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 de Lyon, INRA UMR 203 Biologie Fonctionnelle Insectes et Interactions, 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, a modification which in some other plant pathogenic bacteria contributes to virulence.

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 important 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 pectinases [3], of the regulators controlling their production (*kdgR*, *pecS*, *pecT*, *hns*, *gacA*) , [4-8] of the genes whose expression is coregulated with that of the secreted enzyme genes [9, 10], and of the mechanism of their secretion by the Out type II secretion system [11]. Although of a lesser importance for *Dickeya* virulence, the same type of approach has been used to identify Hrp type III secretion system regulators and effectors [12] [13] [14]. Competition for iron within the plant is strong. *D. dadantii* acquires this metal through production of two siderophores, chrysobactin and achromobactin [15] [16] [17]. More recently, omics approaches have also been used to identify genes induced during plant infection [18] [19] [20]. These studies now give a view on a complex network of factors required for *D. dadantii* virulence [2, 21]. However, these approaches may have missed some important factors not targeted by these analyses. More global screens need to be performed to identify these factors. Libraries of transposon-induced mutants were tested on plants to find mutants showing reduced virulence with *Pectobacterium carotovorum* and *atrosepticum*, two other soft rot enterobacteria [22, 23]. These studies identified auxotrophs, mutants defective in production or secretion of exoenzymes and in motility. Other mutants with a more

complex phenotype were not characterized at this time. Moreover, the number of tested mutants was limited by the necessity to test individually each mutant on plant. This type of work has never been performed on *Dickeya* strains. To have a more complete view of the genes required for the virulence of *Dickeya*, we used a high-throughput sequencing of a saturated transposon library (Tn-seq) to screen tens of thousands random insertion mutants of *D. dadantii* in laboratory medium and during infection of chicory. Tn-seq has been extensively used to uncover essential genes required for mouse colonization by human pathogens *Vibrio cholerae* [24], *Pseudomonas aeruginosa* [25] and *Streptococcus pneumoniae* [26]. Thus, Tn-seq is a very powerful method to identify genes required for 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 obtention 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 (Table 1). The average number of reads per TA is 88 and 75, respectively. The results were reproducible with a Pearson correlation coefficient of 72% (Fig. S1) The location of the unique insertions showed an even distribution around the chromosome (Fig. 1A). For each gene, we calculated a $\log_2$FC corresponding to a ratio between the measured number of reads and the expected number of reads. The density plot (Fig. 1B) indicates that essential and non-essential genes are easily distinguishable, confirming the good quality of our Tn-seq libraries. Then, gene essentiality of the Tn-seq input libraries was determined by using the TRANSIT software [29]. We decided to use the Hidden Markov Model (HMM) which predicts essentiality and non-essentiality for individual insertion sites because it has been shown to give good prediction in datasets with density as low as 20% [29]. The HMM analysis led to the identification of 665 genes essential for growth in LB (ES), representing 14% of the genes of *D. dadantii* 3937, a number in the range of those found for this type of analysis with bacteria. 552 genes were categorized as Growth Defect genes (GD, i.e. mutations in these genes lead to loss of fitness), 125 as growth advantage genes (GA, i.e mutations in these genes lead to gain of fitness) and 3320 as non-essential genes (NE) (table S2).

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 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

[a] The number of reads containing the sequence of a Tn end were normalized for each sample according to the number of reads for the sample Chicory #1

[b] *Dickeya dadantii* 3937 genome has 171,791 TA sites. The density is the % of TAs for which mapped reads has been assigned by the TPP software.

[c] the mean value of mapped reads per TA.

Genes necessary for chicory leaf maceration. We used chicory leaf infection as a model to identify *D. dadantii* genes required for growth in plant tissues. Biological duplicates were performed to insure the reproducibility of the results. Each chicory was inoculated with 10^7 bacteria from the mutant pool and after 2 days more than 10^{10} bacteria were collected from the rotten tissue. Sequencing transposon insertion sites in these bacteria followed by the TPP analysis indicated a density of unique insertion in TAs comparable to that of the input datasets (23-24%). Surprisingly, the results were more highly reproducible than in LB with a very high Pearson correlation coefficient of 98% (Fig. S1).

In order to identify genes of *D. dadantii* conferring loss or gain of fitness *in planta*, we performed the RESAMPLING analysis of the TRANSIT software. The RESAMPLING method is a variation of the classical permutation test in statistics that sums the reads at all TA sites for each gene in each condition. It then calculates the difference of the sum of read-counts between the input (LB) and output (chicory) datasets. The advantage of this statistical method is to attribute for each gene an adjusted p-value (q-value). Genes with a significant difference between total read-counts in LB and chicory achieve a q-value ≤ 0.05. The method also calculates a $\log_2$ fold-change ($\log_2$FC) for each gene based on the ratio of the sum of

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

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

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

monophosphate (UMP) biosynthesis pathway that converts L-glutamine to UMP, a precursor of uracyl, is very important *in planta* since *carAB*, *pyrB*, *pyrC* and *pyrE*, involved in this enzymatic pathway, are all required for growth *in planta* (Fig. 3). This pyrimidine biosynthesis pathway is specific to bacteria. It is noteworthy that in the human pathogen *S. pneumoniae*, mutants of this pathway have a fitness defect in the nasopharynx of infected mice [26]. Hence, it looks that the pyrimidine biosynthesis pathway is particularly important for multiplication of some bacterial species in the host.

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

Degradation of cell wall pectin by a battery of extracellular enzymes is the main determinant of *Dickeya* pathogenicity. Mutants unable to produce or to secrete these enzymes by the type II Out secretion system were not disfavored in chicory since these mutants could use for their growth the pectin degradation compounds produced by enzymes secreted by other bacteria. The redundancy of oligogalacturonate specific porins (KdgM and KdgN) and inner membrane transporters (TogT and TogMNABC) allow entry of these compounds into the bacteria even in a mutant in one of these transport systems. However, *kduI* mutants, blocked

in the intracellular part of the pectin degradation pathway, have a limited growth *in planta*, confirming the importance of the pectin degradation pathway in the disease progression.

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

(iii) Iron uptake. *D. dadantii* produces two types of siderophores, achromobactin and chrysobactin, that are required for the development of maceration symptoms in the iron limited environment of plant hosts [38]. Once iron loaded, the siderophores are imported into the bacteria. Import through the outer membrane requires a specific outer membrane channel and the energy transducing complex formed by TonB ExbB and ExbD. While the absence of synthesis of one of the siderophores can be compensated by the presence of siderophore secreted by other bacteria in the growth medium, mutants of the TonB complex are totally unable to acquire iron and thus are unable to grow in the plant. In accordance, *tonB* was essential in chicory while the genes coding for siderophore synthesis or secretion were not. Similarly a mutant devoid of the iron-loaded chrysobactin transport gene (*fct)* is non-competitive.

(iv) Regulation. Mutants in several genes controlling virulence factor production have a growth defect in the plant. The master regulator FlhDC acts as a regulator of both flagella and virulence factor synthesis in many bacteria such as *Yersinia ruckeri*, *Edwardsiella tarda* and *Ralstonia solanacearum* [39-41]. In *D. dadantii* FlhDC has recently been shown to control, in addition to flagellar motility, type III secretion system and virulence factor synthesis through several pathways [42]. We observed that *flhC* gives a growth advantage in chicory. In addition, we uncovered that some genes regulating *flhDC* in other bacteria regulate *D. dadantii* virulence, probably by controlling *flhDC* expression. *rsmC* is a poorly characterized gene in *D. dadantii* but that has been studied in *Pectobacterium carotovorum*. It negatively controls motility and extracellular enzyme production through modulating transcriptional activity of FlhCD [43]. HdfR is a poorly characterized LysR family regulator that controls the *std* fimbrial operon in *S. enterica* and FlhDC expression in *E. coli* [44]. *rsmC* mutants were overrepresented in the chicory (Fig. 4B), indicating a gain of virulence for these mutants.

hdfR conferred fitness benefits during growth in chicory and could also act in *D. dadantii* as activator of *flhDC* expression.

The GGDEF proteins are c-di-GMP synthase. Their gene are often located next to their cognate EAL diguanylate phosphodiesterase gene. *ecpC (yhjH)* encodes an EAL protein that was shown to activate virulence factor production in *D. dadantii* [45]. *gcpA*, which is located next to *ecpC* encodes a GGDEF protein. However, a *gcpA* mutant could not be constructed and analysed in this previous study. We observed that *gcpA* mutants (Dda_03858) were overrepresented in chicory (Fig. 2). This increased virulence is a phenotype opposite to that described for the *ecpC* mutants, indicating that overproduction of c-di-GMP could reduce *D. dadantii* virulence.

Of the eighteen regulators of the LacI family present in *D. dadantii*, four of them were found to be involved in plant infection [46]. One of those, LfcR, which has been found important for infection of chicory, Saintpaulia and Arabidopsis, was identified as important for chicory infection in our experiment. LfcR is a repressor of adjacent genes [46]. Surprisingly none of these genes appeared to play a role for chicory infection suggesting that other targets of LfcR probably remain to be discovered.

Finally, it is noteworthy to mention that the *ackA* and *pta* genes are GD *in planta*. These genes constitute the reversible Pta-AckA pathway. The steady-state concentration of acetyl-phosphate (acetyl-P), a signaling molecule in bacteria, depends upon the rate of its formation catalyzed by Pta and of its degradation catalyzed by AckA [47]. The GD phenotype of *D. dadantii ackA* and *pta* mutants during infection suggests that acetyl-P might play a crucial signaling role in the adaptation of *D. dadantii* to the plant tissue.

(v) Motility. Motility is an essential virulence factor of *D. dadantii* required to move on the surface of the leaf, enter the wounds and propagate into the plant tissue [48-50]. Accordingly, all the genes required for flagella synthesis, the flagella motor and genes regulating their

synthesis (*flhC*, *flhD*, *fliA*) (see above) are necessary for fitness during chicory infection (Fig. 4C and 5A). All the genes responsible for the transduction of the chemotaxis signal (*cheA*, *B*, *R*, *W*, *X*, *Y* and *Z*) also confer a benefit in planta (Fig. 2). No methyl-accepting chemoreceptor gene mutant was found. Like other environmental bacteria, *D. dadantii* encodes many such proteins (47). They probably present some redundancy in the recognized signal which prevented their detection by our screen.

D. dadantii flagellin is modified by glycosylation

A group of six genes located between *fliA* and *fliC* retained our interest since insertions in these genes led to a growth defect in chicory (Fig. 5A). Dda3937_03424 encodes an O-linked N-acetylglucosamine transferase and Dda3937_03419 encode a protein with a nucleotide diphospho sugar transferase predicted activity. The other ones could be involved in the modification of sugars (predicted function of Dda3937_03423: nucleotide sugar transaminase, Dda3937_03422: carbamoyl phosphate synthase, Dda3937_03421: oxidoreductase; Dda3937_03420: methyltransferase). Their location let suppose that this group of genes could be involved in flagellin glycosylation. Analysis by SDS-PAGE of FliC produced by the wild type, and the A4277 and A3422 strains (Dda3937_03424 and Dda3937_03419 mutants, respectively) showed that in the two latter strains the molecular weight of the protein diminished (Fig. 5B). The molecular weight determined by mass spectroscopy was 28911 Da for FliCA4277, 31034 Da for FliCA3422 and 32258 Da for the WT FliC. Thus, in the wild type strain FliC is modified by the products of the genes Dda_03424 to Dda_03419, probably by multiple glycosylation with a disaccharide. Absence of modification did not modify *D. dadantii* motility (data not shown). The flagellin of the plant pathogens *Pseudomonas syringae* pv *tabaci* and *Burkholderia cenocepacia* are also glycosylated and absence of this modification lowered the ability of these bacteria to cause

disease on tobacco and arabidopsis, respectively [51, 52]. Accordingly, in *D. dadantii*, FliC modification appears important for multiplication of the bacteria in plant.

Additional genes could be involved in virulence

Several genes have a $\log_2$FC >4 or <-4 but do not satisfy the statistical permutation test adjusted by the false discovery rate method (q-value) (table S4). However, most of them belong to the categories described above and could be required for growth in planta. Among those with a $\log_2$FC< -4 can be found genes involved in amino acid and nucleic acid synthesis (*cysH, ilvC, pyrF, pyrD, purC, thrC, metA, cysK, lysC*), flagella and motility (*flgJ, fliO, flgC, fliS, flgG, flgA, flgL, cheW, fliN, fliP, fliK, fliG, fliL*), pectin and glucose metabolism (*kduD, pgi*), EPS synthesis (*gmd*), flagella glycosylation (*vioA*) and regulation (*zur*, *ecpC* and the general RNA chaperone *hfq*).

Among the genes with a $\log_2$FC > 4, three regulators can be noticed: *pecS*, *pecT* and *Dda3937_00840*. *pecS* and *pecT* are known regulators of *D. dadantii* controlling the expression of many factors involved in virulence. Thus, their mutation could confer an increased fitness of the bacteria in chicory. *D. dadantii* possesses a functional *expI-expR* quorum sensing system which does not seem to control plant virulence factor production (Nasser). However, several LuxR family regulator genes which are not associated with a *luxI* gene are present in the genome of the bacteria. Mutants of one of them (*Dda3937_00840*) are overrepresented in the chicory. Its product is probably a repressor of genes conferring an increased fitness i*n planta*.

Validation of the Tn-seq results.

To validate the Tn-seq results, we performed coinoculation experiments in chicory leaves with the wild type strain and various mutants in genes conferring a growth advantage, a growth defect or no fitness benefit in a 1/1 ratio. We calculated a competitivity index (CI) by counting the number of each type of bacteria in the rotten tissue after 24 h. We found a correlation between the competitivity index of mutants with the $\log_2$FC of the corresponding genes with a Pearson coefficient of 0.50 (Fig. 6), indicating that Tn-seq is a reliable technique to identify genes involved in plant colonization and virulence. Additional validations were performed by inoculation of uncharacterized leucine and cytosine auxotroph mutants in chicory leaves which confirmed that these mutants are unable to grow in plant and are thus avirulent (Fig S4).

Conclusion

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

Our results give also some information on the metabolic status of the plant. The amount of nucleic acids and of the amino acids cysteine, leucine, methionine, threonine and isoleucine is too low in chicory to allow the multiplication of mutant bacteria. Some enzymatic steps involved in their synthesis are specific to bacteria and fungi. Thus, they could be good target for the development of specific inhibitors [53] to fight *D. dadantii*.

While Tn-seq has been used to study genes required for the infection of animals, there has been no genome-wide study of factors necessary for a plant pathogen to develop and provoke disease on a plant. Besides the genes of known function described in the Result section, this study allowed the identification of several genes of unknown function required for chicory rotting. Repetition of this experiment with other strains or on other plants will tell if these genes encode strain or host specific virulence factors.

Methods

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

Construction of the transposon library

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

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

DNA preparation for high-throughput sequencing

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

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

Bioinformatics analysis:

Raw reads from the fastQ files were first filtered using cutadapt v1.11 [56] and only reads containing the *mariner* inverted left repeat (ACAGGTTGGATGATAAGTCCCCGGTCTT)

were trimmed and considered *bona fide* transposon-disrupted genes. Trimmed reads were then analyzed using a modified version of the TPP script available from the TRANSIT software v2.0.2 [29]. The mapping step was modified to select only reads mapping uniquely and without mismatch in the *D. dadantii* 3937 genome (Genbank CP002038.1). Then, the counting step was modified to accurately count the reads mapping to each TA site in the reference genome according to the Tn-seq protocol used in this study. Read counts per insertion were normalized using the LOESS method as described in [57]. We next used the TRANSIT software (version 2.0) to compare the Tn-seq datasets.

Strain construction. To construct the A4277 strain, gene Dda3937_03424 was amplified with the oligonucleotides 19732+ and 19732-. The resulting fragment was inserted into the pGEM-T plasmid (Promega). A *uidA*-kan^R cassette [58]was inserted into the unique AgeI site of the fragment. The construct was recombined into the *D. dadantii* chromosome according to [59]. Recombination was checked by PCR.

Protein techniques. Flagella were prepared from overnight LB grown cells. Bacteria were pelleted, resuspended in 1/10 volume of water and passed 20 fold through a needle on a syringe. Cells and cells debris were removed by centrifugation 5 min at 20 000 x g [55]. Proteins were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Coinoculation experiments. To determine the competitivity index, the wild type strain and the mutant to test, marked with an antibiotic resistance gene, were grown overnight in M63 + glycerol medium. Bacteria were washed in M63 medium and the OD_{600} was adjusted to 1.0. Bacteria were mixed to a 1:1 ratio and diluted 10-fold. 10 uL of the mixture were

inoculated into chicory leaves. The wound was covered with mineral oil and the leaves were incubated at 30 °C at high humidity. After 24 h the rotten tissue was collected, homogenized, diluted in M63 and spread onto LB and LB + antibiotic plates. After 48 h at 30°C, colonies were counted. The competitivity index is the ratio (number of mutant bacteria/number of WT bacteria) in the rotten tissue / (number of mutant bacteria/number of WT bacteria) in the inoculum.

Legend of figures

Fig. 1. **Quality control of the Tn-seq *D. dadantii* 3937 libraries**.

(A) Frequency and distribution of transposon sequence reads across the entire *D. dadantii 3937* genome. The localization of transposon insertions shows no bias throughout the genome of *D. dadantii 3937*. B) Density plot of $\log_2$FC (measured reads/expected reads per gene).

Fig 2. Genes identified by Tn-seq exhibiting a fitness change in chicory compared to LB.

Data were obtained with the TRANSIT software**.** From left to right : (a) classification of the gene with the HMM method performed in the LB dataset. (b) Number of TA sites left after removing 10% of 5' and 3' extremities. (c) Mean read number per gene in the LB or chicory dataset. (d) Ratio of read number between chicory and LB expressed in $\log_2$. (e) p-values adjusted for multiple comparisons using the false-discovery rate method. Genes for which a role in *D. dadantii* virulence has been described before are in bold.

Fig 3. Scheme of the purine and pyrimidine biosynthesis pathways in *D. dadantii* that

produce XMP (purine metabolism) and UMP (pyrimidine metabolism) from L-glutamine.

In red are indicated the growth defect genes in chicory that pass the permutation test (q-value ≤ 0.05). The log_2FC of read numbers between chicory and LB for each gene is indicated in bracket. Some genes do not pass the permutation test (in black) but have a strong negative log_2FC. PRPP: 5-phosphoribosyl-1-pyrophosphate ; GAR: 5'-phosphoribosyl-1-glycinamide ; FGAM: 5'-phosphoribosyl-*N*-formylglycinamide ; AIR: 5'-phosphoribosyl-5-aminoimidazole ; CAIR: 5'-phosphoribosyl-5-aminoimidazole carboxylic acid ; SAICAR: 5'-phosphoribosyl-4-(*N*-succino-carboxamide)-5-aminoimidazole ; AICAR: 5-aminoimidazole-4-carboxamide ribonucleotide ; IMP: inosine monophosphate ; XMP: xanthine monophosphate ; UMP: uridine monophosphate.

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

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

Fig 5. Modification of FliC revealed by Tn-seq analysis and SDS-PAGE. (A) Importance of 6 genes located between *fliA* and *fliC* for growth in chicory. Log_2FC are indicated in bracket. Dda3937_03425 and Dda3937_03426 are duplicated transposase genes that have been removed from the analysis. Black arrow: GD in chicory (q-value ≤ 0.05) ; white arrow:

genes that do not pass the permutation test (q-value > 0.05). (B) Analysis by SDS-PAGE of FliC produced by the wild type (lane 1), the A3422 (lane 2) and A4277 (lane 3) strains.

Fig 6. Correlation between the competitivity index (IC) of several mutant strains with the $\log_2$FC of the corresponding genes. IC values were determined in chicory leaves as described in Methods. The IC values for *dltB*, *anrB*, *phoS* and *phoH* are from [60]. Each value is the mean of 5 experiments.

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

Pairs of Tn-seq assay results are compared, with the total number of reads per gene plotted. Analysis of DNA samples corresponding to two independent cultures of the mutant pool grown (A) in LB medium (correlation coefficient R = 0.72) and (B) in chicory (correlation coefficient R = 0.98). Values represent average numbers of reads per gene from the pairs of biological replicates.

Fig S2. Volcano plot of resampling results comparing replicates grown in chicory versus in LB. Significant hits have q < 0.05 or $-\log_{10}$ q > 1.3. Growth defect (GD) or growth advantage (GA) genes are indicated by a red frame.

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

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

mutants of *D. dadantii*.

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

References

1. Adeolu M, Alnajar S, Naushad S, R SG. Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. Int J Syst Evol Microbiol. 2016;66(12):5575-99. Epub 2016/09/14. doi: 10.1099/ijsem.0.001485. PubMed PMID: 27620848.
2. Charkowski A, Blanco C, Condemine G, Expert D, Franza T, Hayes C, et al. The Role of Secretion Systems and Small Molecules in Soft-Rot Enterobacteriaceae Pathogenicity. Annu Rev Phytopathol. 2012;50:425-49. PubMed PMID: 22702350.
3. Hugouvieux-Cotte-Pattat N, Condemine G, Nasser W, Reverchon S. Regulation of pectinolysis in *Erwinia chrysanthemi*. Annu Rev Microbiol. 1996;50:213-57. PubMed PMID: 8905080.
4. Condemine G, Robert-Baudouy J. Analysis of an *Erwinia chrysanthemi* gene cluster involved in pectin degradation. Mol Microbiol. 1991;5(9):2191-202. PubMed PMID: 1766386.
5. Reverchon S, Nasser W, Robert-Baudouy J. *pecS*: a locus controlling pectinase, cellulase and blue pigment production in *Erwinia chrysanthemi*. Mol Microbiol. 1994;11(6):1127-39. Epub 1994/03/01. PubMed PMID: 8022282.

6. Surgey N, Robert-Baudouy J, Condemine G. The Erwinia chrysanthemi pecT gene regulates pectinase gene expression. J Bacteriol. 1996;178(6):1593-9. PubMed PMID: 8626286.

7. Nasser W, Faelen M, Hugouvieux-Cotte-Pattat N, Reverchon S. Role of the nucleoid-associated protein H-NS in the synthesis of virulence factors in the phytopathogenic bacterium *Erwinia chrysanthemi.* Mol Plant Microbe Interact. 2001;14(1):10-20. Epub 2001/02/24. doi: 10.1094/mpmi.2001.14.1.10. PubMed PMID: 11194867.

8. Lebeau A, Reverchon S, Gaubert S, Kraepiel Y, Simond-Cote E, Nasser W, et al. The GacA global regulator is required for the appropriate expression of *Erwinia chrysanthemi* 3937 pathogenicity genes during plant infection. Environ Microbiol. 2008;10(3):545-59. PubMed PMID: 18177376.

9. Condemine G, Castillo A, Passeri F, Enard C. The PecT repressor coregulates synthesis of exopolysaccharides and virulence factors in *Erwinia chrysanthemi*. Mol Plant Microbe Interact. 1999;12(1):45-52. PubMed PMID: 9885192.

10. Reverchon S, Rouanet C, Expert D, Nasser W. Characterization of indigoidine biosynthetic genes in *Erwinia chrysanthemi* and role of this blue pigment in pathogenicity. J Bacteriol. 2002;184(3):654-65. Epub 2002/01/16. PubMed PMID: 11790734; PubMed Central PMCID: PMCPMC139515.

11. Condemine G, Dorel C, Hugouvieux-Cotte-Pattat N, Robert-Baudouy J. Some of the *out* genes involved in the secretion of pectate lyases in *Erwinia chrysanthemi* are regulated by *kdgR*. Mol Microbiol. 1992;6(21):3199-211. PubMed PMID: 1453958.

12. Yang CH, Gavilanes-Ruiz M, Okinaka Y, Vedel R, Berthuy I, Boccara M, et al. *hrp* genes of *Erwinia chrysanthemi* 3937 are important virulence factors. Mol Plant Microbe Interact. 2002;15(5):472-80. Epub 2002/05/31. doi: 10.1094/mpmi.2002.15.5.472. PubMed PMID: 12036278.

13. Li Y, Hutchins W, Wu X, Liang C, Zhang C, Yuan X, et al. Derivative of plant phenolic compound inhibits the type III secretion system of *Dickeya dadantii* via HrpX/HrpY two-component signal transduction and Rsm systems. Molecular plant pathology. 2015;16(2):150-63. Epub 2014/07/06. doi: 10.1111/mpp.12168. PubMed PMID: 24986378.

14. Yang S, Peng Q, Zhang Q, Zou L, Li Y, Robert C, et al. Genome-wide identification of HrpL-regulated genes in the necrotrophic phytopathogen *Dickeya dadantii* 3937. PLoS One. 2010;5(10):e13472. Epub 2010/10/27. doi: 10.1371/journal.pone.0013472. PubMed PMID: 20976052; PubMed Central PMCID: PMCPMC2957411.

15. Franza T, Mahe B, Expert D. *Erwinia chrysanthemi* requires a second iron transport route dependent of the siderophore achromobactin for extracellular growth and plant infection. Mol Microbiol. 2005;55(1):261-75. Epub 2004/12/23. doi: 10.1111/j.1365-2958.2004.04383.x. PubMed PMID: 15612933.

16. Franza T, Sauvage C, Expert D. Iron regulation and pathogenicity in *Erwinia chrysanthemi* 3937: role of the Fur repressor protein. Mol Plant Microbe Interact. 1999;12(2):119-28. Epub 1999/02/02. doi: 10.1094/mpmi.1999.12.2.119. PubMed PMID: 9926414.

17. Franza T, Expert D. The virulence-associated chrysobactin iron uptake system of *Erwinia chrysanthemi* 3937 involves an operon encoding transport and biosynthetic functions. J Bacteriol. 1991;173(21):6874-81. Epub 1991/11/01. PubMed PMID: 1657869; PubMed Central PMCID: PMCPMC209040.

18. Okinaka Y, Yang CH, Perna NT, Keen NT. Microarray profiling of *Erwinia chrysanthemi* 3937 genes that are regulated during plant infection. Mol Plant Microbe Interact. 2002;15(7):619-29. Epub 2002/07/18. doi: 10.1094/mpmi.2002.15.7.619. PubMed PMID: 12118877.

19. Yang S, Perna NT, Cooksey DA, Okinaka Y, Lindow SE, Ibekwe AM, et al. Genome-wide identification of plant-upregulated genes of *Erwinia chrysanthemi* 3937 using a GFP-based IVET leaf array. Mol Plant Microbe Interact. 2004;17(9):999-1008. Epub 2004/09/24. doi: 10.1094/mpmi.2004.17.9.999. PubMed PMID: 15384490.

20. Chapelle E, Alunni B, Malfatti P, Solier L, Pedron J, Kraepiel Y, et al. A straightforward and reliable method for bacterial in planta transcriptomics: application to the *Dickeya dadantii*/*Arabidopsis thaliana* pathosystem. Plant J. 2015;82(2):352-62. Epub 2015/03/06. doi: 10.1111/tpj.12812. PubMed PMID: 25740271.

21. Reverchon S, Muskhelisvili G, Nasser W. Virulence Program of a Bacterial Plant Pathogen: The *Dickeya* Model. Progress in molecular biology and translational science. 2016;142:51-92. Epub 2016/08/31. doi: 10.1016/bs.pmbts.2016.05.005. PubMed PMID: 27571692.

22. Hinton JC, Sidebotham JM, Hyman LJ, Perombelon MC, Salmond GP. Isolation and characterisation of transposon-induced mutants of *Erwinia carotovora* subsp. *atroseptica* exhibiting reduced virulence. Mol Gen Genet. 1989;217(1):141-8. Epub 1989/05/01. PubMed PMID: 2549365.

23. Pirhonen M, Saarilahti H, Karlsson M-B, Palva ET. Identification of pathogenicity determinants of *Erwinia carotovora* subsp. *carotovora* by transposon mutagenesis. Molecular Plant-Microbe Interaction. 1991;4:276-83.

24. Fu Y, Waldor MK, Mekalanos JJ. Tn-Seq analysis of *Vibrio cholerae* intestinal colonization reveals a role for T6SS-mediated antibacterial activity in the host. Cell host & microbe. 2013;14(6):652-63. Epub 2013/12/18. doi: 10.1016/j.chom.2013.11.001. PubMed PMID: 24331463; PubMed Central PMCID: PMCPMC3951154.

25. Skurnik D, Roux D, Aschard H, Cattoir V, Yoder-Himes D, Lory S, et al. A comprehensive analysis of in vitro and in vivo genetic fitness of *Pseudomonas aeruginosa*

using high-throughput sequencing of transposon libraries. PLoS Pathog. 2013;9(9):e1003582. Epub 2013/09/17. doi: 10.1371/journal.ppat.1003582. PubMed PMID: 24039572; PubMed Central PMCID: PMCPMC3764216.

26. van Opijnen T, Camilli A. A fine scale phenotype-genotype virulence map of a bacterial pathogen. Genome research. 2012;22(12):2541-51. Epub 2012/07/25. doi: 10.1101/gr.137430.112. PubMed PMID: 22826510; PubMed Central PMCID: PMCPMC3514683.

27. van Opijnen T, Camilli A. Transposon insertion sequencing: a new tool for systems-level analysis of microorganisms. Nat Rev Microbiol. 2013;11(7):435-42. Epub 2013/05/29. doi: 10.1038/nrmicro3033. PubMed PMID: 23712350; PubMed Central PMCID: PMCPMC3842022.

28. Wiles TJ, Norton JP, Russell CW, Dalley BK, Fischer KF, Mulvey MA. Combining quantitative genetic footprinting and trait enrichment analysis to identify fitness determinants of a bacterial pathogen. PLoS Genet. 2013;9(8):e1003716. Epub 2013/08/31. doi: 10.1371/journal.pgen.1003716. PubMed PMID: 23990803; PubMed Central PMCID: PMCPMC3749937.

29. DeJesus MA, Ambadipudi C, Baker R, Sassetti C, Ioerger TR. TRANSIT--A Software Tool for Himar1 TnSeq Analysis. PLoS computational biology. 2015;11(10):e1004401. Epub 2015/10/09. doi: 10.1371/journal.pcbi.1004401. PubMed PMID: 26447887; PubMed Central PMCID: PMCPMC4598096.

30. Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res. 1999;27(1):29-34. Epub 1998/12/10. PubMed PMID: 9847135; PubMed Central PMCID: PMCPMC148090.

31. Reverchon S, Nasser W. *Dickeya* ecology, environment sensing and regulation of virulence programme. Environ Microbiol Rep. 2013;5(5):622-36. PubMed PMID: 24115612.

32. Ravirala RS, Barabote RD, Wheeler DM, Reverchon S, Tatum O, Malouf J, et al. Efflux pump gene expression in *Erwinia chrysanthemi* is induced by exposure to phenolic acids. Mol Plant Microbe Interact. 2007;20(3):313-20. Epub 2007/03/24. doi: 10.1094/mpmi-20-3-0313. PubMed PMID: 17378434.

33. Malinverni JC, Silhavy TJ. An ABC transport system that maintains lipid asymmetry in the gram-negative outer membrane. Proc Natl Acad Sci U S A. 2009;106(19):8009-14. Epub 2009/04/23. doi: 10.1073/pnas.0903229106. PubMed PMID: 19383799; PubMed Central PMCID: PMCPMC2683108.

34. Page F, Altabe S, Hugouvieux-Cotte-Pattat N, Lacroix JM, Robert-Baudouy J, Bohin JP. Osmoregulated periplasmic glucan synthesis is required for *Erwinia chrysanthemi* pathogenicity. J Bacteriol. 2001;183(10):3134-41. PubMed PMID: 11325942.

35. Bouchart F, Boussemart G, Prouvost AF, Cogez V, Madec E, Vidal O, et al. The virulence of a *Dickeya dadantii* 3937 mutant devoid of osmoregulated periplasmic glucans is restored by inactivation of the RcsCD-RcsB phosphorelay. J Bacteriol. 2010;192(13):3484-90. Epub 2010/04/27. doi: 10.1128/jb.00143-10. PubMed PMID: 20418397; PubMed Central PMCID: PMCPMC2897653.

36. Madec E, Bontemps-Gallo S, Lacroix JM. Increased phosphorylation of the RcsB regulator of the RcsCDB phosphorelay in strains of *Dickeya dadantii* devoid of osmoregulated periplasmic glucans revealed by Phos-tag gel analysis. Microbiology. 2014;160(Pt 12):2763-70. Epub 2014/10/17. doi: 10.1099/mic.0.081273-0. PubMed PMID: 25320363.

37. Wu X, Zeng Q, Koestler BJ, Waters CM, Sundin GW, Hutchins W, et al. Deciphering the components that coordinately regulate virulence factors of the soft rot pathogen *Dickeya dadantii*. Mol Plant Microbe Interact. 2014;27(10):1119-31. Epub 2014/09/03. doi: 10.1094/mpmi-01-14-0026-r. PubMed PMID: 25180688.

38. Franza T, Expert D. Role of iron homeostasis in the virulence of phytopathogenic bacteria: an 'a la carte' menu. Molecular plant pathology. 2013;14(4):429-38. Epub 2012/11/23. doi: 10.1111/mpp.12007. PubMed PMID: 23171271.

39. Tans-Kersten J, Brown D, Allen C. Swimming motility, a virulence trait of *Ralstonia solanacearum,* is regulated by FlhDC and the plant host environment. Mol Plant Microbe Interact. 2004;17(6):686-95. Epub 2004/06/16. doi: 10.1094/mpmi.2004.17.6.686. PubMed PMID: 15195951.

40. Jozwick AK, Graf J, Welch TJ. The flagellar master operon *flhDC* is a pleiotropic regulator involved in motility and virulence of the fish pathogen *Yersinia ruckeri*. J Appl Microbiol. 2016. Epub 2016/12/17. doi: 10.1111/jam.13374. PubMed PMID: 27981729.

41. Xu T, Su Y, Xu Y, He Y, Wang B, Dong X, et al. Mutations of flagellar genes *fliC12*, *fliA* and *flhDC* of *Edwardsiella tarda* attenuated bacterial motility, biofilm formation and virulence to fish. J Appl Microbiol. 2014;116(2):236-44. Epub 2013/10/15. doi: 10.1111/jam.12357. PubMed PMID: 24118854.

42. Yuan X, Khokhani D, Wu X, Yang F, Biener G, Koestler BJ, et al. Cross-talk between a regulatory small RNA, cyclic-di-GMP signalling and flagellar regulator FlhDC for virulence and bacterial behaviours. Environ Microbiol. 2015;17(11):4745-63. Epub 2015/10/16. doi: 10.1111/1462-2920.13029. PubMed PMID: 26462993.

43. Chatterjee A, Cui Y, Chatterjee AK. RsmC of *Erwinia carotovora* subsp. *carotovora* negatively controls motility, extracellular protein production, and virulence by binding FlhD and modulating transcriptional activity of the master regulator, FlhDC. J Bacteriol. 2009;191(14):4582-93. Epub 2009/05/19. doi: 10.1128/jb.00154-09. PubMed PMID: 19447906; PubMed Central PMCID: PMCPMC2704716.

44. Ko M, Park C. H-NS-Dependent regulation of flagellar synthesis is mediated by a LysR family protein. J Bacteriol. 2000;182(16):4670-2. Epub 2000/07/27. PubMed PMID: 10913108; PubMed Central PMCID: PMCPMC94646.

45. Yi X, Yamazaki A, Biddle E, Zeng Q, Yang CH. Genetic analysis of two phosphodiesterases reveals cyclic diguanylate regulation of virulence factors in *Dickeya dadantii*. Mol Microbiol. 2010;77(3):787-800. Epub 2010/06/30. doi: 10.1111/j.1365-2958.2010.07246.x. PubMed PMID: 20584146.

46. Van Gijsegem F, Wlodarczyk A, Cornu A, Reverchon S, Hugouvieux-Cotte-Pattat N. Analysis of the LacI family regulators of *Erwinia chrysanthemi* 3937, involvement in the bacterial phytopathogenicity. Mol Plant Microbe Interact. 2008;21(11):1471-81. PubMed PMID: 18842096.

47. Wolfe AJ. The acetate switch. Microbiol Mol Biol Rev. 2005;69(1):12-50. Epub 2005/03/10. doi: 10.1128/mmbr.69.1.12-50.2005. PubMed PMID: 15755952; PubMed Central PMCID: PMCPMC1082793.

48. Antunez-Lamas M, Cabrera-Ordonez E, Lopez-Solanilla E, Raposo R, Trelles-Salazar O, Rodriguez-Moreno A, et al. Role of motility and chemotaxis in the pathogenesis of *Dickeya dadantii* 3937 (ex *Erwinia chrysanthemi* 3937). Microbiology. 2009;155(Pt 2):434-42. Epub 2009/02/10. doi: 10.1099/mic.0.022244-0. PubMed PMID: 19202091.

49. Rio-Alvarez I, Munoz-Gomez C, Navas-Vasquez M, Martinez-Garcia PM, Antunez-Lamas M, Rodriguez-Palenzuela P, et al. Role of *Dickeya dadantii* 3937 chemoreceptors in the entry to Arabidopsis leaves through wounds. Molecular plant pathology. 2015;16(7):685-98. Epub 2014/12/10. doi: 10.1111/mpp.12227. PubMed PMID: 25487519.

50. Jahn CE, Willis DK, Charkowski AO. The flagellar sigma factor fliA is required for *Dickeya dadantii* virulence. Mol Plant Microbe Interact. 2008;21(11):1431-42. Epub 2008/10/10. doi: 10.1094/mpmi-21-11-1431. PubMed PMID: 18842093.

51. Taguchi F, Yamamoto M, Ohnishi-Kameyama M, Iwaki M, Yoshida M, Ishii T, et al. Defects in flagellin glycosylation affect the virulence of *Pseudomonas syringae* pv. *tabaci* 6605. Microbiology. 2010;156(Pt 1):72-80. Epub 2009/10/10. doi: 10.1099/mic.0.030700-0. PubMed PMID: 19815579.

52. Khodai-Kalaki M, Andrade A, Fathy Mohamed Y, Valvano MA. *Burkholderia cenocepacia* Lipopolysaccharide Modification and Flagellin Glycosylation Affect Virulence but Not Innate Immune Recognition in Plants. MBio. 2015;6(3):e00679. Epub 2015/06/06. doi: 10.1128/mBio.00679-15. PubMed PMID: 26045541; PubMed Central PMCID: PMCPMC4462625.

53. Thangavelu B, Bhansali P, Viola RE. Elaboration of a fragment library hit produces potent and selective aspartate semialdehyde dehydrogenase inhibitors. Bioorg Med Chem. 2015;23(20):6622-31. Epub 2015/09/26. doi: 10.1016/j.bmc.2015.09.017. PubMed PMID: 26404410; PubMed Central PMCID: PMCPMC4601562.

54. Résibois A, Colet M, Faelen M, Schoonejans E, Toussaint A. phiEC2, a new generalized transducing phage of *Erwinia chrysanthemi*. Virology. 1984;137(1):102-12. PubMed PMID: 18639822.

55. Shevchik VE, Condemine G, Robert-Baudouy J. Characterization of DsbC, a periplasmic protein of *Erwinia chrysanthemi* and *Escherichia coli* with disulfide isomerase activity. Embo J. 1994;13(8):2007-12. PubMed PMID: 8168497.

56. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnetjournal. 2011;17:10-2.

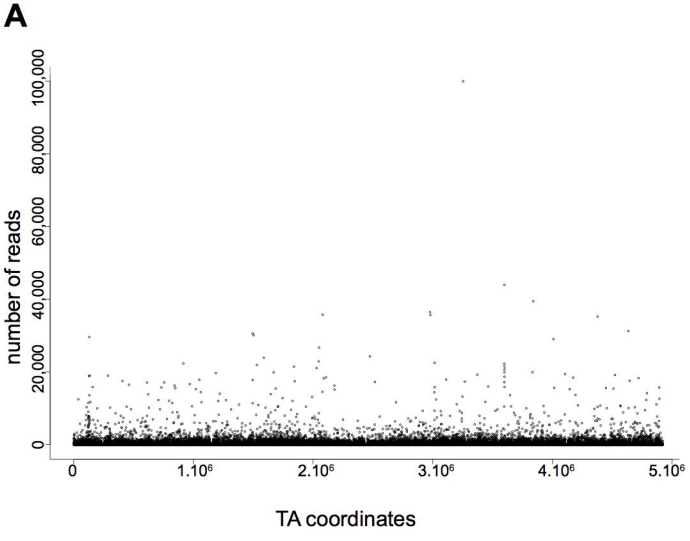
57. Zomer A, Burghout P, Bootsma HJ, Hermans PW, van Hijum SA. ESSENTIALS: software for rapid analysis of high throughput transposon insertion sequencing data. PLoS One. 2012;7(8):e43012. Epub 2012/08/18. doi: 10.1371/journal.pone.0043012. PubMed PMID: 22900082; PubMed Central PMCID: PMCPMC3416827.

58. Bardonnet N, Blanco C. Improved vectors for transcriptional signal screening in corynebacteria. FEMS Microbiol Lett. 1991;68(1):97-102. PubMed PMID: 1722768.

59. Roeder DL, Collmer A. Marker-exchange mutagenesis of a pectate lyase isozyme gene in *Erwinia chrysanthemi*. J Bacteriol. 1985;164(1):51-6. PubMed PMID: 2995324.

60. Pandin C, Caroff M, Condemine G. Antimicrobial Peptide Resistance Genes in the Plant Pathogen *Dickeya dadantii.* Appl Environ Microbiol. 2016;82(21):6423-30. Epub 2016/08/28. doi: 10.1128/aem.01757-16. PubMed PMID: 27565623; PubMed Central PMCID: PMCPMC5066359.

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

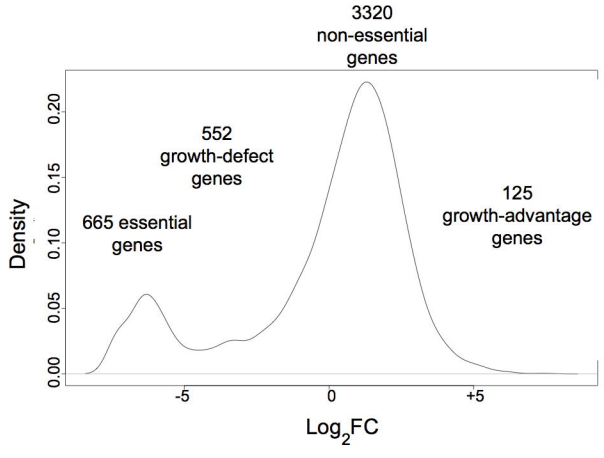

B
3320
non-essential
genes
552
growth-defect
genes
665 essential
genes
125
growth-advantage
genes
Density
0.00
0.05
0.10
0.15
0.20
-5
0
+5
Log2FC

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

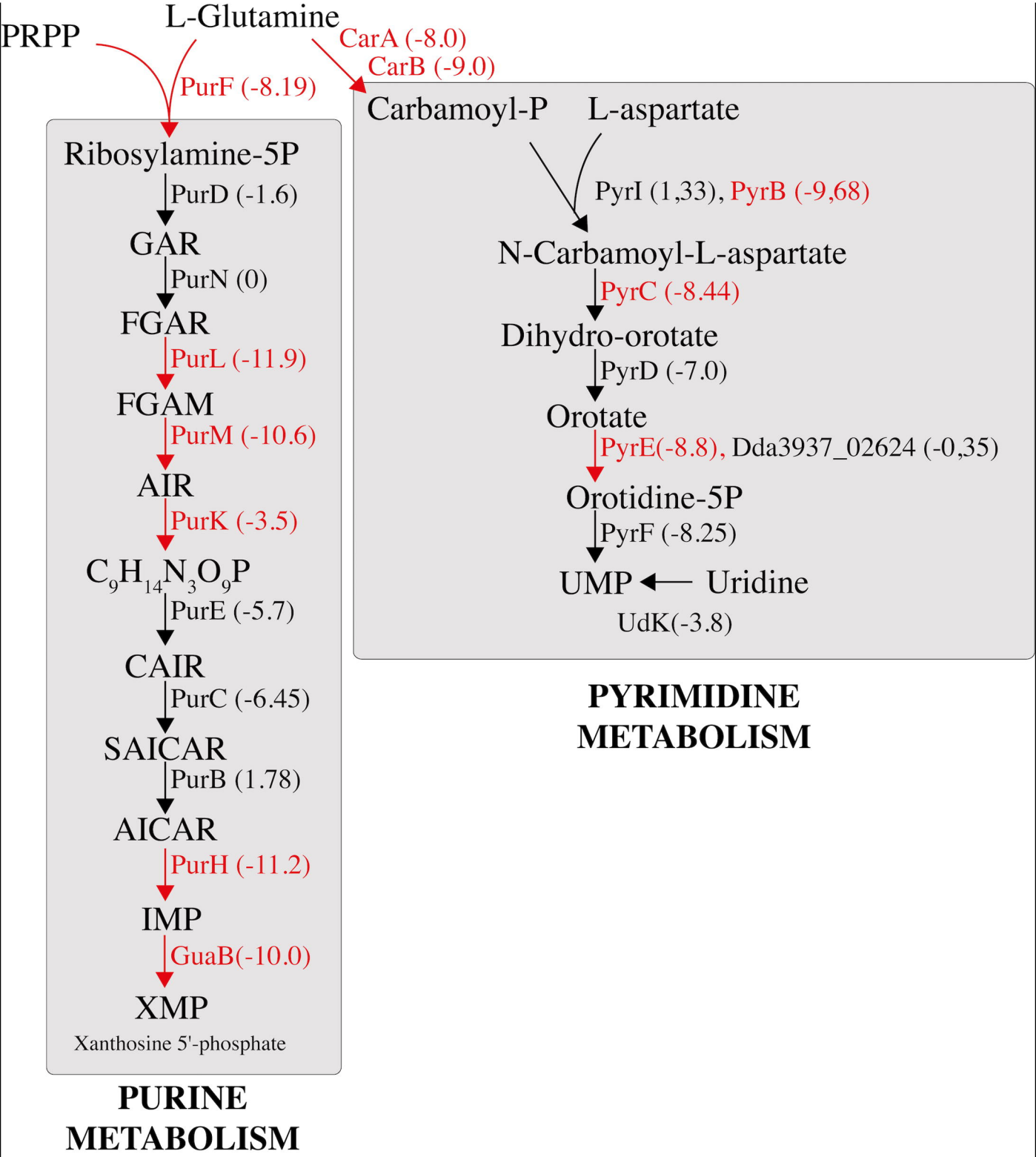

PRPP
L-Glutamine
PurF (-8.19)
CarA (-8.0)
CarB (-9.0)
Ribosylamine-5P
PurD (-1.6)
GAR
PurN (0)
FGAR
PurL (-11.9)
FGAM
PurM (-10.6)
AIR
PurK (-3.5)
$C_9H_{14}N_3O_9P$
PurE (-5.7)
CAIR
PurC (-6.45)
SAICAR
PurB (1.78)
AICAR
PurH (-11.2)
IMP
GuaB(-10.0)
XMP
Xanthosine 5'-phosphate
PURINE METABOLISM
Carbamoyl-P
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

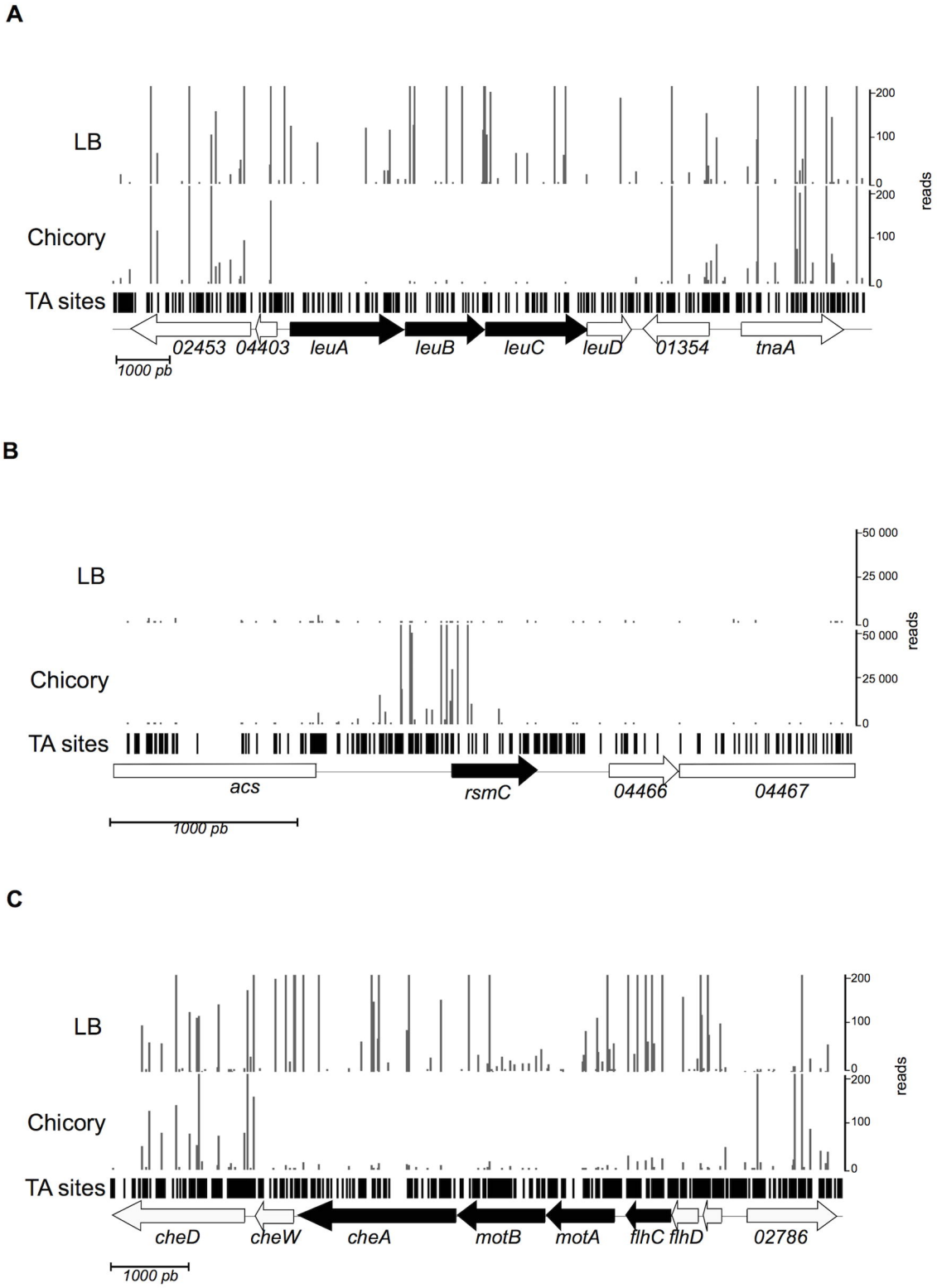
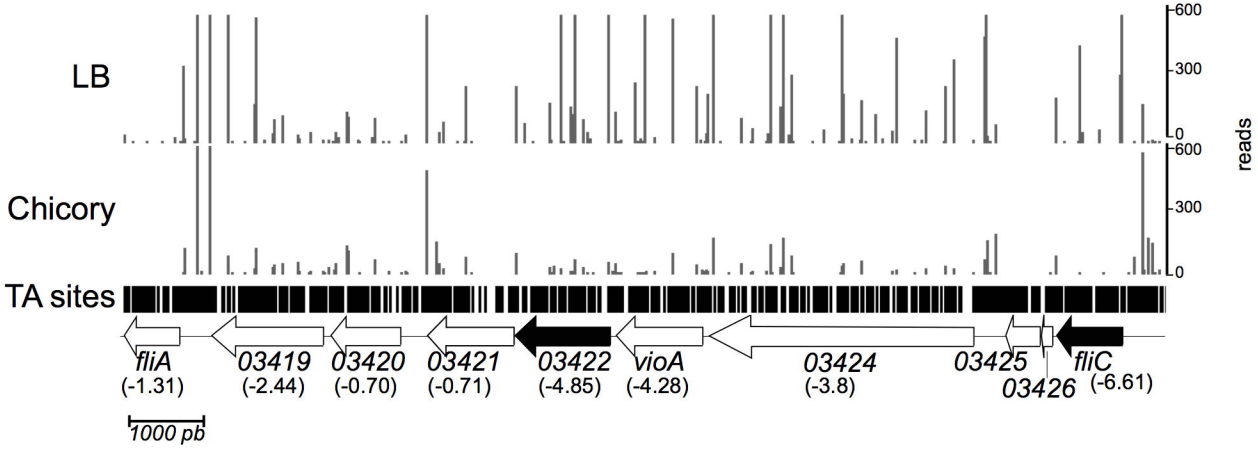

A
LB
Chicory
TA sites
reads
200
100
0
02453
04403
leuA
leuB
leuC
leuD
01354
tnaA
1000 pb
B
LB
Chicory
TA sites
reads
50 000
25 000
0
acs
rsmC
04466
04467
1000 pb
C
LB
Chicory
TA sites
reads
200
100
0
cheD
cheW
cheA
motB
motA
flhC
flhD
02786
1000 pb

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

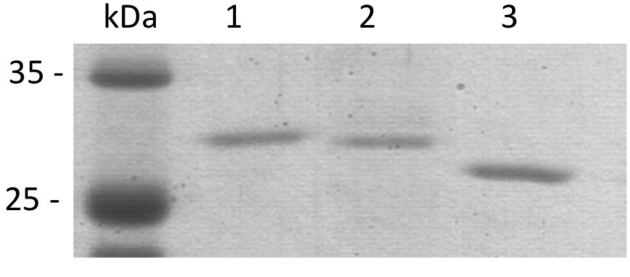
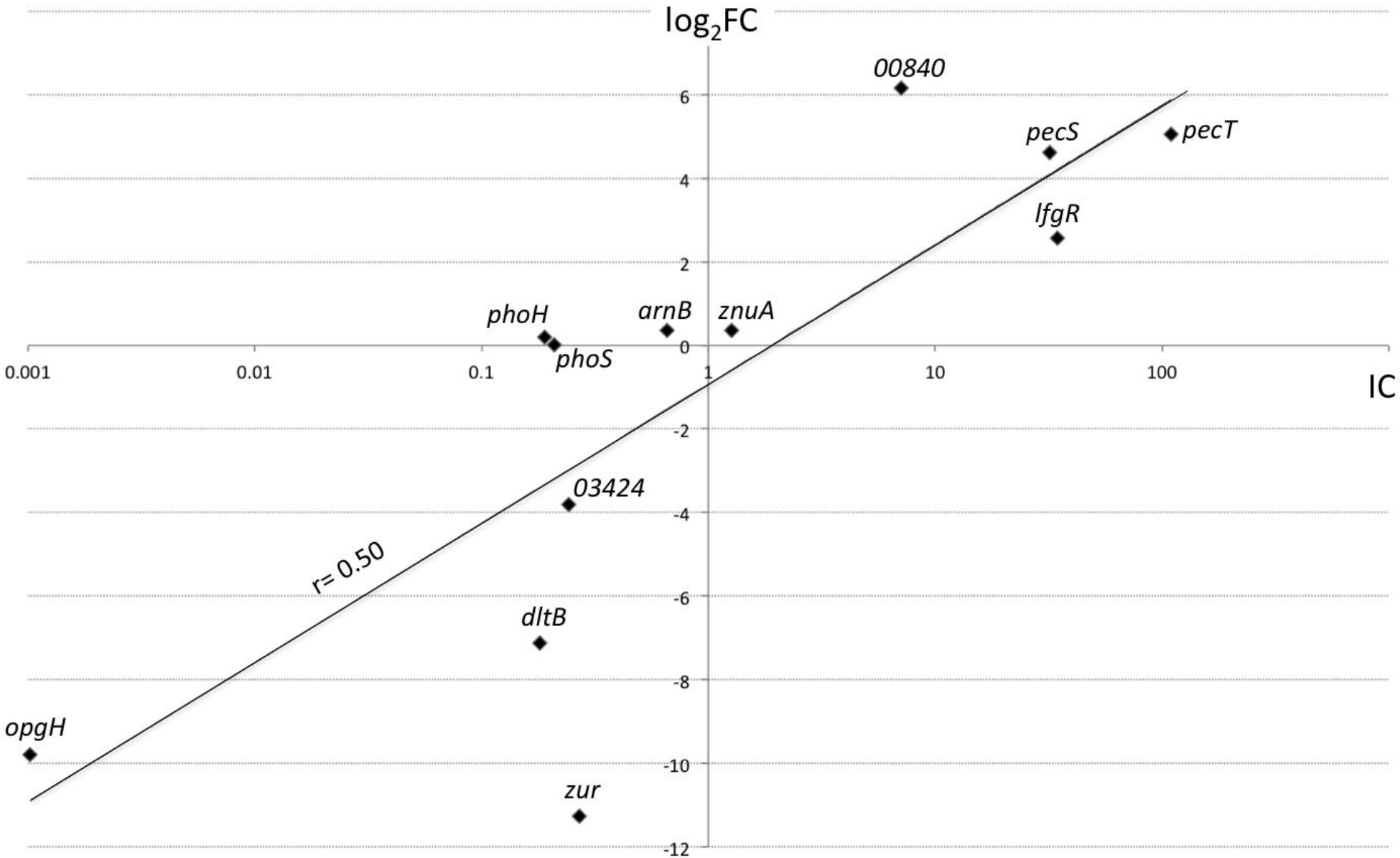

kDa
1
2
3
35 -
25 -

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