Full Title:

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

Short title: *Dickeya dadantii* virulence genes in chicory

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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, a modification which in some other plant pathogenic bacteria contributes to virulence.

40 **Introduction**

41 *Dickeya* are broad-host range phytopathogenic bacteria belonging to the
42 Pectobacteriaceae family [1] that provoke the soft rot disease on many plant species. They
43 are the cause of important losses on economically important crops such as potato, chicory and
44 ornamentals. Identification and studies on the virulence factors of these bacteria have been
45 performed mostly on the model strain *D. dadantii* 3937 and focused mainly on three
46 domains/aspects, known to be important for disease development: plant cell wall degrading
47 enzymes, the type III secretion system and iron metabolism [2]. Secretion of plant cell wall
48 degrading enzymes has long ago been identified as the bacteria main virulence factor. Many
49 studies focused on the identification and characterization of these secreted enzymes, mostly
50 pectinases [3], of the regulators controlling their production (*kdgR*, *pecS*, *pecT*, *hns*, *gacA*) ,
51 [4-8] of the genes whose expression is coregulated with that of the secreted enzyme genes [9,
52 10], and of the mechanism of their secretion by the Out type II secretion system [11].
53 Although of a lesser importance for *Dickeya* virulence, the same type of approach has been
54 used to identify Hrp type III secretion system regulators and effectors [12] [13] [14].
55 Competition for iron within the plant is strong. *D. dadantii* acquires this metal through
56 production of two siderophores, chrysobactin and achromobactin [15] [16] [17]. More
57 recently, omics approaches have also been used to identify genes induced during plant
58 infection [18] [19] [20]. These studies now give a view on a complex network of factors
59 required for *D. dadantii* virulence [2, 21]. However, these approaches may have missed some
60 important factors not targeted by these analyses. More global screens need to be performed to
61 identify these factors. Libraries of transposon-induced mutants were tested on plants to find
62 mutants showing reduced virulence with *Pectobacterium carotovorum* and *atrosepticum*, two
63 other soft rot enterobacteria [22, 23]. These studies identified auxotrophs, mutants defective
64 in production or secretion of exoenzymes and in motility. Other mutants with a more

65 complex phenotype were not characterized at this time. Moreover, the number of tested
66 mutants was limited by the necessity to test individually each mutant on plant. This type of
67 work has never been performed on *Dickeya* strains. To have a more complete view of the
68 genes required for the virulence of *Dickeya*, we used a high-throughput sequencing of a
69 saturated transposon library (Tn-seq) to screen tens of thousands random insertion mutants of
70 *D. dadantii* in laboratory medium and during infection of chicory. Tn-seq has been
71 extensively used to uncover essential genes required for mouse colonization by human
72 pathogens *Vibrio cholerae* [24], *Pseudomonas aeruginosa* [25] and *Streptococcus*
73 *pneumoniae* [26]. Thus, Tn-seq is a very powerful method to identify genes required for
74 bacterial growth in their host. However, this technology has not yet been employed to unveil
75 genes required for the multiplication of a phytopathogen in a plant host. By applying this
76 technique to screen a *D. dadantii* mutant library in chicory, we identified metabolic pathways
77 and bacterial genes required for growth *in planta*. Among them, we found a cluster of genes
78 required for flagellin glycosylation, a modification known to be important for several plant
79 pathogenic bacteria virulence.
80
81
82 **Results and discussion**
83
84 **Characterization of *D. dadantii* 3937 *Himar1* transposon library**
85 Many tools are available to perform Tn-seq [27]. In order to perform a Tn-seq experiment
86 with *D. dadantii* 3937, we used a *Himar9* mariner transposon derivative carrying MmeI
87 restriction sites in the inverted repeats (IR) and a kanamycin resistance cassette between the
88 IRs [28]. We realized a biparental mating between *E. coli* and *D. dadantii* on M63 agar
89 medium without carbon source and amino acids. We obtained approximately 300 000

90 colonies that were pooled. Subsequent DNA sequencing (see below) showed the presence of
91 transposon insertions in amino acid biosynthesis pathways, demonstrating that mating on
92 M63 minimal medium does not prevent the obtention of auxotroph mutants. To identify
93 essential genes, mutants were grown in LB medium during several generations. Two DNA
94 libraries were prepared from two cultures and subjected to high-throughput sequencing. The
95 mariner transposon inserts into TA dinucleotides. The TPP software [29] was used to
96 determine the number of reads at each TA site for each biological replicate. *D. dadantii*
97 genome has 171 791 TA sites that can be targeted by the *Himar9* transposase. Pairs of
98 biological replicates were compared. 37 794 and 48 101 unique insertions in TAs were
99 detected in each sample, which corresponds to 22 and 28% density of insertion respectively
100 (Table 1). The average number of reads per TA is 88 and 75, respectively. The results were
101 reproducible with a Pearson correlation coefficient of 72% (Fig. S1) The location of the
102 unique insertions showed an even distribution around the chromosome (Fig. 1A). For each
103 gene, we calculated a $\log_2$FC corresponding to a ratio between the measured number of reads
104 and the expected number of reads. The density plot (Fig. 1B) indicates that essential and non-
105 essential genes are easily distinguishable, confirming the good quality of our Tn-seq libraries.
106 Then, gene essentiality of the Tn-seq input libraries was determined by using the TRANSIT
107 software [29]. We decided to use the Hidden Markov Model (HMM) which predicts
108 essentiality and non-essentiality for individual insertion sites because it has been shown to
109 give good prediction in datasets with density as low as 20% [29]. The HMM analysis led to
110 the identification of 665 genes essential for growth in LB (ES), representing 14% of the genes
111 of *D. dadantii* 3937, a number in the range of those found for this type of analysis with
112 bacteria. 552 genes were categorized as Growth Defect genes (GD, i.e. mutations in these
113 genes lead to loss of fitness), 125 as growth advantage genes (GA, i.e mutations in these
114 genes lead to gain of fitness) and 3320 as non-essential genes (NE) (table S2).

115 **TABLE 1** Tn-Seq analysis of *Dickeya dadantii* 3937
116

Mutant pool	Total no. of reads	No. of reads containing Tn end	No. of reads normalized[a]	No. of mapped reac to unique TA sites	No. of mapped reads to unique TA sites after LOESS correction	Density (%)[b]	Mean read count[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

117 [a] The number of reads containing the sequence of a Tn end were normalized for each sample
118 according to the number of reads for the sample Chicory #1
119 [b] *Dickeya dadantii* 3937 genome has 171,791 TA sites. The density is the % of TAs for which mapped
120 reads has been assigned by the TPP software.
121 [c] the mean value of mapped reads per TA.
122

123 **Genes necessary for chicory leaf maceration.** We used chicory leaf infection as a model to
124 identify *D. dadantii* genes required for growth in plant tissues. Biological duplicates were
125 performed to insure the reproducibility of the results. Each chicory was inoculated with 10^7
126 bacteria from the mutant pool and after 2 days more than 10^{10} bacteria were collected from
127 the rotten tissue. Sequencing transposon insertion sites in these bacteria followed by the TPP
128 analysis indicated a density of unique insertion in TAs comparable to that of the input
129 datasets (23-24%). Surprisingly, the results were more highly reproducible than in LB with a
130 very high Pearson correlation coefficient of 98% (Fig. S1).
131 In order to identify genes of *D. dadantii* conferring loss or gain of fitness *in planta*, we
132 performed the RESAMPLING analysis of the TRANSIT software. The RESAMPLING
133 method is a variation of the classical permutation test in statistics that sums the reads at all
134 TA sites for each gene in each condition. It then calculates the difference of the sum of read-
135 counts between the input (LB) and output (chicory) datasets. The advantage of this statistical
136 method is to attribute for each gene an adjusted p-value (q-value). Genes with a significant
137 difference between total read-counts in LB and chicory achieve a q-value ≤ 0.05. The method
138 also calculates a $\log_2$ fold-change ($\log_2$FC) for each gene based on the ratio of the sum of

139 read counts in the output datasets (chicory) versus the sum of read counts in the input (LB)
140 datasets [29]. Applied to our Tn-seq datasets and selecting only genes achieving a q-value ≤
141 0.05, we identified 122 genes out of 4666 required for fitness *in planta*, as shown with the
142 volcano plot of RESAMPLING results comparing replicates grown in LB versus *in planta*
143 (Fig. S2). From this 122 genes, we applied an additional cutoff by removing 20 genes with a
144 mean read count in LB <5 (less than 5 reads in average / TA). These genes were categorized
145 as ES or GD in LB. We also removed from the analysis 6 genes with a $\log_2$FC comprised
146 between -2 and 2. By applying all these criteria, we retained only 96 genes for a further
147 analysis (Fig. 2). 92 of them were identified as GD genes in the chicory ($\log_2$FC ≤2), the 4
148 left as GA genes in the chicory ($\log_2$FC ≥2). Some of these genes, in bold in Fig 2, were
149 already known to play a role in *D. dadantii* virulence, confirming the validity of the Tn-seq
150 approach. Using the Kyoto Encyclopedia of Genes and Genomes (KEGG) [30], we
151 discovered that certain metabolic pathways and biological functions are very important for
152 growth in chicory (Table S3). We highlight some of them in the next sections of the article.
153

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

155 **(i) Metabolism.** Chicory appears as an environment in which amino acids, nucleic acids and
156 some vitamins (pyridoxal) are scarce. Of the 92 genes identified as GD genes *in planta*, 8 are
157 involved in purine and 7 in pyrimidine metabolisms (table S3). In the purine metabolism
158 pathway, the inosine monophosphate (IMP) biosynthesis pathway that produces IMP from L-
159 glutamine and 5-phosphoribosyl diphosphate is particularly important for *D. dadantii in*
160 *planta* since 5 out of the 10 genes of this pathway are significantly GD genes *in planta* (Fig.
161 3). IMP is the precursor of adenine and guanine. Next, IMP can be converted in xanthosine
162 5'-phosphate (XMP) by the IMP dehydrogenase GuaB. *guaB* gene is also a GD gene *in*
163 *planta*, with a strong $\log_2$FC of -10.06 (Fig. 3). In the pyrimidine synthesis, the uridine

164 monophosphate (UMP) biosynthesis pathway that converts L-glutamine to UMP, a precursor
165 of uracyl, is very important *in planta* since *carAB*, *pyrB*, *pyrC* and *pyrE*, involved in this
166 enzymatic pathway, are all required for growth *in planta* (Fig. 3). This pyrimidine
167 biosynthesis pathway is specific to bacteria. It is noteworthy that in the human pathogen *S.*
168 *pneumoniae*, mutants of this pathway have a fitness defect in the nasopharynx of infected
169 mice [26]. Hence, it looks that the pyrimidine biosynthesis pathway is particularly important
170 for multiplication of some bacterial species in the host.
171 Mutants in genes involved in the synthesis of sulfur-containing amino acids (*cysIJQ, metB),*
172 lysine (*lysA*) and leucine (*leuABC)* are disadvantaged in chicory (Fig. 2 and Fig. 4A). These
173 amino acids are known to be present in low concentration in plant tissues. Other amino acids
174 seem to be present in quantity sufficient for growth of *D. dadantii* auxotrophs. Low level of
175 certain amino acids probably induces the stringent response in the bacteria. Reduced growth
176 in the plant of the *relA* mutant, unable to synthesize the alarmone ppGpp, supports this
177 hypothesis. Glucose is the main sugar present in plant tissue, present as a circulating sugar or
178 a cellulose degradation product. Mutants in the PTS glucose transport system genes *ptsI* and
179 *ptsG* have a reduced growth in bacteria (Fig. 2) showing its importance as a carbon source in
180 planta.
181 Degradation of cell wall pectin by a battery of extracellular enzymes is the main determinant
182 of *Dickeya* pathogenicity. Mutants unable to produce or to secrete these enzymes by the type
183 II Out secretion system were not disfavored in chicory since these mutants could use for their
184 growth the pectin degradation compounds produced by enzymes secreted by other bacteria.
185 The redundancy of oligogalacturonate specific porins (KdgM and KdgN) and inner
186 membrane transporters (TogT and TogMNABC) allow entry of these compounds into the
187 bacteria even in a mutant in one of these transport systems. However, *kduI* mutants, blocked

188 in the intracellular part of the pectin degradation pathway, have a limited growth *in planta*,
189 confirming the importance of the pectin degradation pathway in the disease progression.
190 **(ii) Stress resistance**. Plant is an hostile environment for the bacteria that has to cope with
191 antimicrobial peptides, ROS, toxic compounds and acidic pH [31]. We observed that the
192 pump AcrABTolC, that can efflux a wide range of compounds [32], is important for survival
193 in chicory (Fig. S3). Stress can lead to the accumulation of phospholipids in the outer
194 membrane. This accumulation makes the bacteria more sensitive to small toxic molecules
195 [33]. Such a phospholipid accumulation probably occurs when the bacteria infect chicory
196 since *mlaC* and *mlaF* mutants, which are unable to prevent phospholipid accumulation in the
197 outer membrane, have a reduced growth in plant. Production of exopolysaccharides (EPS)
198 was shown to protect the bacteria during the first steps of infection [9]. We observed that
199 *rffG* and *wzx* mutants unable to synthesize EPS have a growth defect in chicory. A set of
200 genes required to repair or degrade altered proteins (*clpA, degQ, trxB*) are also important for
201 survival *in planta*. No gene directly involved in detoxification of ROS was detected in our
202 analysis. However, ROS can create DNA damage. The two helicases involved in DNA repair,
203 UvrD and HelD, give growth advantage in plant. Osmoregulated periplasmic glycans (OPG)
204 are polymers of glucose found in the periplasm of α, β and γ proteobacteria. Their exact role
205 is unknown but their absence leads to avirulence in certain bacteria such as *D. dadantii* [34].
206 This absence induces a membrane stress that is sensed and transduced by the Rcs envelope
207 stress response system. This system controls the expression of many genes, including those
208 involved in motility, and those encoding plant cell wall degrading enzymes through the
209 RsmA-RsmB system [35-37]. Thus, mutants defective in OPG synthesis are expected to have
210 a reduced virulence. Indeed, in our experiment, mutants in the two genes involved in OPG
211 synthesis, *opgG* and *opgH* were non competitive in chicory (Fig. 2).

212 **(iii) Iron uptake**. *D. dadantii* produces two types of siderophores, achromobactin and
213 chrysobactin, that are required for the development of maceration symptoms in the iron
214 limited environment of plant hosts [38]. Once iron loaded, the siderophores are imported into
215 the bacteria. Import through the outer membrane requires a specific outer membrane channel
216 and the energy transducing complex formed by TonB ExbB and ExbD. While the absence of
217 synthesis of one of the siderophores can be compensated by the presence of siderophore
218 secreted by other bacteria in the growth medium, mutants of the TonB complex are totally
219 unable to acquire iron and thus are unable to grow in the plant. In accordance, *tonB* was
220 essential in chicory while the genes coding for siderophore synthesis or secretion were not.
221 Similarly a mutant devoid of the iron-loaded chrysobactin transport gene (*fct)* is non-
222 competitive.
223 **(iv) Regulation.** Mutants in several genes controlling virulence factor production have a
224 growth defect in the plant. The master regulator FlhDC acts as a regulator of both flagella and
225 virulence factor synthesis in many bacteria such as *Yersinia ruckeri*, *Edwardsiella tarda* and
226 *Ralstonia solanacearum* [39-41]. In *D. dadantii* FlhDC has recently been shown to control, in
227 addition to flagellar motility, type III secretion system and virulence factor synthesis through
228 several pathways [42]. We observed that *flhC* gives a growth advantage in chicory. In
229 addition, we uncovered that some genes regulating *flhDC* in other bacteria regulate *D.*
230 *dadantii* virulence, probably by controlling *flhDC* expression. *rsmC* is a poorly characterized
231 gene in *D. dadantii* but that has been studied in *Pectobacterium carotovorum*. It negatively
232 controls motility and extracellular enzyme production through modulating transcriptional
233 activity of FlhCD [43]. HdfR is a poorly characterized LysR family regulator that controls the
234 *std* fimbrial operon in *S. enterica* and FlhDC expression in *E. coli* [44]. *rsmC* mutants were
235 overrepresented in the chicory (Fig. 4B), indicating a gain of virulence for these mutants.

236 *hdfR* conferred fitness benefits during growth in chicory and could also act in *D. dadantii* as
237 activator of *flhDC* expression.
238 The GGDEF proteins are c-di-GMP synthase. Their gene are often located next to their
239 cognate EAL diguanylate phosphodiesterase gene. *ecpC (yhjH)* encodes an EAL protein that
240 was shown to activate virulence factor production in *D. dadantii* [45]. *gcpA*, which is located
241 next to *ecpC* encodes a GGDEF protein. However, a *gcpA* mutant could not be constructed
242 and analysed in this previous study. We observed that *gcpA* mutants (Dda_03858) were
243 overrepresented in chicory (Fig. 2). This increased virulence is a phenotype opposite to that
244 described for the *ecpC* mutants, indicating that overproduction of c-di-GMP could reduce *D.*
245 *dadantii* virulence.
246 Of the eighteen regulators of the LacI family present in *D. dadantii*, four of them were found
247 to be involved in plant infection [46]. One of those, LfcR, which has been found important
248 for infection of chicory, Saintpaulia and Arabidopsis, was identified as important for chicory
249 infection in our experiment. LfcR is a repressor of adjacent genes [46]. Surprisingly none of
250 these genes appeared to play a role for chicory infection suggesting that other targets of LfcR
251 probably remain to be discovered.
252 Finally, it is noteworthy to mention that the *ackA* and *pta* genes are GD *in planta*. These
253 genes constitute the reversible Pta-AckA pathway. The steady-state concentration of acetyl-
254 phosphate (acetyl-P), a signaling molecule in bacteria, depends upon the rate of its formation
255 catalyzed by Pta and of its degradation catalyzed by AckA [47]. The GD phenotype of *D.*
256 *dadantii ackA* and *pta* mutants during infection suggests that acetyl-P might play a crucial
257 signaling role in the adaptation of *D. dadantii* to the plant tissue.
258 **(v) Motility**. Motility is an essential virulence factor of *D. dadantii* required to move on the
259 surface of the leaf, enter the wounds and propagate into the plant tissue [48-50]. Accordingly,
260 all the genes required for flagella synthesis, the flagella motor and genes regulating their

261 synthesis (*flhC*, *flhD*, *fliA*) (see above) are necessary for fitness during chicory infection (Fig.
262 4C and 5A). All the genes responsible for the transduction of the chemotaxis signal (*cheA*, *B*,
263 *R*, *W*, *X*, *Y* and *Z*) also confer a benefit in planta (Fig. 2). No methyl-accepting chemoreceptor
264 gene mutant was found. Like other environmental bacteria, *D. dadantii* encodes many such
265 proteins (47). They probably present some redundancy in the recognized signal which
266 prevented their detection by our screen.
267
268 ***D. dadantii* flagellin is modified by glycosylation**
269 A group of six genes located between *fliA* and *fliC* retained our interest since insertions in
270 these genes led to a growth defect in chicory (Fig. 5A). Dda3937_03424 encodes an O-linked
271 N-acetylglucosamine transferase and Dda3937_03419 encode a protein with a nucleotide
272 diphospho sugar transferase predicted activity. The other ones could be involved in the
273 modification of sugars (predicted function of Dda3937_03423: nucleotide sugar
274 transaminase, Dda3937_03422: carbamoyl phosphate synthase, Dda3937_03421:
275 oxidoreductase; Dda3937_03420: methyltransferase). Their location let suppose that this
276 group of genes could be involved in flagellin glycosylation. Analysis by SDS-PAGE of FliC
277 produced by the wild type, and the A4277 and A3422 strains (Dda3937_03424 and
278 Dda3937_03419 mutants, respectively) showed that in the two latter strains the molecular
279 weight of the protein diminished (Fig. 5B). The molecular weight determined by mass
280 spectroscopy was 28911 Da for FliCA4277, 31034 Da for FliCA3422 and 32258 Da for the
281 WT FliC. Thus, in the wild type strain FliC is modified by the products of the genes
282 Dda_03424 to Dda_03419, probably by multiple glycosylation with a disaccharide. Absence
283 of modification did not modify *D. dadantii* motility (data not shown). The flagellin of the
284 plant pathogens *Pseudomonas syringae* pv *tabaci* and *Burkholderia cenocepacia* are also
285 glycosylated and absence of this modification lowered the ability of these bacteria to cause

286 disease on tobacco and arabidopsis, respectively [51, 52]. Accordingly, in *D. dadantii*, FliC
287 modification appears important for multiplication of the bacteria in plant.
288
289 **Additional genes could be involved in virulence**
290 Several genes have a $\log_2$FC >4 or <-4 but do not satisfy the statistical permutation test
291 adjusted by the false discovery rate method (q-value) (table S4). However, most of them
292 belong to the categories described above and could be required for growth in planta. Among
293 those with a $\log_2$FC< -4 can be found genes involved in amino acid and nucleic acid synthesis
294 (*cysH*, *ilvC*, *pyrF*, *pyrD*, *purC*, *thrC*, *metA*, *cysK*, *lysC*), flagella and motility *(flgJ*, *fliO*, *flgC*,
295 *fliS*, *flgG*, *flgA*, *flgL*, *cheW*, *fliN*, *fliP*, *fliK*, *fliG*, *fliL*), pectin and glucose metabolism (*kduD*,
296 *pgi*), EPS synthesis (*gmd*), flagella glycosylation (*vioA*) and regulation (*zur*, *ecpC* and the
297 general RNA chaperone *hfq*).
298 Among the genes with a $\log_2$FC > 4, three regulators can be noticed: *pecS*, *pecT* and
299 *Dda3937_00840*. *pecS* and *pecT* are known regulators of *D. dadantii* controlling the
300 expression of many factors involved in virulence (ref, ref). Thus, their mutation could confer
301 an increased fitness of the bacteria in chicory. *D. dadantii* possesses a functional *expI-expR*
302 quorum sensing system which does not seem to control plant virulence factor production
303 (Nasser). However, several LuxR family regulator genes which are not associated with a *luxI*
304 gene are present in the genome of the bacteria. Mutants of one of them (*Dda3937_00840*) are
305 overrepresented in the chicory. Its product is probably a repressor of genes conferring an
306 increased fitness i*n planta*.
307
308
309 **Validation of the Tn-seq results**.

310 To validate the Tn-seq results, we performed coinoculation experiments in chicory leaves
311 with the wild type strain and various mutants in genes conferring a growth advantage, a
312 growth defect or no fitness benefit in a 1/1 ratio. We calculated a competitivity index (CI) by
313 counting the number of each type of bacteria in the rotten tissue after 24 h. We found a
314 correlation between the competitivity index of mutants with the log_2FC of the corresponding
315 genes with a Pearson coefficient of 0.50 (Fig. 6), indicating that Tn-seq is a reliable technique
316 to identify genes involved in plant colonization and virulence. Additional validations were
317 performed by inoculation of uncharacterized leucine and cytosine auxotroph mutants in
318 chicory leaves which confirmed that these mutants are unable to grow in plant and are thus
319 avirulent (Fig S4).
320
321
322 **Conclusion**
323 This Tn-seq experiment highlights new factors required for *D. dadantii* successful rotting of
324 chicory. Many genes known to be important for pathogenesis were not found in this screen
325 because their products are secreted and can be shared with other strains in the community.
326 This includes all the proteins secreted by the type II secretion system and small molecules
327 such as siderophores and butanediol. Other categories of genes were not found: for example,
328 no genes involved in response to acidic or oxidative stresses were identified. Chicory has
329 been described as an inadequate model to study the response of *D. dadantii* to oxidative stress
330 (touati, expert). Similarly, the type III *hrp* genes were not identified in our study. The Hrp
331 system is not necessary for *D. dadantii* virulence and in our experimental conditions (high
332 inoculum on isolated chicory leaves) the necrotrophic capacities of *D. dadantii* (production of
333 plant cell wall degrading enzymes) is probably sufficient to provoke the disease. Studies on
334 other host plants will allow to determine all the full virulence repertoire of the bacteria.

335 Our results give also some information on the metabolic status of the plant. The amount of
336 nucleic acids and of the amino acids cysteine, leucine, methionine, threonine and isoleucine is
337 too low in chicory to allow the multiplication of mutant bacteria. Some enzymatic steps
338 involved in their synthesis are specific to bacteria and fungi. Thus, they could be good target
339 for the development of specific inhibitors [53] to fight *D. dadantii.*
340 While Tn-seq has been used to study genes required for the infection of animals, there has
341 been no genome-wide study of factors necessary for a plant pathogen to develop and provoke
342 disease on a plant. Besides the genes of known function described in the Result section, this
343 study allowed the identification of several genes of unknown function required for chicory
344 rotting. Repetition of this experiment with other strains or on other plants will tell if these
345 genes encode strain or host specific virulence factors.
346

347 **Methods**

348 **Bacterial strains and growth conditions.** Bacterial strains, phages, plasmids and
349 oligonucleotides used in this study are described in Table S1. *D. dadantii* and *E. coli* cells
350 were grown at 30 and 37°C respectively in LB medium or M63 minimal medium
351 supplemented with a carbon source (2 g/L). When required antibiotics were added at the
352 following concentration: ampicillin, 100 µg/L, kanamycin and chloramphenicol, 25 µg/L.
353 Media were solidified with 1.5 g/L agar. Transduction with phage PhiEC2 was performed
354 according to [54]. The motility of each mutant was compared with that of the wild-type strain
355 on semisolid (0.4%) LB agar plates as previously described [55].

356 **Construction of the transposon library**

357 Five mL of an overnight culture of *D. dadantii* strain A350 and of *E. coli* MFDpir/pSamEC
358 were mixed and centrifuged 2 min at 6000 g. The bacteria were resuspended in 1 mL of M63

359 medium and spread onto a 0.45 µm cellulose acetate filter placed on a M63 medium agar
360 plate. After 8h, bacteria were resuspended in 1 mL M63 medium. An aliquot was diluted and
361 spread onto LB agar + kanamycin plates to estimate the efficiency of mutagenesis. The other
362 part was inoculated in 100 mL of LB medium + kanamycin and grown for 24 h at 30°C. To
363 confirm that the bacteria that grew were *D. dadantii* strains with a transposon but without
364 plasmid pSamEC, we checked that all the grown bacteria were kan^R, amp^S and
365 diaminopimelate (DAP) prototrophs (MFDpir is DAP^-). The bacteria were frozen in 40%
366 glycerol at -80°C and represent a library of about 300 000 mutants.

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

368 An aliquot of the mutant library was grown overnight in LB medium + kanamycin. To
369 identify essential genes in LB, the culture was diluted 100-fold in LB and grown for 6 h. To
370 infect chicory, the overnight culture was centrifuged and resuspended at OD_{600} = 1 in M63
371 medium. Chicories cut in half were inoculated with 10 µL of this bacterial suspension and
372 incubated at 30°C with maximum moist. After 60 h, the rotten tissue was collected and
373 filtered through a cheesecloth. The bacteria were collected by centrifugation and washed
374 twice in M63 medium. DNA was extracted from 1.5 mL aliquots of bacterial suspension
375 adjusted to OD_{600}1.5 with the Promega Wizard Genomic DNA purification kit. Next steps of
376 the DNA preparation methods were adapted from [25]. All DNA gel-extraction were
377 performed onto a blue-light transilluminator of DNA stained with gel-green (Biotium) to
378 avoid DNA mutation and double-stranded breaks. 50 µg of DNA samples were digested with
379 50 U MmeI in a total volume of 1.2 mL for one hour at 37°C according to manufacturer's
380 instructions, then heat-inactivated for 20 minutes at 80°C, purified (QIAquick, PCR
381 purification kit Qiagen) and concentrated using a vacuum concentrator to a final volume of
382 25 µL. Digested DNA samples were run on a 1% agarose gel, the 1.0–1.5 kb band containing
383 the transposon and adjacent DNA was cut out and DNA was extracted from the gel according

384 to manufacturer's instructions (Qiaquik Gel Extraction Kit, Qiagen). This allowed recovery
385 of all the fragments containing genomic DNA adjacent to transposons (1201 bp of
386 transposable element with 32-34 bp of genomic DNA). A pair of single-stranded
387 complementary oligonucleotides containing an unique 5-nt barcode sequence (LIB_AdaptT
388 and LIB_AdaptB) was mixed and heated to 100°C, then slowly cooled down in a water bath
389 to obtain double-stranded adaptors with two-nucleotide overhangs. 1 µg DNA of each sample
390 was ligated to the barcoded adaptors (0.44 mM) with 2000 U T4 DNA ligase in a final
391 volume of 50 µL at 16°C overnight. Five identical PCR reactions from the ligation product
392 were performed to amplify the transposon adjacent DNA. One reaction contained 100 ng of
393 DNA, 1 unit of Q5 DNA polymerase (Biolabs), 1X Q5 Buffer, 0.2 mM dNTPs, 0.4 µM of the
394 forward primer (LIB_PCR_5, which anneals to the P7 Illumina sequence of the transposon)
395 and the reverse primer (LIB_PCR_3, which anneals to the P5 adaptor). Only 18 cycles were
396 performed to keep a proportional amplification of the DNA. Samples were concentrated
397 using a vacuum concentrator to a final volume of 25 µL. Amplified DNA was run on a 1.8%
398 agarose gel and the 125 bp band was cut-out and gel extracted (QIAquick, PCR purification
399 kit Qiagen). DNA was finally dialysed (MF-Millipore™ Membrane Filters) for 4 hours.
400 Quality control of the Tn-seq DNA libraries (size of the fragments and concentration) and
401 High-throughput sequencing on HiSeq 2500 (Illumina) was performed by MGX (CNRS
402 sequencing service, Montpellier). 6 DNA libraries were multiplexed on one flow-cell. After
403 demultiplexing, the total number of reads was comprised between 18 and 31 millions (Table
404 1).
405
406 **Bioinformatics analysis:**
407 Raw reads from the fastQ files were first filtered using cutadapt v1.11 [56] and only reads
408 containing the *mariner* inverted left repeat (ACAGGTTGGATGATAAGTCCCCGGTCTT)

409 were trimmed and considered *bona fide* transposon-disrupted genes. Trimmed reads were
410 then analyzed using a modified version of the TPP script available from the TRANSIT
411 software v2.0.2 [29]. The mapping step was modified to select only reads mapping uniquely
412 and without mismatch in the *D. dadantii* 3937 genome (Genbank CP002038.1). Then, the
413 counting step was modified to accurately count the reads mapping to each TA site in the
414 reference genome according to the Tn-seq protocol used in this study. Read counts per
415 insertion were normalized using the LOESS method as described in [57]. We next used the
416 TRANSIT software (version 2.0) to compare the Tn-seq datasets.
417
418 **Strain construction.** To construct the A4277 strain, gene Dda3937_03424 was
419 amplified with the oligonucleotides 19732+ and 19732-. The resulting fragment was
420 inserted into the pGEM-T plasmid (Promega). A *uidA*-kan^R cassette [58]was inserted
421 into the unique AgeI site of the fragment. The construct was recombined into the *D.*
422 *dadantii* chromosome according to [59]. Recombination was checked by PCR.
423
424 **Protein techniques.** Flagella were prepared from overnight LB grown cells.
425 Bacteria were pelleted, resuspended in 1/10 volume of water and passed 20 fold through
426 a needle on a syringe. Cells and cells debris were removed by centrifugation 5 min at 20
427 000 x g [55]. Proteins were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-
428 PAGE).
429
430 **Coinoculation experiments**. To determine the competitivity index, the wild type strain
431 and the mutant to test, marked with an antibiotic resistance gene, were grown overnight in
432 M63 + glycerol medium. Bacteria were washed in M63 medium and the OD_{600} was adjusted
433 to 1.0. Bacteria were mixed to a 1:1 ratio and diluted 10-fold. 10 uL of the mixture were

434 inoculated into chicory leaves. The wound was covered with mineral oil and the leaves were
435 incubated at 30 °C at high humidity. After 24 h the rotten tissue was collected, homogenized,
436 diluted in M63 and spread onto LB and LB + antibiotic plates. After 48 h at 30°C, colonies
437 were counted. The competitivity index is the ratio (number of mutant bacteria/number of WT
438 bacteria) in the rotten tissue / (number of mutant bacteria/number of WT bacteria) in the
439 inoculum.
440
441 **References**
442
443
444
445 **Legend of figures**
446
447 **Fig. 1**. **Quality control of the Tn-seq *D. dadantii* 3937 libraries**.
448 (A) Frequency and distribution of transposon sequence reads across the entire *D. dadantii*
449 *3937* genome. The localization of transposon insertions shows no bias throughout the genome
450 of *D. dadantii 3937*. B) Density plot of $\log_2$FC (measured reads/expected reads per gene).
451
452 **Fig 2. Genes identified by Tn-seq exhibiting a fitness change in chicory compared to LB.**
453 Data were obtained with the TRANSIT software**.** From left to right : (a) classification of the
454 gene with the HMM method performed in the LB dataset. (b) Number of TA sites left after
455 removing 10% of 5' and 3' extremities. (c) Mean read number per gene in the LB or chicory
456 dataset. (d) Ratio of read number between chicory and LB expressed in $\log_2$. (e) p-values
457 adjusted for multiple comparisons using the false-discovery rate method. Genes for which a
458 role in *D. dadantii* virulence has been described before are in bold.

459 **Fig 3. Scheme of the purine and pyrimidine biosynthesis pathways in *D. dadantii* that**
460 **produce XMP (purine metabolism) and UMP (pyrimidine metabolism) from L-**
461 **glutamine.**
462 In red are indicated the growth defect genes in chicory that pass the permutation test (q-value
463 ≤ 0.05). The log_2FC of read numbers between chicory and LB for each gene is indicated in
464 bracket. Some genes do not pass the permutation test (in black) but have a strong negative
465 log_2FC. PRPP: 5-phosphoribosyl-1-pyrophosphate ; GAR: 5'-phosphoribosyl-1-glycinamide
466 ; FGAM: 5'-phosphoribosyl-*N*-formylglycinamide ; AIR: 5'-phosphoribosyl-5-
467 aminoimidazole ; CAIR: 5'-phosphoribosyl-5-aminoimidazole carboxylic acid ; SAICAR: 5'-
468 phosphoribosyl-4-(*N*-succino-carboxamide)-5-aminoimidazole ; AICAR: 5-aminoimidazole-
469 4-carboxamide ribonucleotide ; IMP: inosine monophosphate ; XMP: xanthine
470 monophosphate ; UMP: uridine monophosphate.
471
472 **Fig 4. Examples of essential and important genes revealed by Tn-seq**.
473 Number of reads at each transposon location in the sample grown in LB or in chicory. Data
474 are averaged from biological replicates and normalized as described in the method section.
475 Three regions of the genome representative of Tn-seq results are shown, with the predicted
476 genes represented at the bottom of each panel. Peaks represent read number at TA sites.
477 Black arrows represent genes that pass the permutation test (q-value ≤ 0.05). (A) Essentiality
478 of leucine biosynthetic genes in chicory. (B) Insertions in the 5' region of *rsmC* generate
479 growth advantage for the bacteria in chicory. (C) Importance of genes involved in motility for
480 growth in chicory.

481 **Fig 5. Modification of FliC revealed by Tn-seq analysis and SDS-PAGE. (**A) Importance
482 of 6 genes located between *fliA* and *fliC* for growth in chicory. Log_2FC are indicated in
483 bracket. Dda3937_03425 and Dda3937_03426 are duplicated transposase genes that have

484 been removed from the analysis. Black arrow: GD in chicory (q-value ≤ 0.05) ; white arrow:
485 genes that do not pass the permutation test (q-value > 0.05). (B) Analysis by SDS-PAGE of
486 FliC produced by the wild type (lane 1), the A3422 (lane 2) and A4277 (lane 3) strains.
487
488 **Fig 6. Correlation between the competitivity index (IC) of several mutant strains with**
489 **the log_2FC of the corresponding genes.** IC values were determined in chicory leaves as
490 described in Methods. The IC values for *dltB*, *anrB*, *phoS* and *phoH* are from [60]. Each
491 value is the mean of 5 experiments.
492
493 **Fig S1. Biological reproducibility of the Tn-seq results**.
494 Pairs of Tn-seq assay results are compared, with the total number of reads per gene plotted.
495 Analysis of DNA samples corresponding to two independent cultures of the mutant pool
496 grown (A) in LB medium (correlation coefficient R = 0.72) and (B) in chicory (correlation
497 coefficient R = 0.98). Values represent average numbers of reads per gene from the pairs of
498 biological replicates.
499
500 **Fig S2. Volcano plot of resampling results comparing replicates grown in chicory versus**
501 **in LB**. Significant hits have q < 0.05 or $-\log_{10} q > 1.3$. Growth defect (GD) or growth
502 advantage (GA) genes are indicated by a red frame.

503 **Fig S3. *acrAB* are essential in chicory.** Number of Tn-seq reads at each insertion site in the
504 *acrA acrB* region in samples grown in LB or in chicory. Data are averaged from biological
505 replicates and normalized as described in Methods. *dnaX* which encodes both the tau and
506 gamma subunits of DNA polymerase is represented by a grey arrow. *dnaX* is essential gene in
507 LB. *acrAB* represented by grey arrows are GD in chicory (q-value ≤ 0.05).

508 **Fig S4. Pathogenicity tests on chicory with leucine (A5969) and cytosine (A5968)**
509 **mutants of *D. dadantii*.**
510 Each leaf was inoculated with 10^6 bacteria. Length of rotten tissue was observed after 24h.

511

512

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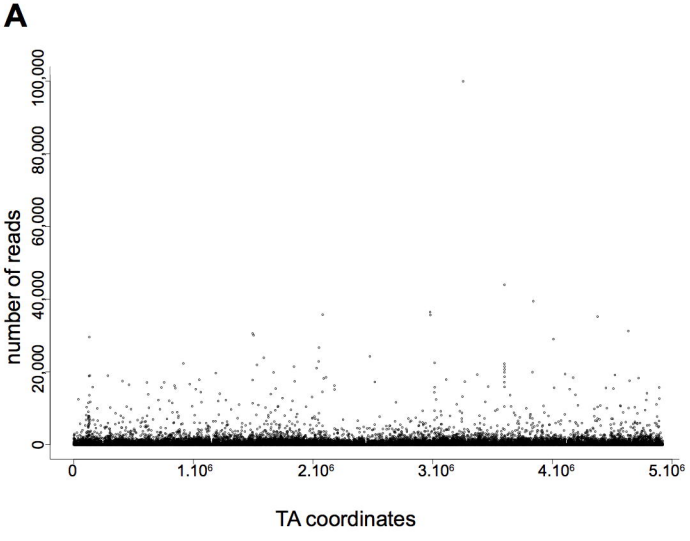
Figure 1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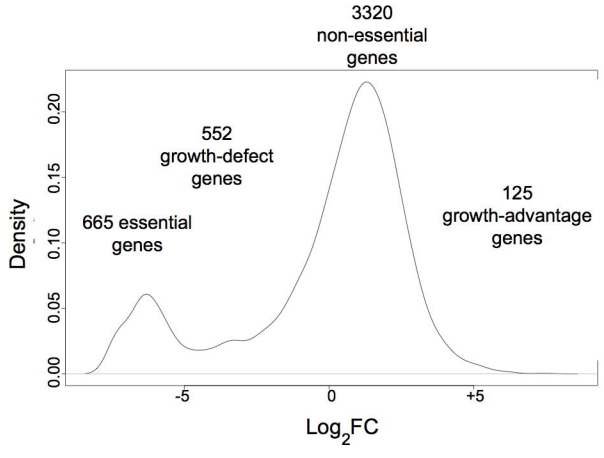
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genes
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growth-defect
genes
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genes
125
growth-advantage
genes
Density
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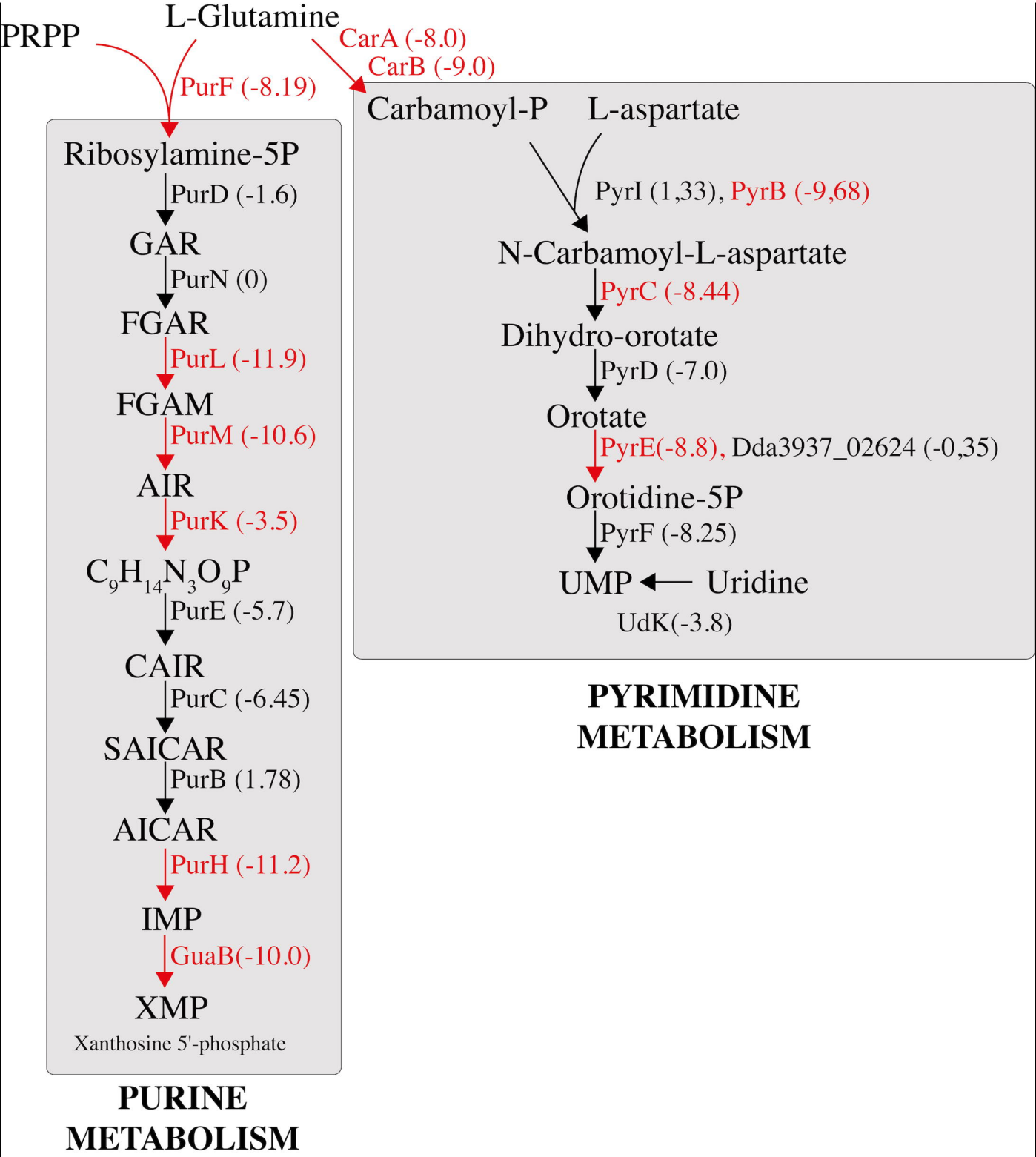
0.10
0.15
0.20
-5
0
+5
Log2FC

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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PurL (-11.9)
FGAM
PurM (-10.6)
AIR
PurK (-3.5)
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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
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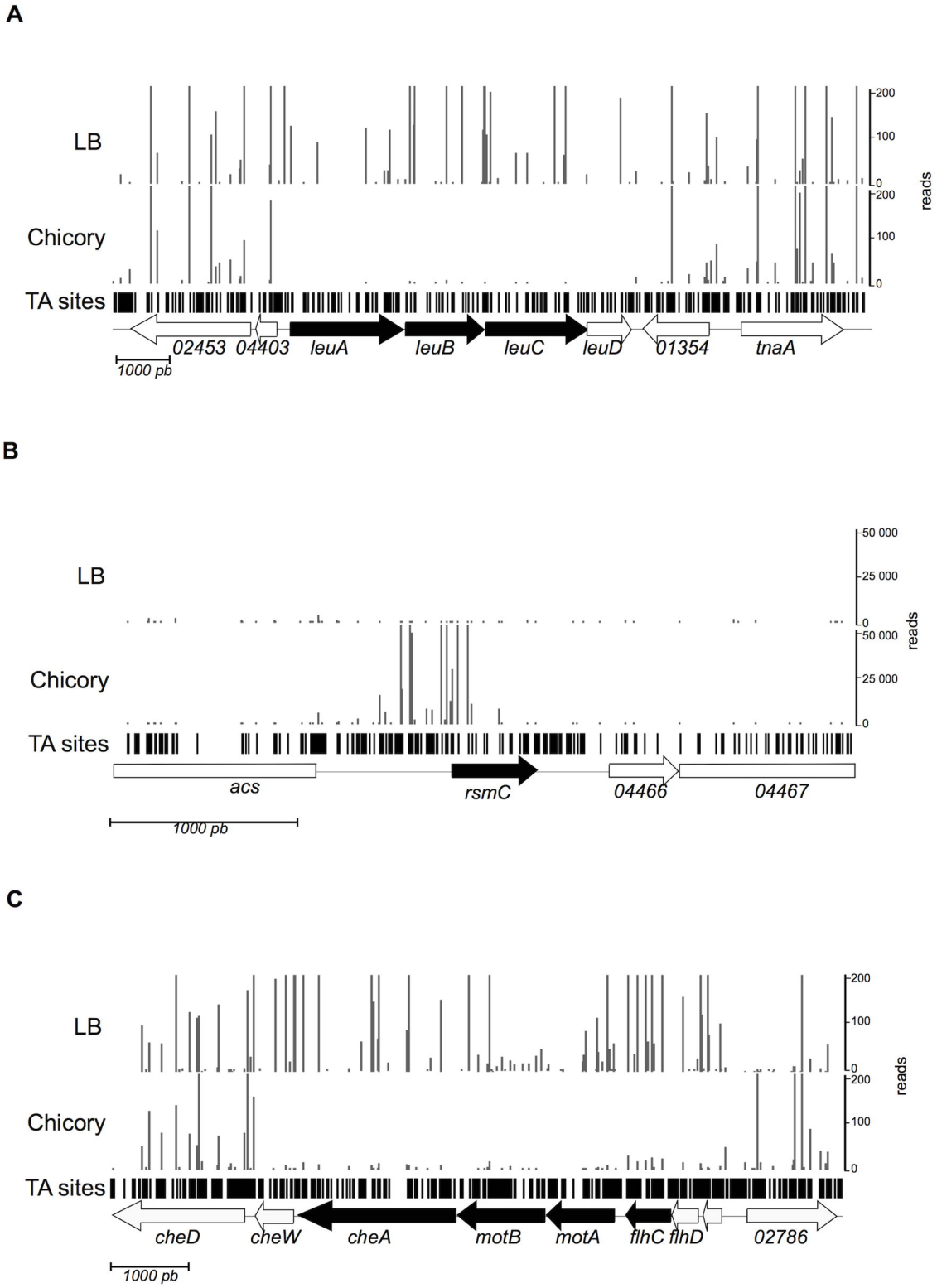
Uridine
UdK(-3.8)
PYRIMIDINE METABOLISM

Figure 4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B
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TA sites
reads
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25 000
0
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rsmC
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04467
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C
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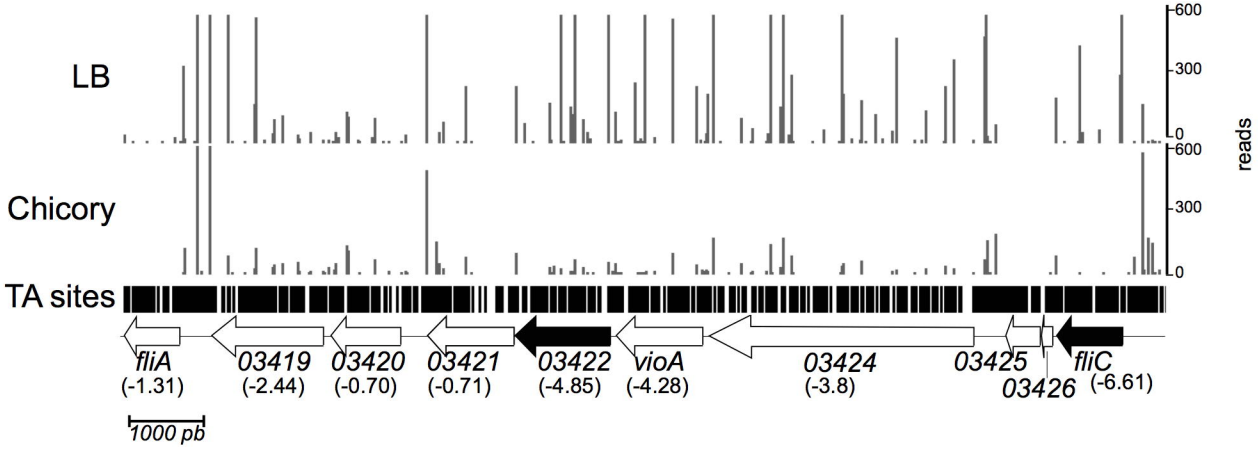
Figure 5

A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B

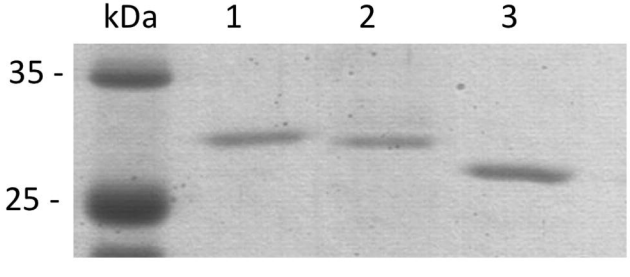
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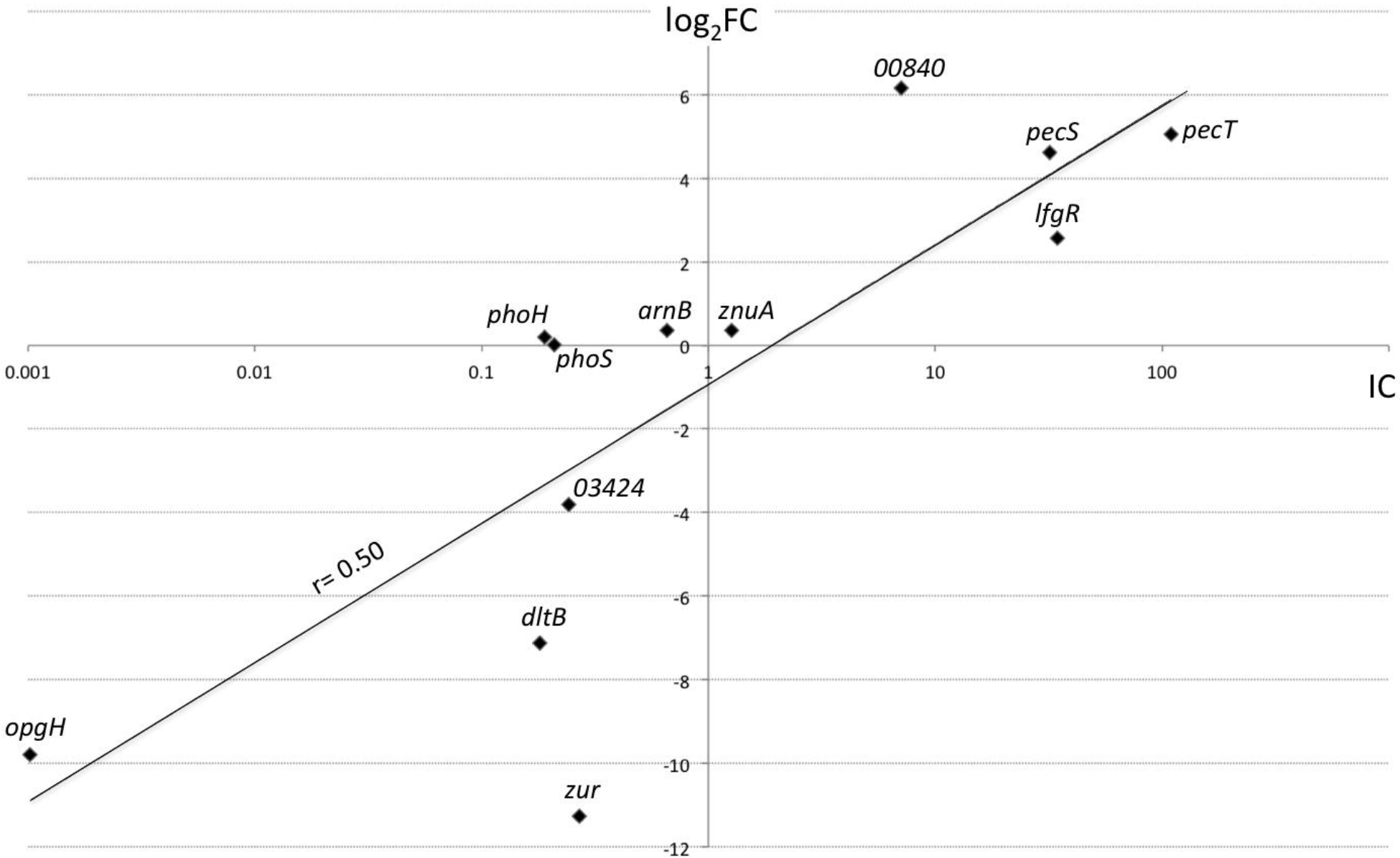
Figure 6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