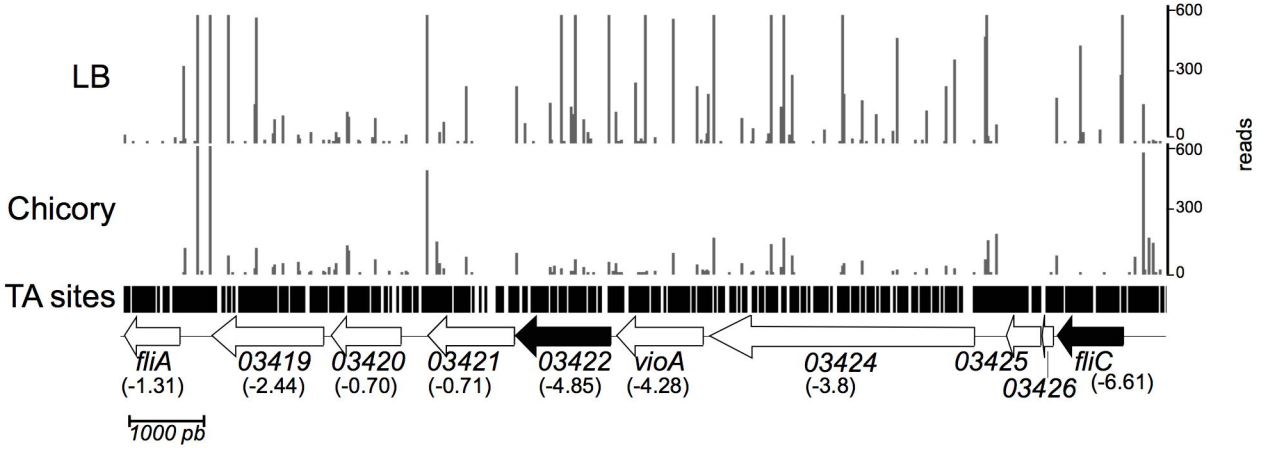
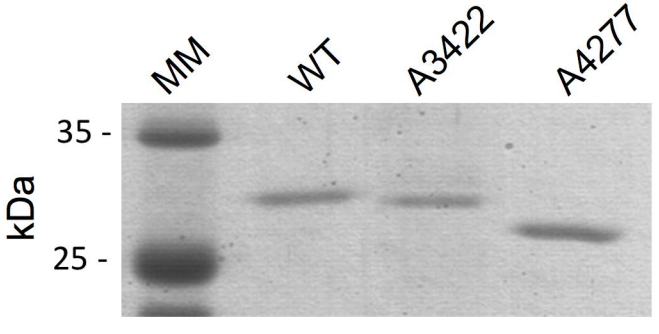

A
LB
Chicory
TA sites
reads
200
100
0
02453
04403
leuA
leuB
leuC
leuD
01354
tnaA
1000 pb
B
LB
Chicory
TA sites
reads
50 000
25 000
0
acs
rsmC
04466
04467
1000 pb
C
LB
Chicory
TA sites
reads
200
100
0
cheD
cheW
cheA
motB
motA
flhC
flhD
02786
1000 pb

Figure 5

A


LB
Chicory
TA sites
600
300
0
reads
fliA
(-1.31)
03419
(-2.44)
03420
(-0.70)
03421
(-0.71)
03422
(-4.85)
vioA
(-4.28)
03424
(-3.8)
03425
03426
fliC
(-6.61)
1000 pb

B


MM
WT
A3422
A4277
35 -
25 -
kDa

C

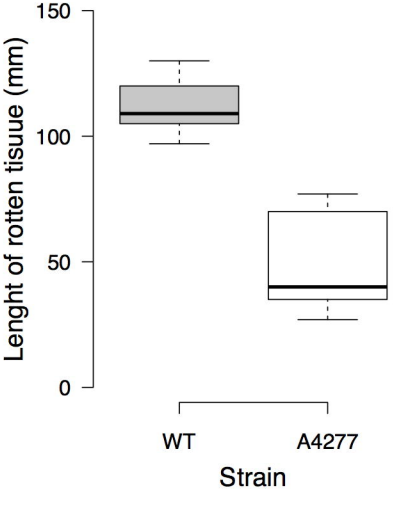
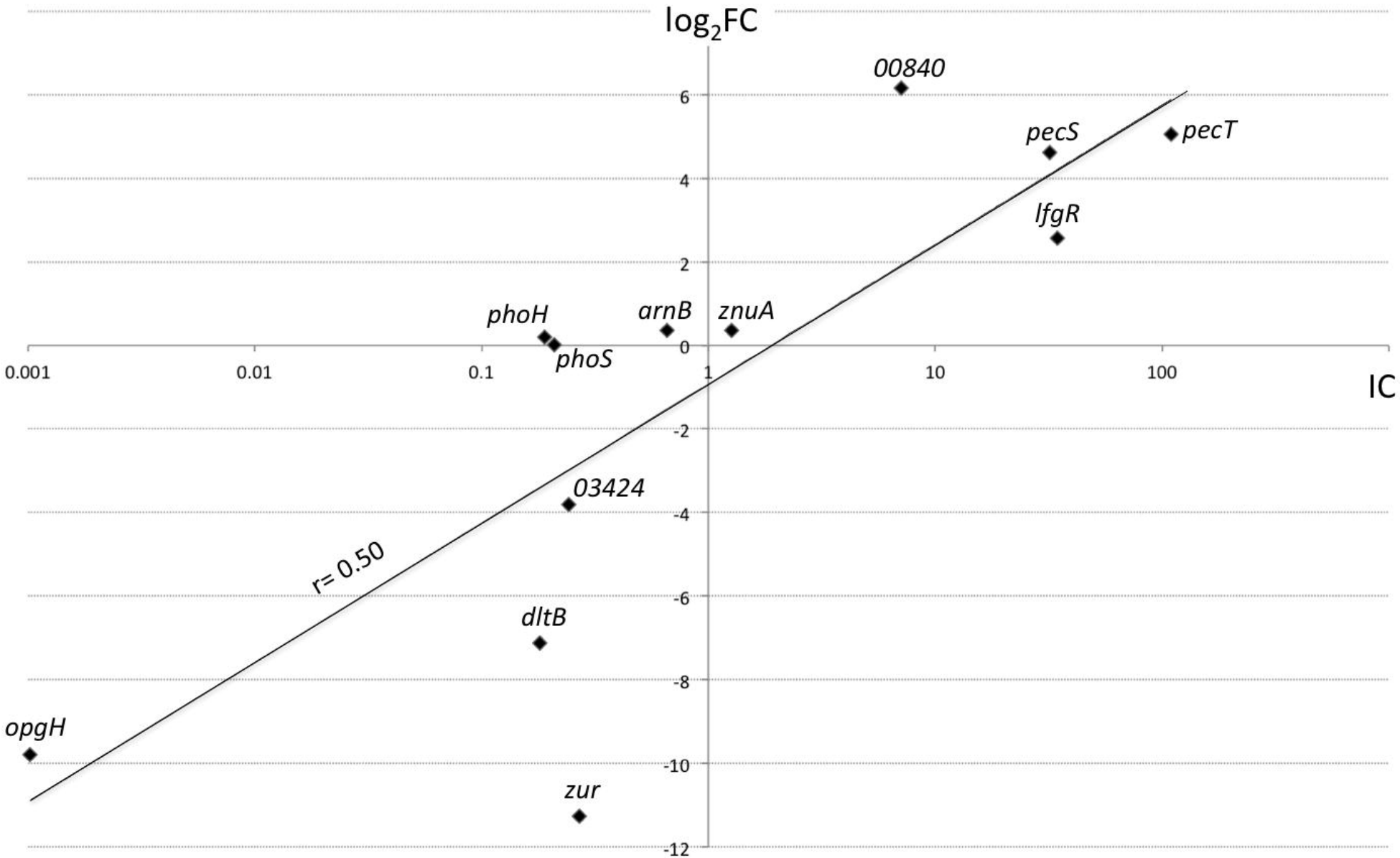

150
100
50
0
Lenght of rotten tisuue (mm)
WT
A4277
Strain

Figure 6


log2FC
IC
00840
pecS
pecT
lfgR
phoH
arnB
znuA
phoS
03424
dltB
opgH
zur
r= 0.50
6
4
2
0
-2
-4
-6
-8
-10
-12
0.001
0.01
0.1
1
10
100