Evolution of the metabolic and regulatory networks associated with oxygen availability in two phytopathogenic enterobacteria

Lavanya Babujee1†, Jennifer Apodaca1†, Venkatesh Balakrishnan1, Paul Liss1, Patricia J Kiley2, Amy O Charkowski3, Jeremy D Glasner1 and Nicole T Perna1,4*

Abstract

Background: Dickeya dadantii and Pectobacterium atrosepticum are phytopathogenic enterobacteria capable of facultative anaerobic growth in a wide range of O2 concentrations found in plant and natural environments. The transcriptional response to O2 remains under-explored for these and other phytopathogenic enterobacteria although it has been well characterized for animal-associated genera including Escherichia coli and Salmonella enterica. Knowledge of the extent of conservation of the transcriptional response across orthologous genes in more distantly related species is useful to identify rates and patterns of regulon evolution. Evolutionary events such as loss and acquisition of genes by lateral transfer events along each evolutionary branch results in lineage-specific genes, some of which may have been subsequently incorporated into the O2-responsive stimulon. Here we present a comparison of transcriptional profiles measured using densely tiled oligonucleotide arrays for two phytopathogens, Dickeya dadantii 3937 and Pectobacterium atrosepticum SCRI1043, grown to mid-log phase in MOPS minimal medium (0.1% glucose) with and without O2.

Results: More than 7% of the genes of each phytopathogen are differentially expressed with greater than 3-fold changes under anaerobic conditions. In addition to anaerobic metabolism genes, the O2 responsive stimulon includes a variety of virulence and pathogenicity-genes. Few of these genes overlap with orthologous genes in the anaerobic stimulon of E. coli. We define these as the conserved core, in which the transcriptional pattern as well as genetic architecture are well preserved. This conserved core includes previously described anaerobic metabolic pathways such as fermentation. Other components of the anaerobic stimulon show variation in genetic content, genome architecture and regulation. Notably formate metabolism, nitrate/nitrite metabolism, and fermentative butanediol production, differ between E. coli and the phytopathogens. Surprisingly, the overlap of the anaerobic stimulon between the phytopathogens is also relatively small considering that they are closely related, occupy similar niches and employ similar strategies to cause disease. There are cases of interesting divergences in the pattern of transcription of genes between Dickeya and Pectobacterium for virulence-associated subsystems including the type VI secretion system (T6SS), suggesting that fine-tuning of the stimulon impacts interaction with plants or competing microbes.

Conclusions: The small number of genes (an even smaller number if we consider operons) comprising the conserved core transcriptional response to O2 limitation demonstrates the extent of regulatory divergence prevalent in the Enterobacteriaceae. Our orthology-driven comparative transcriptomics approach indicates that the adaptive response in the enterobacteria is a result of interaction of core (regulators) and lineage-specific (structural and regulatory) genes. Our subsystems based approach reveals that similar phenotypic outcomes are sometimes achieved by each organism using different genes and regulatory strategies.
Background

Dickeya dadantii and Pectobacterium atrosepticum cause soft-rot diseases characterized by maceration of plant tissues through the action of multiple secreted plant cell wall degrading enzymes [1]. D. dadantii strain 3937 (D. dadantii) was originally isolated from African violet and is better known by its former name Erwinia chrysanthemi 3937, and P. atrosepticum strain SCRI1043 was isolated from potato [2,3], but individual strains and these genera as a whole have broad host range, affecting over 50% of angiosperm plant orders [4]. They are a world-wide problem for economically important crops and ornamental plants [5]. Both D. dadantii and P. atrosepticum are relatively well-studied model organisms for understanding the molecular biology of soft-rot pathogenesis [6,7]. Like most enterobacteria, Dickeya and Pectobacterium are facultative anaerobes that are able to grow with or without O2 by shifting metabolic strategies from aerobic respiration to anaerobic respiration or fermentation [8]. They experience a wide range of O2 concentrations in different plant tissues and natural reservoirs like soil and water [9]. Lack of O2 is thought to be one of the factors that can trigger rapid expansion of latent infections leading to devastating post-harvest destruction of entire crops in storage [5].

Apart from a small number of important virulence factors, such as pectinases PelA, D and E [10], little is known about which genes are regulated by O2 availability in these two soft-rot pathogens. In contrast, O2-regulated genes have been extensively studied in the model animal-associated enterobacteria Escherichia coli and Salmonella enterica, where available data includes genome-scale expression profiling of the anaerobic stimulon of wild-type strains as well as mutants of key regulators FNR, ArcA, NarPQ and NarXL [11-16]. Most of these regulators, and many of the known target genes associated with anaerobic metabolism are conserved across the enterobacteria and among more distantly related gamma-proteobacteria [17]. Thus, we expect a conserved core transcriptional response to O2 limitation that includes the basic cellular machinery required to generate energy in an anaerobic environment. Yet, some of the O2-regulated genes found in E. coli are simply not present in other genera, and other genes that may be O2-responsive in the phytopathogens are not shared with animal-associated organisms. A more complete picture of the anaerobic stimulon of plant-pathogenic enterobacteria requires direct experimentation in these organisms.

Here, we characterize the transcript profiles of P. atrosepticum and D. dadantii grown with and without O2 under controlled laboratory conditions. We performed our experiments in defined media to illuminate solely the O2-responsive regulatory network. These conditions are not expected to mirror the complex, dynamic, and largely undefined environment of a plant host. Rather, we seek to identify components of the anaerobic stimulon for follow-up experimentation, provide a framework for identification of characteristics of the response to O2 in more complex datasets, and investigate the conservation and divergence in O2-mediated regulation among the enterobacteria.

Results and discussion

A large number of genes are in the O2-response stimulon

Using a conditional false-discovery rate (cFDR) of 0.01 (permissive criterion for differential expression), EBarrays [18] detects over 2204 differentially expressed genes in D. dadantii (48.5%), and 599 in P. atrosepticum (13.4%). Particularly in D. dadantii where the extremely good agreement between replicates improves the sensitivity, many of these are genes that show small changes between the aerobic and anaerobic conditions. Requiring the changes (anaerobic/aerobic) be at least 3-fold reduces the numbers to 443 differentially expressed genes in D. dadantii (9.8%) and 320 genes in P. atrosepticum (7.3%). Thus, a substantial fraction of each genome is involved in the anaerobic stimulon even using our stringent criteria (cFDR = 0.01 and fold change > 3), consistent with published reports for E. coli K-12 [12] despite differences in array platforms and analysis methods. The most extreme and conserved transcriptional responses are associated with anaerobic metabolism indicating that these organisms are responding to O2 availability.

Differences in the genetic architecture of the O2-responsive stimulon are illuminated by a biological subsystems approach

Pectobacterium and Dickeya (with Brenneria) form a monophyletic clade of phytopathogens distinct from other genera of enterobacteria, like Escherichia and Salmonella [4], where the transcriptional response to O2 has been extensively studied. Nevertheless, all free-living enterobacteria with sequenced genomes share a substantial fraction of ancestral genes that are thought to reflect clonal or vertical descent. Gene losses, duplications, and lateral gene transfers lead to content differences among genomes. Little is known about the extent to which these types of events factor into variation in the response of different enterobacteria to O2 availability.

We used OrthoMCL [19] to cluster protein-coding genes from D. dadantii, P. atrosepticum, and E. coli, and used these ortholog groups to compare transcript profiles across organisms. Genes for some of the well-characterized components of the E. coli anaerobic energy metabolism architecture are entirely missing from one or both of the phytopathogens. Some
functional equivalents within and between organisms are carried out by genes that are not orthologous. For these reasons, we find it useful to approach the comparison from a biological subsystem-oriented perspective that accommodates the complexity of the evolutionary history and functional redundancy, and considers related gene products, such as genes associated with a single molecular complex or biological process [20]. In the following sections, we report our findings from a largely statistical perspective. We discuss the possible biological significance in later sections following a subsystem-oriented approach that groups orthologous, paralogous and even analogous genes with functionally related products.

Transcriptional response to O₂ limitation for genes orthologous in the two phytopathogens

In total, our OrthoMCL analysis clustered 3110 D. dadantii genes and 3094 P. atrosepticum genes into groups that contained at least one gene from both phytopathogens. Of these, 2889 groups are simple 1-1 cases and their expression patterns are summarized in Figure 1. Overall, far more of the 1-1 orthologs differentially expressed under aerobic and anaerobic conditions exhibit congruent rather than divergent expression patterns. This observation is not surprising since the two pathogens supposedly share a common ancestor and occupy similar ecological niches. Among genes that are detected as differentially expressed using stringent criteria (cFDR = 0.01 and fold change > 3), 247 D. dadantii genes and 196 P. atrosepticum genes have predicted orthologs in the other genome (Additional File 1). Of these, 96 show fold changes > 3 for both orthologs. Eighty-one ortholog sets show congruent changes in expression in both organisms; transcripts for 51 are “up-regulated” (transcript abundance is higher under anaerobic conditions) and 30 are “down-regulated” (transcript abundance is lower under anaerobic conditions). Together these represent a minimal conserved transcriptional response shared by both plant pathogens (Table 1).

Not surprisingly, the majority of the up-regulated genes encode essential cellular functions under anaerobic conditions. Beside these, 15 ortholog sets change expression in opposite directions in D. dadantii and P. atrosepticum (Table 2). Interestingly, 12 of the 15 ortholog sets belong to a single biological system, namely type VI secretion system.

If we include simple ortholog groups where both orthologs are detected as differentially expressed (cFDR = 0.01), but one or the other, or even both have fold changes < 3, then we identify 222 ortholog groups in total that are differentially expressed in a congruent direction, and 51 that are differentially expressed in divergent directions across the two phytopathogens (Additional File 2). This provides a more generous estimate of the conserved core and divergently expressed members of the stimulon. This more permissive congruent set includes operons associated with anaerobiosis in other organisms and closer inspection shows that in these cases, some genes do meet our stringent criteria. This observation provides evidence that our more permissive congruent set includes real members of the anaerobic stimulon. Below, where we detail the genes and biological processes that are implicated in the transcriptional response to O₂, we guide inclusion by the stringent set (96 genes), but do not limit discussion to genes meeting the stringent significance criteria.

Comparison of the expression patterns for orthologs shared by D. dadantii, P. atrosepticum and E. coli

D. dadantii and P. atrosepticum are more closely related to each other than to E. coli. Our OrthoMCL analysis identified 2231 groups that include at least one gene from each of the three organisms (totaling 2309 E. coli genes, 2283 D. dadantii genes, and 2263 P. atrosepticum genes). Of these, 2124 ortholog groups include a single gene from each organism. 1124 of the 2124 groups have a differentially expressed gene (permissive criteria) in at least one of the three organisms, and 261 groups have at least one gene that shows fold change > 3 (114 genes in P. atrosepticum, 111 in E. coli, and 153 in D. dadantii, see Additional File 3). Only 20 ortholog groups contain genes that show a congruent expression pattern with fold change greater than 3 for all three orthologs (Table 1, bold gene names), suggesting that the conserved response to O₂ is small in terms of the number of genes involved, or that the conserved response lies in orthologs with smaller magnitude changes. This set is mainly comprised of genes known to function in cellular metabolism under anaerobic conditions namely, frdABCD, dcdB, adhE, hycP, focA, hybO, yfID, nrdG, nrdD, besides others such as the collagenase encoding genes yhbUV, a peptidase coding gene pepT, and two other genes ynfK and ycbJ which are all up-regulated. The stringent congruent set also includes four down-regulated genes. These are exbB that encodes a component of the TonB-exbBD complex, yceI that encodes a cytochrome b561 and two other genes (yceF, yigI), which encode uncharacterized proteins.

Relaxing the analysis stringency to consider all 1-1-1 orthologs that are differentially expressed (no fold change threshold) in all three organisms results in only a small increase to 39 ortholog groups with congruent expression pattern (see Additional File 2, bold gene names). This suggests that the magnitude of the response is not the primary reason the conserved stimulon is so small. Even our most permissive analysis suggests that there are more genes in 1-1 ortholog groups that are differentially expressed in a subset of the organisms than congruent across all three.
Transcriptional response to O₂ limitation for genes orthologous in the two phytopathogens and not shared with E. Coli

*D. dadantii* and *P. atrosepticum* share lifestyle characteristics, including a plant-host environment, not common to *E. coli*. They are also more closely related to each other and have acquired genes by lateral transfer events along the shared branch since their divergence from *E. coli*. We examined the set of orthologs shared by the two phytopathogens, but absent from *E. coli* to examine which if any of these lineage-specific genes are O₂-responsive. A total of 780 OrthoMCL groups include genes from both phytopathogens and none from *E. coli*, and 716 of these are simple 1-1 ortholog groups. Only 22 genes without *E. coli* orthologs are differentially expressed with fold changes greater than 3 in both *D. dadantii* and *P. atrosepticum* (see Table 1). Of these 5 are up-regulated and encode butanediol dehydrogenase, lactoylglutathione lyase, a nickel transporter (different from the *E. coli nickABCDE* nickel transport system), a putative membrane protein and a hypothetical protein. All of the 17 down-regulated genes encode proteins that
Table 1 Minimal conserved anaerobic transcriptional response shared by *D. dadantii* 3937 and *P. atrosepticum* SCR1043 and its comparison to *E. coli*.

| ASAP Feature ID | Gene Name | Product | D. dadantii Fold change | P. atrosepticum Fold change | E. coli Fold change |
|-----------------|-----------|---------|-------------------------|-----------------------------|-------------------|
| **A. Orthologs down-regulated ≥3-fold in both *D. dadantii* and *P. atrosepticum*** |
| ABF-0016541     | exbB      | membrane spanning protein in TonB-ExbB-ExbD complex | -6.1                       | -3.5                        | -3.3              |
| ABF-0020799     | ndaA      | ribonucleotide reductase of class Ia (aerobic), alpha subunit | -4.9                       | -4.7                        | -2.2*             |
| ABF-0020798     | nddA      | ribonucleotide reductase of class Ia (aerobic), beta subunit | -3.4                       | -3.9                        | -2.0*             |
| ABF-0017172     | sdcA      | serine transporter | -3.1                       | -3.2                        | 1.3*              |
| ABF-0020323     | sfaA      | iron-binding periplasmic protein | -9.1                       | -26.9                       |
| ABF-0019943     | yceI      | secreted protein | -3.1                       | -5.0                        | -6.0              |
| ABF-0019942     | yceJ      | predicted cytochrome b561 | -4.7                       | -4.3                        | -8.6              |
| ABF-0020068     | ydiU      | hypothetical protein | -3.4                       | -4.2                        | -2.2*             |
| ABF-0015019     | yigl      | conserved protein | -2.9                       | -7.4                        | -3.1              |
| ABF-0015019     | ykgM      | predicted ribosomal protein | -9.0                       | -16.5                       | -1.4*             |
| ABF-0019536     | ykgO      | predicted ribosomal protein | -6.7                       | -15.3                       |
| ABF-0017084     | zuuA      | zinc ABC transporter, periplasmic-binding protein ZnuA | -4.3                       | -17.5                       | -1.2*             |
| ABF-0017082     | zuuB      | high-affinity zinc transport system membrane protein | -3.3                       | -2.9                        | 1.4*              |
| ABF-0017083     | zuuC      | high-affinity zinc transport system ATP-binding protein | -3.1                       | -2.8                        | -1.1*             |
| ABF-0018178     | sdcA      | iron dicitrato-binding protein | -10.6                      | -8.4                        |
| ABF-0018571     | sfaA      | putative iron ABC transporter permease protein | -5.9                       | -23.3                       |
| ABF-0018572     | sfaA      | putative iron ABC transporter, periplasmic-binding protein | -7.9                       | -117.8                      |
| ABF-0018573     | sfaA      | putative iron ABC transporter ATP-binding protein | -7.3                       | -104.7                      |
| ABF-0018665     | sfaA      | TonB-dependent ferric achromobactin receptor | -31.6                      | -4.9                        |
| ABF-0019222     | ydiU      | putative ABC transporter substrate-binding protein | -16.8                      | -106.2                      |
| ABF-0019223     | ydiU      | putative ABC transporter substrate-binding protein | -22.8                      | -47.8                       |
| ABF-0019568     | ydiU      | putative transport system permease protein | -10.1                      | -32.4                       |
| ABF-0019569     | ydiU      | putative ABC transporter substrate-binding protein | -23.1                      | -31.3                       |
| ABF-0019570     | ydiU      | putative ABC transporter substrate-binding protein | -16.7                      | -20.8                       |
| ABF-0019572     | ydiU      | putative ABC transporter substrate-binding protein | -17.0                      | -13.9                       |
| ABF-0020094     | ydiU      | ABC-type transporter, periplasmic component | -4.5                       | -6.3                        |
| ABF-0020095     | ydiU      | ABC transporter, permease protein | -5.9                       | -4.3                        |
| ABF-0020096     | ydiU      | ABC transporter, permease protein | -3.0                       | -4.4                        |
| ABF-0020097     | ydiU      | ABC transporter ATP-binding protein | -3.4                       | -3.2                        |
| ABF-0046525     | ydiU      | ABC transporter substrate-binding protein | -81.6                      | -37.0                       |
| **B. Orthologs up-regulated ≥3-fold in both *D. dadantii* and *P. atrosepticum*** |
| ABF-0020642     | adhE      | iron-dependent alcohol dehydrogenase | 16.8                       | 3.6                         | 3.9               |
| ABF-0018570     | ahpC      | alkyl hydroperoxide reductase, C22 subunit | 2.8                        | 6.1                         | -2.2*             |
| ABF-0019339     | budC      | 2,3-butanediol dehydrogenase | 36.8                       | 4.1                         |
| ABF-0018628     | dcuB      | C4-dicarboxylate transporter DcuB | 16.4                       | 66.3                        | 8.5               |
| ABF-0018914     | dps       | Fe-binding and storage protein | 3.2                        | 2.9                         | -2.5*             |

*Babujee et al. BMC Genomics 2012, 13:110 http://www.biomedcentral.com/1471-2164/13/110*
Table 1 Minimal conserved anaerobic transcriptional response shared by *D. dadantii* 3937 and *P. atrosepticum* SCRI1043 and its comparison to *E. coli*.

| Accession | Accession | Accession | Description                                      | D. dadantii | P. atrosepticum | E. coli |
|-----------|-----------|-----------|--------------------------------------------------|-------------|-----------------|--------|
| ABF-0019603 | ABL-0062860 | ABE-0003073 | focA formate transporter                          | 8.1         | 4.8             | 3.1    |
| ABF-0017842 | ABL-0064288 | ABE-0013604 | frdA fumarate reductase (anaerobic) NAD/flavoprotein subunit | 7.5         | 11.2            | 3.5    |
| ABF-0017841 | ABL-0064289 | ABE-0013602 | frdB fumarate reductase (anaerobic), Fe-S subunit | 8.8         | 8.6             | 5.3    |
| ABF-0017839 | ABL-0064290 | ABE-0013598 | frdC fumarate reductase (anaerobic), membrane anchor subunit | 7.9         | 9.6             | 3.7    |
| ABF-0017837 | ABL-0064291 | ABE-0013595 | frdD fumarate reductase (anaerobic), membrane anchor subunit | 7.0         | 7.8             | 4.5    |
| ABF-0019825 | ABL-0062949 | ABE-0002893 | grxA glutaredoxin 1, coenzyme for ribonucleotide reductase | 4.3         | 3.1             | 1.0*   |
| ABF-0017078 | ABL-0061498 | ABE-0003960 | hoxN high-affinity nickel transport protein       | 6.7         | 17.6            |        |
| ABF-0017349 | ABL-0061473 | ABE-0008930 | hybA predicted hydrogenase 2 cytochrome b type component | 5.4         | 18.8            | 2.6*   |
| ABF-0017353 | ABL-0061476 | ABE-0008924 | hybC2 hydrogenase 2-specific chaperone             | 5.7         | 15.3            | 2.1*   |
| ABF-0017346 | ABL-0061471 | ABE-0008922 | hybD2 hydrogenase 2, small subunit                | 3.4         | 18.1            | 14.6   |
| ABF-0015747 | ABL-0061483 | ABE-0008931 | hycD protease involved in processing C-terminal end of HycE | 9.6         | 25.8            | 1.4*   |
| ABF-0015752 | ABL-0061495 | ABE-0008919 | hydN formate dehydrogenase-H, ferredoxin subunit   | 55.3        | 183.5           | 1.7*   |
| ABF-0015735 | ABL-0061493 | ABE-0008917 | hyfA hydrogenase 4, 4Fe-4S subunit                | 32.2        | 128.0           | MO     |
| ABF-0015736 | ABL-0061492 | ABE-0008916 | hyfB hydrogenase 4, membrane subunit              | 26.7        | 163.1           | MO     |
| ABF-0015737 | ABL-0061491 | ABE-0008915 | hyfC hydrogenase 4, membrane subunit              | 10.9        | 123.6           | MO     |
| ABF-0015734 | ABL-0061490 | ABE-0008914 | hyfD hydrogenase 4, membrane subunit              | 26.2        | 173.6           | -1.7*  |
| ABF-0015733 | ABL-0061489 | ABE-0008913 | hyfE hydrogenase 4, membrane subunit              | 29.4        | 72.0            | 1.5*   |
| ABF-0015740 | ABL-0061488 | ABE-0008912 | hyfF hydrogenase 4, membrane subunit              | 15.8        | 57.3            | 2.7*   |
| ABF-0015741 | ABL-0061487 | ABE-0008911 | hyfG hydrogenase 4, membrane subunit              | 26.7        | 75.1            | MO     |
| ABF-0015742 | ABL-0061486 | ABE-0008910 | hyfH hydrogenase 4, Fe-S subunit                  | 17.0        | 64.9            | 1.9*   |
| ABF-0015744 | ABL-0061485 | ABE-0008909 | hyfI hydrogenase 4, Fe-S subunit                  | 17.5        | 62.2            | MO     |
| ABF-0015745 | ABL-0061484 | ABE-0008908 | hyfJ predicted processing element hydrogenase 4   | 31.3        | 46.9            | MO     |
| ABF-0017358 | ABL-0061480 | ABE-0008907 | hyfK GTP hydrolase involved in nickel liganding into hydrogenases | 5.8         | 41.4            | 2.3*   |
| ABF-0020729 | ABL-0061479 | ABE-0008906 | hyfL predicted processing element hydrogenase 4   | 21.1        | 18.4            | 3.8    |
| ABF-0017360 | ABL-0061478 | ABE-0008905 | hyfM [NiFe] hydrogenase metallocenter assembly protein HybG | 3.6         | 31.6            | 2.4*   |
| ABF-0047122 | ABL-0062652 | ABE-0006058 | manZ mannose-specific enzyme IID component of PTS  | 3.9         | 4.1             | -2.1*  |
| ABF-0016556 | ABL-0060593 | ABE-0013865 | nrdD anaerobic ribonucleoside-triphosphate reductase | 17.8        | 24.4            | 4.4    |
| ABF-0016554 | ABL-0060592 | ABE-0013860 | nrdG anaerobic ribonucleotide reductase activating protein | 4.9         | 6.2             | 4.5    |
| ABF-0017768 | ABL-0062712 | ABE-0003800 | pepT peptidase T                                  | 31.3        | 19.7            | 3.6    |
| ABF-0019604 | ABL-0062861 | ABE-0003227 | pfkB pyruvate formate lyase I                     | 103.2       | 3.4             | MO     |
| ABF-00174126 | ABL-0064936 | ABE-0003226 | rnf ribosome modulation factor                    | 8.3         | 3.2             | 1.9*   |
| ABF-0015967 | ABL-0063825 | ABE-0008501 | tpxC thioredoxin 2                                | 7.0         | 21.3            | -3.8   |
| ABF-0016966 | ABL-0061596 | ABE-0002386 | ybfA predicted protein                            | 6.7         | 3.9             | 2.2*   |
| ABF-0019390 | ABL-0062816 | ABE-0003125 | ychI conserved protein                            | 6.1         | 4.9             | 3.3    |
| ABF-0018000 | ABL-0062073 | ABE-0003740 | ycfP conserved protein                            | 3.1         | 3.2             | 1.7*   |
| ABF-0020593 | ABL-0063322 | ABE-0007565 | ytbS predicted transporter                        | 3.5         | 4.6             | 1.0*   |
| ABF-0020590 | ABL-0063324 | ABE-0007571 | ytbU conserved protein                            | 3.2         | 7.3             | 1.4*   |
| ABF-0020347 | ABL-0063577 | ABE-0008489 | yfD pyruvate formate lyase subunit                | 18.8        | 3.1             | 5.9    |

Babujee et al. BMC Genomics 2012, 13:110
http://www.biomedcentral.com/1471-2164/13/110
Table 1 Minimal conserved anaerobic transcriptional response shared by *D. dadantii* 3937 and *P. atrosepticum* SCR1043 and its comparison to *E. coli*.

(Continued)

| OrthoMCL ID1 | OrthoMCL ID2 | OrthoMCL ID3 | Gene Symbol | Product Description | Fold Change *D. dadantii* | Fold Change *P. atrosepticum* | Fold Change *E. coli* |
|--------------|--------------|--------------|-------------|----------------------|--------------------------|----------------------------|----------------------|
| ABF-0018102  | ABL-0060954  | ABE-0010380  | yhbU        | predicted peptidase (collagenase-like) | 8.5                      | 25.5                       | 5.9                  |
| ABF-0018103  | ABL-0060953  | ABE-0010382  | yhbV        | predicted protease     | 9.9                      | 18.0                       | 3.7                  |
| ABF-0015647  | ABL-0060483  | ABE-0010667  | yhdH        | predicted oxidoreductase, Zn-dependent and NAD(P)-binding | 4.9                      | 3.0                        | 1.8*                 |
| ABF-0020757  | ABL-0062521  | ABE-0005319  | ynlK        | predicted dethiobiotin synthetase | 9.6                      | 16.3                       | 5.3                  |
| ABF-0017163  | ABL-0063441  |              |             | putative membrane protein | 7.8                      | 8.8                        |                     |
| ABF-0018208  | ABL-0060635  |              |             | hypothetical protein   | 14.1                     | 30.7                       |                     |
| ABF-0018787  | ABL-0063809  |              |             | lactoylglutathione lyase-like lyase | 53.8                     | 3.8                        |                     |
| ABF-0019032  | ABL-0061661  | ABE-0004921  |             | formate dehydrogenase, cytochrome B56 subunit | 24.3                     | 27.1                       | 1.5*                 |

This list of orthologous phytopathogen genes which have a 1-1 relationship according to OrthoMCL are differentially expressed with a 3- fold or more change in gene expression for both orthologs. Where there is a single *E. coli* ortholog in the group, the fold change is included, and, statistically insignificant values are marked with an asterisk. Several groups have multiple orthologs in *E. coli*, (indicated by MO) and their expression values are not included. Orthologs showing congruent expression pattern with fold changes > 3 across all three organisms have gene names in bold. The gene names and products were selected from among the three organisms to favor the most correct or most potentially informative and edited slightly to unify the format.
constitute transport systems, and many of them likely transport iron. These are discussed in greater detail in later sections.

Transcriptional response of genes shared with E. Coli and only one of the phytopathogens

Forty P. atrosepticum genes shared with E. coli but not with D. dadantii are O2 responsive in P. atrosepticum in our experiments, and for 26 of them transcript levels change more than 3-fold between the conditions. Similarly, 70 D. dadantii genes that have orthologs in E. coli but not in P. atrosepticum are differentially expressed in D. dadantii, of which 18 show fold changes > 3 (see Additional File 4).

Each phytopathogen shows a distinct response to O2 limitation - differential expression of genes without orthologs in the other organism

D. dadantii and P. atrosepticum each encode a substantial number of genes that are not predicted to have orthologs in the other phytopathogen or in E. coli. There are 1267 D. dadantii protein-coding genes in the ASAP database that were not found in OrthoMCL groups. Of these, 501 are differentially expressed and 142 show fold changes greater than 3. These 142 genes include several which were previously implicated in virulence or growth and survival in plant hosts (Additional File 5), and other recognizable biological processes, but 57 genes encode proteins of unknown function underscoring our incomplete understanding of the response to O2 limitation.

In P. atrosepticum, there are 1130 ungrouped protein-coding genes, of which 144 are differentially expressed and 73 show fold changes greater than 3. The 73 genes include the coronofacic acid synthesis genes all of which are > 3-fold up-regulated, three genes that encode putative oxidoreductases, at least ten genes that encode putative exported proteins, and 13 genes of unknown function (Additional File 6).

We attribute the larger number of unique O2-regulated genes from D. dadantii to the smaller variance among replicates. But even using the numbers from P. atrosepticum, the number of genes (144 and 73) in the organism-specific transcriptional response is comparable to the number of genes (81) in the conserved transcriptional response, or greatly exceeds it, if we limit the core to the 20 differentially expressed genes with fold changes greater than 3 that are shared across all three organisms.

The anaerobic stimulon

The enterobacteria have considerable flexibility, both regulatory and enzymatically, in adapting their metabolism to changing environments. Orthologous genes that demonstrate a response to O2 limitation, many of which have 3-fold or more differences in gene expression, are presented according to biological subsystems. The anaerobic growth conditions used in our experiments have limited amounts of alternative electron acceptors (i.e.-nitrate) and are expected to favor fermentation as opposed to anaerobic respiration. Data in the following sections detail the similarities and differences in gene expression patterns of

---

**Table 2 Comparison of divergent and differentially expressed genes in the phytopathogens to E. coli.**

| ASAP Feature ID | D. dadantii | P. atrosepticum | E. coli | D. dadantii | P. atrosepticum | E. coli |
|-----------------|-------------|-----------------|--------|-------------|-----------------|--------|
| ABF-0014955     | ABL-0064548 | ABE-0003424     | putA   | 5.3         | -3.7            | -1*    |
| ABF-0015852     | ABL-0063738 | ABE-0002201     | putative lipoprotein | -3.4 | 6.3 | 1.2* |
| ABF-0015853     | ABL-0063739 | ABE-0003424     | putative membrane protein | -5.1 | 6.5  |
| ABF-0015854     | ABL-0063740 | ABE-0003424     | icmF-related protein | -4.9 | 7  |
| ABF-0015858     | ABL-0063744 | ABE-0003424     | putative chaperone | -4.3 | 10  |
| ABF-0015859     | ABL-0063745 | ABE-0003424     | putative membrane protein | -5.8 | 8.8  |
| ABF-0015860     | ABL-0063746 | ABE-0003424     | hypothetical protein | -3.6 | 9.9  |
| ABF-0015861     | ABL-0063747 | ABE-0003424     | putative lipoprotein | -4.4 | 8.6  |
| ABF-0015862     | ABL-0063748 | ABE-0003424     | hypothetical protein | -4.5 | 9.4  |
| ABF-0015864     | ABL-0063749 | ABE-0003424     | hypothetical protein | -4.4 | 6.8  |
| ABF-0015865     | ABL-0063750 | ABE-0003424     | hypothetical protein | -5.6 | 6.8  |
| ABF-0015866     | ABL-0063751 | ABE-0003424     | hypothetical protein | -4.6 | 9.1  |
| ABF-0015868     | ABL-0063752 | ABE-0003424     | hypothetical protein | -3.8 | 6.6  |
| ABF-0018340     | ABL-0061543 | ABE-0002155     | lpa     | 3 | -3.3 | -1.4* |
| ABF-0018771     | ABL-0062484 | ABE-0002155     | cybC    | 3.3 | -17.6 |

This list of orthologous phytopathogen genes have a 1-1 relationship according to OrthoMCL and are differentially expressed with a 3 fold or more change in gene expression for both orthologs, but in opposite directions. Where there is a single E. coli ortholog in the group, the fold change is included, and, statistically insignificant values are marked with an asterisk. The gene names and products were selected from among the three organisms to favor the most correct or most potentially informative and edited slightly to unify the format.
metabolic subsystems conserved across all three organisms (Figure 2) as well as those within and between phytopathogen species (Additional File 7). Additionally, in examining the genomes of these species, we have found many genes associated with the anaerobic stimulon have also undergone considerable changes in genomic architecture. These changes may have an influence on transcriptional differences across each species. These data also demonstrate how the anaerobic stimulon has evolved to include interactions not specifically associated with anaerobic metabolism.

Global transcriptional regulators associated with anaerobiosis

In *E. coli*, the key O2 responsive transcriptional regulators include FNR, ArcAB, NarXL, and NarPQ. FNR is an iron-sulfur cluster-containing protein that dimerizes in the absence of O2 and can act as either a transcriptional activator or repressor [21]. The amino acid sequence of FNR from the two phytopathogens is 97.6% identical to the *E. coli* protein with no differences in important functional domains. Surprisingly, *fur* is more down regulated in *D. dadantii* under anaerobic conditions than either

![Figure 2 Metabolic overview of conserved pathways in *E. coli*, *P. atrosepticum* and *D. dadantii*. Changes in gene expression under anaerobic conditions are shown for all three organisms and are represented by different colors. Fold change patterns and genome order are as follows: *E. coli*, *P. atrosepticum* and *D. dadantii* (see key within the figure). Each orthologous group of genes is represented by three blocks colored by fold change (dark blue: down-regulated, fold change > 3; light blue: down-regulated, fold change < 3; bright yellow: up-regulated, fold change > 3; dirty yellow: up-regulated, fold change < 3; black: no change in expression, X: ortholog absent in that organism). Fold change values for polycistronic operons are averaged across genes. The transcriptional regulators FNR, ArcA, NarP, NarL and FhIA, for which there are known targets, are denoted in the figure based on their mode of regulation (△) up regulated (▲) down-regulated. Several components in this figure, such as fermentation and respiratory chains are adapted from Unden and Dunnwald and Sawers et al. 2004 [34,46]. A more detailed diagram of the genomic structure for formate hydrogen lyase complex and accompanying hydrogenases (HYD 1-4) is shown in Figure 3.](image-url)
P. atrosepticum or E. coli. If this decreased expression is mediated by FNR as it is in E. coli, it suggests that FNR represses its own synthesis to a much greater extent in D. dadantii than in E. coli.

ArcB is the sensor kinase of the two-component regulatory system, ArcAB, which detects signals emanating from the aerobic respiratory chain, such as the oxidation state of ubiquinones [22]. Under anaerobic conditions ArcB phosphorylates ArcA, activating its site-specific DNA binding activity, which primarily represses genes required for aerobic metabolism [23]. D. dadantii arcB contains a nonsense mutation at codon 383, a change that is predicted to interfere with the multi-step phosphorylation transfer to ArcA [24]. It is possible that ArcA is partnered with a different sensor kinase in D. dadantii, or that this organism does not have a functional ArcA-mediated regulatory system. Curiously, arcA is up-regulated in D. dadantii, while arcA and arcB transcript levels are unaffected in P. atrosepticum and E. coli.

NarPQ and NarXL are paralogous two-component regulatory systems associated with nitrate/nitrite regulation under anaerobic conditions in E. coli. P. atrosepticum encodes NarXL and NarPQ of which the latter is missing in D. dadantii. The sensor kinase NarX responds to higher concentrations of nitrate than its paralog NarQ, which also responds to nitrite and aeration [25,26]. While each sensor kinase can phosphorylate both response regulators, dephosphorylation to the inactive state for NarP is restricted to the cognate partner [27,28]. Both response regulators activate genes associated with nitrate and nitrite catabolism and repress genes involved in other anaerobic respiratory and fermentative pathways. In P. atrosepticum, narPQ is up-regulated, while its ortholog in E. coli, although not detected as differentially expressed in Kang et al., is up-regulated less than 3-fold.

TCA cycle and glycolysis

Many genes associated with the central metabolic enzymes of glycolysis and the aerobic TCA cycle, have simple 1-1-1 orthologous relationships. While the transcription patterns are largely conserved, however, most are congruent between E. coli and P. atrosepticum but different in D. dadantii (Figure 2). For example, all four genes of the TCA cycle enzyme succinate dehydrogenase (sdhCDAB) are > 3-fold down-regulated in E. coli and P. atrosepticum but in D. dadantii only sdhD is down-regulated. These differences may be related to the arcB mutation found in D. dadantii, which may prevent the anaerobic repression of ArcA regulated genes. In genes that encode isozymes, such as aconitase, all three organisms have at least one isozyme with a conserved response. The fumarase paralogs (fumA, fumB) are the only TCA cycle genes that do not share simple orthology relationships according to OrthoMCL, although the fumC isozymes do. Nevertheless, there is some level of anaerobically induced expression in one of the isozymes across all three organisms (Figure 2).

Fermentation

Conserved genes that participate in fermentation include those associated with the reduction of pyruvate to lactate (ldhA) or the non-oxidative conversion to acetyl-coenzyme-A and formate by pyruvate formate lyase (PFL, pfB), and the subsequent conversion to acetate and ethanol (pta, ackA and adhE). In E. coli, fermentative lactate dehydrogenase (ldhA) is induced under low pH [29]; however under our growth conditions and in the Kang et al. data, no significant changes in gene expression were detected in any organism. The remaining genes pfB and adhE are up-regulated in all three, however, only pta is upregulated in E. coli and D. dadantii and ackA in P. atrosepticum (see Figure 2).

There is remarkable genomic and transcriptional variation across all three organisms in the genes associated with formate metabolism. In E. coli, formate, in the absence of a terminal electron acceptor, is further dismutated to CO$_2$ and H$_2$ via the formate hydrogen lyase complex (FHL), a multienzyme complex that includes a cytoplasmic formate dehydrogenase (FDH-H, fdhF), a hydrogenase (Hyd-3, encoded by the hyc operon) and other components. In E. coli, the FHL type hydrogenase groups with genes of the hydrogenase 3 and hydrogenase 4 (hyf) systems. Differences in the structure and content of the hydrogenases in the phytopathogens are shown in Figure 3. D. dadantii encodes a similar complement of hydrogenases to P. atrosepticum, but they are divided into two distinct loci. Expression of the genes encoding hydrogenase 3 in E. coli, and the single FHL type hydrogenases in both D. dadantii and P. atrosepticum are up-regulated.

Expression of fdhF (including a paralog of fdhF [ABL-0061761]) in P. atrosepticum is up-regulated in both phytopathogens, but remains unaffected in E. coli where FHL expression is known to be dependent on formate and an acidic pH under fermentative conditions (Figure 2). FHL is regulated by the formate-dependent regulator (flhA) and sigma-54 [30,31], but are not differentially expressed in E. coli. The E. coli FhlA regulon includes many components (for example, hydrogenases 3, 4) missing in the phytopathogens, and is an obvious example of regulatory divergence where the network is not only smaller in the phytopathogen lineage, but is transcriptionally divergent (Figure 2). Additionally, OrthoMCL groups flhA with the E. coli regulator of hydrogenase 4 (hyfR), together with single genes, which are differentially expressed, in D. dadantii and P. atrosepticum.

Two additional E. coli formate dehydrogenase and hydrogenase isozymes linked to respiration are discussed in the following section. Unlike the phytopathogens,
**Figure 3** Genomic architecture of hydrogenase gene clusters from *E. coli*, *D. dadantii* and *P. atrosepticum*. Gene order and orientation of hydrogenase gene clusters from all three organisms are illustrated, including 4 clusters from *E. coli*, 2 from *D. dadantii* 3937 and 1 from *P. atrosepticum* SCR1043. Direction (forward or reverse complement indicated by (rc)) was selected to maximize collinearity with the single hydrogenase cluster from *P. atrosepticum*. Colors are indicative of OrthoMCL grouping unless otherwise indicated by footnotes such that each color marks the genes associated with *E. coli* clusters and members of orthologous groups from *D. dadantii* 3937 and *P. atrosepticum* SCR1043 labeled with the same name. White genes are singlets with no orthologs in the other two organisms. 1. hypC has an ortholog in *D. dadantii* 3937 that is located elsewhere. 2. hybF and hypB are grouped by OrthoMCL. 3. hyf (E. coli) and hypH (D. dadantii 3937 and P. atrosepticum SCR1043) are grouped by OrthoMCL, but *E. coli* hypH is not part of the cluster (singleton). 4. fhdF has an ortholog in *E. coli* that is located elsewhere. 5. ascBF and ascC have orthologs in *P. atrosepticum* SCR1043 that are located elsewhere, 6. OrthoMCL groups most members of the *E. coli* hydrogenase 3 and 4 systems.

*E. coli* formate dehydrogenases are the only proteins in *E. coli* that require selenocysteine for assembly and maturation [32-34]. The specific requirement for selenocysteine in the oxidation of formate has been shown for *E. coli* [32-34]. The specific requirement for selenocysteine is for assembly and maturation [32-34]. The specific requirement for selenocysteine is for assembly and maturation [32-34]. The specific requirement for selenocysteine is for assembly and maturation [32-34]. The specific requirement for selenocysteine is for assembly and maturation [32-34]. The specific requirement for selenocysteine is for assembly and maturation [32-34].

Both *D. dadantii* and *P. atrosepticum* have a *budAB* operon (encoding alpha-acetolactate decarboxylase and acetolactate synthase) adjacent to a divergently transcribed *budR*-like gene encoding a LysR family transcriptional regulator and *budC* (encoding 2, 3-butanediol dehydrogenase). In our experiments *budC* is up-regulated in both phytopathogens. The *bud* genes enable fermentative butanediol production, a feature that limits the channeling of pyruvate to acid producing pathways and thus counteracts the lethal effects of acidification [35]. It has recently been shown that during soft-rot infection the *bud* genes play the essential role of increasing the pH of the plant apoplast to facilitate activity of pectate lyases [36]. The pathway is not present in *Escherichia* or *Salmonella* but is present in other members of the enterobacteria such as *Serratia, Enterobacter, Erwinia, and Klebsiella* [37]. In these organisms, butanediol is involved in interactions among plant, animal and insect hosts by acting as a signaling molecule. The mechanism of insect-attraction has been described [38]. It has been shown to produce an anti-inflammatory response in endotoxin-induced lung injury in rats [39,40].

**Aerobic and anaerobic respiration**

In the absence of O₂, *E. coli* is able to reduce a variety of alternate electron acceptors, including fumarate, dimethyl sulfoxide (DMSO), trimethylamine N-oxide (TMAO), nitrate and nitrite [41], to conserve energy through anaerobic respiration using electrons from a variety of donors. The ability to respire fumarate, nitrate and nitrite anaerobically has been reported for *D. dadantii* and *P. atrosepticum* [42] and a subset of the pathways are conserved with *E. coli*, for example, fumarate reductase (frdABC) and nitrate reductase (narGHJ). Other nitrate/nitrite reductases, which have a complex evolutionary history, are discussed in more detail below. There are no phytopathogen orthologs of the *E. coli* DMSO reductase genes (*dmsABC*), the two TMAO reductases (*torCAD* and *torYZ*) and their associated regulators (*torR, torS* and *torT*).

*E. coli* has three nitrate reductases and two nitrite reductases, which are part of the NarL and NarP regulons (see Figure 2). These include two membrane-bound
proton-translocating nitrate reductases, (narGHJI and narZYWV operons), the periplasmic nitrate reductase (napFDAGHBC operon), a formate-dependent respiratory nitrite reductase (nrfABCDEFG) and the NADH-dependent nitrite reductase (nirBDC). The E. coli narGHJI operon has orthologs in both phytopathogens, but narZYWV does not. The narGHJI operon is up-regulated in both D. dadantii and P. atrosepticum, but is not differentially expressed in E. coli. However, narGHI in E. coli is known to be induced by FNR during anaerobic growth and further induced by NarL [43,44]. Although there are predicted binding sites for NarL and FNR in the regulatory region of narG in the phytopathogens, the sequence upstream of the conserved FNR binding site, 53 bp from the transcriptional start, has diverged.

Comparative analysis reveals differences in genomic content of genes involved in nitrate/nitrite metabolism (Figure 4). The locus for the periplasmic nitrate reductase (nap), including part of the ccm operon and narP, is conserved between E. coli and P. atrosepticum, but is missing in D. dadantii. In E. coli, the genes for NarQ, and the respiratory nitrite reductase Nrf, which are also missing in D. dadantii, are not grouped in the same locus as found in P. atrosepticum (Figure 4). The napABCDEFGH operon is not differentially expressed in P. atrosepticum or E. coli under the growth conditions used in our experiments. The P. atrosepticum nrf genes are > 3-fold up-regulated, while in E. coli, only nrfB shows a greater than 3-fold change. The E. coli NADH dependent nitrite reductase (nirBDC-cysG operon) is a cytoplasmic enzyme that does not produce a proton

Figure 4 Genomic architecture of genes involved with nitrate/nitrite metabolism in E. coli, D. dadantii and P. atrosepticum. Direction (forward or reverse complement indicated by (rc)) was selected to maximize collinearity. Colors are indicative of OrthoMCL grouping unless otherwise indicated by footnotes such that each color marks the genes associated with E. coli clusters and members of orthologous groups from D. dadantii 3937 and P. atrosepticum SCR1043 labeled with the same name. White genes are singletons with no orthologs in the other two organisms. 1. narPQ and narXL are paralogous components in E. coli and P. atrosepticum SCR1043 with the same name. White genes are singletons with no orthologs in the other two organisms. 2. ccmABCDEFGH, type 1 cytochrome C biogenesis system contains a duplication of ccmH in P. atrosepticum. 3. OrthoMCL cluster that includes the nirF encoded large subunit from E. coli, a single protein from D. dadantii 3937 annotated as nirB, and two paralogs from P. atrosepticum SCR1043, annotated as narB and naiB.
gradient, and is thought to be involved with detoxification of nitrite (Figure 4). We believe nirB gene was erroneously assigned in *D. dadantii*, because this gene is part of a larger cluster conserved among the two phytopathogens that include a transcriptional regulator (*nasR*), an ABC transporter (*nasF*, *nasE* and *nasD*), two subunits of an assimilatory nitrate reductase (*nasB* and *nasA*), and an uroporphyrin-III C-methyltransferase (*nirE*). We hesitate to assign orthology of the *nas* systems between the phytopathogens because the genome context is not conserved beyond the *nas* subsystem itself, but it is clear that the phytopathogen loci are more structurally conserved (order and content) than either is with the *nir* system of *E. coli* (Figure 4).

*E. coli* has two characterized aerobic terminal reductases that are conserved in the phytopathogens. These are: the cytochrome bo3 type quinol oxidase (encoded by *cyoABCD*), which has a low affinity for O2, and the high-affinity bd-type cytochrome oxidase (encoded by *cydABCD*). The *cyoABCD* operon is down-regulated in *E. coli* and *P. atrosepticum*, but shows very little change in *D. dadantii*. The *cydABCD* operon is only up-regulated in *D. dadantii*. The cytochrome oxidase loci are regulated in part by ArcAB in *E. coli*, which may explain why *D. dadantii* shows the most divergent expression patterns. Additional Cyt bd type oxidases are encoded in *E. coli* (*appCB*) and *P. atrosepticum*, which may be strain-specific, as suggested by their genomic context and OrthoMCL clustering.

*E. coli* encodes additional cytochromes with predicted roles in the electron transport chain. One of which, *ycfJ*, is > 3-fold down-regulated in all three organisms under anaerobic conditions. The others show divergent patterns of gene expression. The gene *cybBD* (cytochrome b561) groups with *yodB* in *E. coli*, and single orthologs from both phytopathogens, of which only the *D. dadantii* ortholog is differentially expressed. The *E. coli* *cybC*, a pseudogene in strain MG1655, is a soluble cytochrome b562 of unknown function [45] but has orthologs that are divergently expressed in the phytopathogens (Additional File 8).

In *E. coli* there are 15 known dehydrogenases that donate electrons to the respiratory chain [46]. Some of these have already been mentioned, but most are conserved in the phytopathogen lineage (*sdhCDAB, mgo, glpD, ndh, nuo*). Only the operon encoding NADH dehydrogenase I, *nuo*, is differentially expressed in the phytopathogens while *ndh* remains unaffected by O2.

The dehydrogenases involved in formate metabolism and reuse of dihydrogen have diverged in the phytopathogen lineage. These include the formate dehydrogenase N (Fdh-N, *fdnGHI*), which functions in the formate-nitrate respiratory chain and the structurally related formate dehydrogenase O (Fdh-O, *fdoGHI*). Fdh-O is expressed at relatively low levels independent of either O2 or nitrate availability and is thought to provide this critical activity during the transition to anaerobic growth [47]. *D. dadantii* encodes two formate dehydrogenases homologous to Fdh-O and Fdh-N, while *P. atrosepticum* has only one formate dehydrogenase. There is insufficient conservation of genome context to confidently assign orthology of these genes to either Fdn-N or Fdn-O. Phylogenetic analysis (Figure 5) of the major subunit genes of the two formate dehydrogenases showed that all three phytopathogen loci are more similar to each other than either of those found in *E. coli*, suggesting that none of them are orthologous to the *E. coli* loci. Furthermore, all the formate dehydrogenases in the phytopathogens are up-regulated even though nitrate is not present in the medium. In *E. coli*, expression of these genes is below detectable levels. An alignment of phytopathogen and *E. coli* sequences upstream of *fdnG* revealed a conserved promoter and an FNR binding site relative to the -42.5 position, however there are no NarL

---

**Figure 5** Phylogenetic analysis of the major subunit of formate dehydrogenases from select enterobacteria. Sequences of the major subunit of formate dehydrogenase from Fdh-O and Fdh-N were aligned using CLUSTALW. The tree was constructed using NJ with default parameters of MEGA 4.0.
heptamer sites relative to the -77,-100,-109, or -124 positions in the phytopathogens.

There are two systems for the oxidation of hydrogen under anaerobic conditions in *E. coli*: the Tat dependent periplasmic uptake [48] hydrogenases hydrogenase 1 and hydrogenase 2 that are involved in hydrogen oxidation coupled to quinone reduction under anaerobic conditions. Like the FHL-type hydrogenases there are differences in the structure and content of these loci between *E. coli* and the phytopathogens (Figure 3). There are no phytopathogen orthologs for any genes of the *E. coli* hydrogenase 1 operon, *hyaABCDEF*. In *P. atrosepticum*, all hydrogenase related genes are clustered in a single chromosomal locus that OrthoMCL groups with *E. coli* hydrogenase 2. In *E. coli*, several genes of the hydrogenase 1 and 2 are up-regulated with others changing in a congruent though not statistically significant way. In *D. dadantii* and *P. atrosepticum*, all genes associated with the hydrogenase 2-like system are up-regulated.

It appears that much of the transcriptional variation between the phytopathogens and *E. coli* with respect to metabolic responses to O2, is mainly the result of changes in gene content, genome rearrangements of both important regulatory and metabolic components of the anaerobic stimulon, specifically in relation to the loss of the NarP regulon and a mutation in ArcB in *D. dadantii* and also of genes involved in nitrate/nitrite metabolism. Variation in the components of the respiratory formate dehydrogenases and hydrogenases in both phytopathogens, exhibit more complex evolutionary histories with equivalent functions carried out by paralogous or analogous systems, and many of these exhibit differential responses to O2 across these organisms. The only obvious energy metabolism subsystem present in the phytopathogens that is missing from *E. coli* involves butanediol fermentation.

Sequences of the *arcB* locus from *D. dadantii* strains in our lab confirm the *arcB* nonsense mutation (data not shown), but we have yet to confirm whether this mutation is found in *D. dadantii* 3937 strains from other labs, although it is not present in any of the recently sequenced *Dickeya* species. Whether this mutation is laboratory derived or is a lineage specific event remains to be determined. Regardless, this strain has been successfully used to identify and test various pathogenicity related phenotypes in *planta* suggesting that it may not affect its ability to macerate host tissues.

**Stress responses**

Analysis of the expression pattern of genes associated with various types of stress responses reveals interesting similarities and differences among the three organisms. Overall, the patterns are suggestive of a phytopathogen-specific oxidative stress response. For example, *ahlP*, *trxC*, *sodC* and *dps* encode bona fide virulence factors in bacterial pathogens [49-53] and they counteract damage due to reactive O2 species. Counterintuitively, these genes are up-regulated in the phytopathogens under anaerobic conditions and as expected, remain unaffected in *E. coli*. It is possible that prolonged growth in an O2 limited environment simulates a situation that soft-rotting bacteria experience prior to encountering the host oxidative burst. Our data suggest the possibility that both *P. atrosepticum* and *D. dadantii* may be able to anticipate and respond to a host induced oxidative environment before its onset, leading to the speculation that in these two phytopathogens the regulatory networks that govern responses to two opposite stressors (anaerobic stress and oxidative stress) may be linked to increase chances of survival in the plant environment. Such anticipatory responses are proposed to occur in organisms living in environments that change in predictable ways [54]. The phytopathogen-lineage specific up-regulation of both the *narGHI* genes and the nitrate-dependent formate dehydrogenase genes (*fdnGHI*) involved in respiratory nitrate reduction, despite the absence of nitrate in our growth media, may also be regarded as an anticipatory response in view of the fact that nitrate is an abundant anion in plant. Similar to *E. coli*, it is possible that a complex regulatory network that includes PecS [55], OxyR [56], FNR, (represses *sodC* in *E. coli*), RpoS (induces *sodC*) as well as regulatory elements of nitrate metabolism may be involved.

Some oxidative stress responsive genes that are down-regulated in the phytopathogens, as expected in an anaerobic environment, are either lineage-specific (*ohrR and ohr*) or strain-specific (*indABC, vfmABCDE*) and are not present in *E. coli*. The expression pattern of several other genes associated with oxidative stress is similar in *E. coli* and *P. atrosepticum*, but differs in *D. dadantii* and for two of these genes, *sodA* and *tpx*, ArcA-mediated regulation has been demonstrated for the *E. coli* ortholog [57]. Orthologs of universal stress proteins and cold shock proteins, which are involved in the response to a variety of environmental stresses in enterobacteria [58-62], are up-regulated in one or both phytopathogens but remain unaffected in *E. coli* (see Figure 2)

**Metal transport systems**

A variety of transition metals including iron, manganese, nickel, zinc and copper are required by bacteria for the activity and stability of proteins, including FNR and most of the respiratory enzymes. A large number of genes are devoted to their acquisition, uptake, storage and efflux in order to maintain homeostasis. Since several of the anaerobic respiratory enzymes require a different complement of metals (e.g. nickel) than the aerobic respiratory enzymes, and because unbound iron oxidation states is
readily influenced by the O2 status of the growth media, we are not surprised to observe transcriptional changes for associated genes, and some of them are described below.

Each of the three organisms up-regulates at least one nickel uptake system (nikABCDE in E. coli, hoxN in the phytopathogens) indicating an increased requirement for this metal during anaerobiosis. In E. coli the NikABCDE system transports nickel for the NiFe hydrogenases. Genes encoding transport systems for copper and zinc largely show phytopathogen-specific responses. For example, the Cus metal efflux system (cusCFBA operon) is up-regulated in both phytopathogens and is down-regulated in E. coli whereas, the copA gene is down-regulated in both phytopathogens, but not in E. coli. The Cus system and CopA are associated with copper homeostasis under anaerobic and aerobic conditions respectively, in E. coli [63]. Similarly, zinc uptake systems (znuABC) are down-regulated only in the phytopathogens. Additionally, both phytopathogens down-regulate transcripts (> 3-fold) for an ABC transport system predicted to transport zinc (according to the D. dadantii annotations). Genes for this system are not present in E. coli. All three organisms encode a zinc uptake regulator (Zur), the gene for which is down-regulated only in D. dadantii.

All three organisms have a substantial number of genes involved in synthesis and transport of iron chelating siderophores and other iron-containing compounds. Overall, these subsystems are down-regulated in the phytopathogens, and to a lesser extent in E. coli suggesting that the phytopathogens may have a reduced demand for iron during anaerobiosis or may reduce iron levels to avoid damage in the anticipated oxidative environment of the host. Many of these genes are likely regulated by Fur in all three organisms.

Genes that belong to the same ortholog group do not necessarily synthesize the same siderophore although they might be able to transport some of them. For example, OrthoMCL clusters genes for synthesis of the D. dadantii siderophore, chrysobactin, with enterobactin synthesis genes of E. coli, but the siderophores are distinct [64]. D. dadantii does not synthesize enterobactin, although it is capable of uptake and utilization of exogenous enterobactin [65]. The OrthoMCL clusters also include single members from P. atrosepticum, but none of the genes share extended conserved genomic context across organisms, and there is no data on whether P. atrosepticum produces enterobactin, chrysobactin or another siderophore using these genes. It is also established that some bacteria can "steal" siderophores from their neighbors as seen in P. atrosepticum which is unlikely to produce achromobactin, but may be able to uptake and transport it via genes orthologous to the D. dadantii cbABCD [66]. A detailed comparison of the D. dadantii and P. atrosepticum iron homeostasis systems is found in Franzia and Expert [64].

Genes for several iron transporting ABC transport systems shared only between the phytopathogens are all largely down-regulated in both organisms (for example OrthoMCL groups 3161-3163, yfeABCD, сфABC [67]). In E. coli, under anaerobic conditions uptake of ferrous iron is expected to increase relative to oxidized ferric iron. In line with this expectation, the feoAB genes that are involved in transport of ferrous iron are up-regulated in D. dadantii. However, in the Kang et al. experiments, neither the efeBOU genes nor the feoAB genes, which encode ferrous iron transporters were up-regulated. Several genes encoding iron storage proteins are up-regulated in the phytopathogens and remain unaffected or are down-regulated in E. coli (see Additional File 7). These include the bfr gene, encoding a bacterioferritin which contributes differentially to the virulence of D. dadantii depending on the host [68] and dps which encodes a ferritin-like protein, Dps, with pleiotropic functions. D. dadantii also up-regulates transcripts for a second strain-specific Dps-like protein (ABF-0015905).

Other transporters
Most of the transporters for amino acids are down-regulated in the phytopathogens, and unaffected in E. coli. Only one gene (sulfate transporter; ybS) shows > 3-fold congruent up-regulation between the phytopathogens and a sodium-serine transporter gene (sst) is up-regulated both in E. coli and P. atrosepticum. Even though OusA has been implicated in anaerobiosis in D. dadantii [69], we did not detect changes in expression for its gene in our experiments.

Cell-wall degradation
In P. atrosepticum and D. dadantii, pathogenicity is largely due to their capacity to depolymerize plant cell wall polymers including cellulose, hemicellulose and pectic substances, as well as other components such as lignin and proteins, through the coordinate production of multiple cell wall degrading enzymes (CWDE). Because of their indispensable role in pathogenicity, expression of CWDE is strictly regulated at the transcriptional level by multiple regulators and further fine tuned by environmental factors including pH, osmolarity and O2 concentrations all of which influence the successful onset of disease symptoms [70]. Anaerobic regulation in the presence of an inducer has been demonstrated for pelA, pelD, pelE and pelL [71] in D. dadantii. Except for pelD none of these four genes has an ortholog in P. atrosepticum (see Additional File 7). As expected for cells grown in non-inducing conditions, all the reported pectate lyase genes (pelA to E, I, Z, f) are repressed in D. dadantii. This trend is not seen in P. atrosepticum where in fact, one gene, pelB, is up-regulated. The only CWDE-encoding gene that is down-regulated in P. atrosepticum is a pectin lyase, pnl, which is a member
of a complex ortholog group. It is interesting to note that a
*D. dadantii* -specific gene, *xyuA* (ABF-0019026), encoding a putative endoxylanase, characterized in a related corn pathogen [72], is > 3-fold up-regulated.

**Phytopathogen secretion systems**

Both *D. dadantii* and *P. atrosepticum* encode a diverse collection of secretion systems, several of which are known to play key roles in interaction with plant hosts. The T6SS shows one of the most dramatic divergent expression patterns observed in our experiments. The T6SS mediates secretion of proteins encoded within repetitive clusters of genes found distributed throughout the genome, often, though not reliably, annotated as *hcp* and *vgrG*. OrthoMCL clusters paralogs of each type into two groups. The *hcp* cluster includes three members from *P. atrosepticum* and two from *D. dadantii*. The *vgrG* cluster includes three members from *D. dadantii* and five from *P. atrosepticum*. The *D. dadantii* T6SS is down-regulated in the absence of O2 and the *P. atrosepticum* T6SS is up-regulated. A previous report demonstrated that the *P. atrosepticum* T6SS cluster and proteins secreted via the T6SS (four *hcp* genes and three *vgrG* genes) are induced by plant host extracts [73,74]. Mutants of two genes, believed to correspond to a structural component of the secretion apparatus (*vasK*) and a sigma-54 dependent regulator (*vasH*), showed increased virulence relative to wild-type, a phenotype attributed to increased growth (higher density) and associated virulence relative to wild-type, a phenotype attributed to sigma-54 dependent regulator ([75].

The T6SS in each organism.

Under the anaerobic conditions used in our experiments, genes for type I secreted proteases (PrtA, PrtB, PrtC, PrtD) and for their accessory proteins (PrtE and PrtF) show a similar trend as the CWDE; they are down regulated in *D. dadantii*, and remain unaffected in *P. atrosepticum*. The Type II secretion system (T2SS) is responsible for secretion of CWDE as well as several other targets [75]. In addition, it has been linked to iron homeostasis in *D. dadantii* with interactions between inner membrane components of the T2SS and the machinery for achromobactin synthesis [76]. *E. coli* has an orthologous secretion system that is not expressed in wild-type *E. coli* strains but is functional in *hns* mutant strains [77], and the corresponding genes are unaffected in *E. coli*.

Genes associated with the T2SS are nearly all down-regulated in *D. dadantii* and unaffected in *P. atrosepticum*, where absolute expression levels remain high regardless of O2 availability. *D. dadantii* also encodes a second locus similar to genes of a T2SS, which is associated with targeting proteins to the outer membrane [78]. Several genes from this system in *D. dadantii* are also down-regulated. Both phytopathogens encode a Type III secretion system (T3SS), a syringe-like apparatus employed by numerous Gram-negative pathogens to inject bacterial proteins into host cells. Although the T3SS is required for full virulence in *D. dadantii* [79] and *P. atrosepticum* [80], far fewer secreted effector proteins have been identified in soft-rot associated pathogens than many other bacteria, and some pathogenic *Pectobacterium* lack a T3SS altogether [81]. The *D. dadantii* T3SS has also been implicated in multicellular behavior and biofilm formation [82]. Genes associated with the T3SS are largely unaffected in both *D. dadantii* and *P. atrosepticum*, with low absolute expression levels with and without O2. However, in *D. dadantii* several related genes are down-regulated including, *hrpN*, which encodes a σ54-enhancer binding regulatory protein, two secreted harpin genes, *hrpP* and *hrpW* and *dspE* encoding a T3 secreted effector. None of these genes show a similar response in *P. atrosepticum*. Genes that encode a putative two partner secreted adhesin and its associated activator/transporter constitute a complex OrthoMCL group that has two *D. dadantii* genes and three *P. atrosepticum* genes (includes *hecA/B*). Only the *P. atrosepticum* orthologs are up-regulated under anaerobiosis. In *E. chrysanthemi* strain EC16 a role for HecA in early pathogenesis has been suggested [83].

**Chemotaxis**

Methyl-accepting chemotaxis proteins (MCPs) transduce environmental and cellular signals to the flagella [84]. The *D. dadantii* genome has 45 genes that encode proteins whose products are annotated as MCPs, while there are 36 such genes in *P. atrosepticum*. The C-terminal signal transduction domain of MCPs is highly conserved across all members of the family, while the N-terminal sensory domain varies extensively. This complicates reliable prediction of orthology. In our OrthoMCL analysis, 12 out of 15 *D. dadantii*-specific MCPs and one of 5 *P. atrosepticum*-specific MCPs are differentially expressed. Of the MCPs shared between the phytopathogens 17 are differentially expressed in *D. dadantii* and 9 in *P. atrosepticum*. Setting aside the potential errors with prediction of orthology, there is clearly an O2-availability regulated motility response in both *D. dadantii* and *P. atrosepticum*.

Though not an MCP, the *E. coli* Aer has been associated with aerotaxis via sensing of cellular redox potential using an FAD cofactor [85]. The phytopathogens each have three homologs that show similarity to Aer throughout the entire length of the alignment (*aer1, aer2* and ABL-0063893 in *P. atrosepticum* and *aer1*, ABF-0014726 and ABF-0014843 in *D. dadantii*). Both phytopathogens include at least one putative aerotaxis receptor
up-regulated and one down-regulated under anaerobic conditions. Transcripts of the *E. coli* aer gene decrease, though not statistically significantly, during anaerobiosis.

Flagella, motility and attachment
In the soft-rot pathogens, the contribution of flagella to motility is important for virulence [86-88]. Genes encoding regulatory elements of flagellar assembly as well as some of the genes of the flagellar apparatus are affected under anaerobic conditions in one or both phytopathogens. None of the differentially expressed genes show a similar trend in both phytopathogens (see Additional File 7) suggesting that these genes may be regulated differently during anaerobiosis in these two phytopathogens.

Polysaccharides
Genes encoding enzymes that produce a range of polysaccharides such as lipopolysaccharides (fda and waa genes), exopolysaccharides/O-antigens (wza and rfd genes), enterobacterial common antigen (wec and eff genes) and membrane derived oligosaccharides such as periplasmic glucans (apg genes) play important roles in virulence, adhesion, resistance to host-derived compounds and are considered virulence factors in *P. atrosepticum* and/or *D. dadantii*. Interestingly, many regulators shared between the phytopathogens are differentially expressed in a lineage-specific pattern that may indicate regulatory divergence for these loci. It is possible that at least some of these regulators respond to host-derived signals under the O2-limiting and inducing conditions encountered within the plant.

Most of the other global regulators of virulence including KdgR [111,112], Cpp [99,101] and Fur (fur shows statistically significant up-regulation but only minimal (~1.7 fold-change), that are highly conserved between the phytopathogens are unaffected in either organism. They all show moderate levels of expression regardless of O2 availability. Although transcription of several KdgR target genes is affected in our experiments, the involvement of KdgR is unlikely to account for the change in expression for these targets since the inducer 2-keto-3-deoxyglucosonate (KDG), a pectin degradation compound was not present in our medium. Furthermore other than CWDE genes mentioned above, which are members of complex overlapping regulons, none of the co-regulated transporter genes such as KdgMN and togMNAB are affected in our experiments.

Toxins
The *P. atrosepticum* genome contains a cluster of 9 cfa and cfl genes that are > 3- fold up-regulated in the absence of O2. They encode a putative polyketide biosynthesis system predicted to synthesize a compound similar to coronafacic acid, a component of the coronatine phytotoxin produced by *P. syringae* [94], and mutations in the *P. atrosepticum* genes dramatically reduces virulence on potato. A similar gene cluster was recently characterized from a phytopathogenic *Streptomyces*, with mutants in the polyketide synthesis system showing reduced virulence on tobacco [95].

*D. dadantii* is pathogenic to pea aphids under laboratory conditions and this trait appears relatively widely distributed among species of enterobacteria [96]. Deleting a cluster of four genes encoding proteins similar to cytolytic delta-endo-toxins from the gram-positive entomopathogen *Bacillus thuringiensis* significantly reduced virulence of *D. dadantii* on aphids. These four genes (typically expressed as a single transcriptional unit) are up-regulated in our experiments. Interestingly these genes were regulated by many of the same regulators that control expression of virulence factors for the plant host but in opposite directions [97].

Pathogenicity-associated transcriptional regulators (TR)
In *P. atrosepticum* and *D. dadantii*, several regulators coordinate expression of virulence factors in response to environmental or physiological conditions [98-103]. O2 dependent modulation has been demonstrated for very few virulence factors or associated regulators.

In our experiments, *D. dadantii* and *P. atrosepticum* each appear to have at least one O2-responsive strain-specific global regulatory gene whose expression is influenced by anaerobiosis. They are a gene for PecM [103-107] in *D. dadantii* and that for RdgB in *P. atrosepticum* [108,109]. Beside these, the PecS repressed *expI* gene [110] coding for the LuxI homolog in *D. dadantii* for the production of AHL, the *expR* gene that activates PecS and which encodes the AHL receptor are down-regulated only in *D. dadantii*, and their orthologs are unaffected in *P. atrosepticum*. Interestingly, many regulators shared between the phytopathogens are differentially expressed in a lineage-specific pattern that may indicate regulatory divergence for these loci. It is possible that at least some of these regulators respond to host-derived signals under the O2-limiting and inducing conditions encountered within the plant.
**Table 3** List of small RNAs that are O$_2$-responsive in at least one of the two phytopathogens *D. dadantii* and *P. atrosepticum*

| ASAP Feature ID | P. atrosepticum | E. coli | Gene Name | Fold Change | P. atrosepticum | E. coli | References |
|-----------------|-----------------|---------|-----------|-------------|-----------------|---------|------------|
| ABF-0061315     | ABL-0064934     |         | arcZ      | 1.9         | -1.2*           |         | [115]      |
| ABF-0061324     | ABL-0061410     | ABE-0001579 | ffs       | 12.8        | 4.3             | 1.4*    | [116-118]  |
| ABF-0174125     | ABL-0064933     |         | fnS       | 28.3        | 52.4            |         | [113,114]  |
| ABF-0061309     | ABL-0064917     |         | glmY      | 3           | 1.4*            |         | [119-123]  |
| ABF-0061313     | ABL-0064935     |         | glmZ      | -0.8*       | 1.9*            |         | [119-123]  |
| ABF-0061316     | ABL-0060542     | ABE-00010260 | mnpB     | 2.7         | 1.2*            | 1.6*    | [12]       |
| ABF-0061322     | ABL-0061263     |         | rsmB      | 3.7         | 1.2*            |         | [124-126]  |
| ABF-0061325     | ABL-0062736     |         | ryeA      | 2.3         | -2.1            |         | [127,128]  |
| ABF-0061326     | ABL-0062735     |         | ryeB      | 5.4         | -2.3            |         | [127,128]  |
| ABF-0061311     | ABL-0063956     |         | rygA      | 6.3         | -2.0*           |         | [129,130]  |
| ABF-0061314     | ABL-0060225     | ABE-00012621 | spf      | 16.3        | -1.1*           | 2.08*   | [131]      |
| ABF-0061318     | ABL-0060877     |         | strA      | 0.6*        | -3              |         | [132]      |
| ABF-0061317     | ABL-0060677     | ABE-0009556 | surS      | 5.2         | 2               | 1.75*   | [133]      |
| ABF-0061323     | ABL-0061273     |         | tff       | -1.8        | -1.1*           |         | [134]      |

This list of small RNA genes are differentially expressed in one or both phytopathogens. Where there is a gene in *E. coli* in the group, the fold change is included. Statistically insignificant values are marked with an asterisk.

**E. coli.** In *E. coli* transcriptional activation of *fnrS* by FNR during anaerobiosis leads to translational repression of cydDC, metE, sodA, sodB. FnRS also activates at least one target gene *(yhaO)*; [113,114]. In the phytopathogens, *fnrS* is > 3-fold up-regulated in both *D. dadantii* and in *P. atrosepticum*. In *E. coli*, a second small RNA, ArcZ, is important under anaerobic conditions and is regulated by ArcAB [115]. In our experiments, arcZ is detected as differentially expressed in *D. dadantii*, but not in *P. atrosepticum.*

Several other small RNA genes show fairly compelling evidence of differential transcriptional regulation between the two phytopathogens including *spf* (Spot 42), *rygA/*omrA and *ryeA*, and possibly *glmY*, *glmZ*, and *rsmB*. In *D. dadantii*, one up-regulated small RNA corresponds to the *spf* gene, an ortholog of the *E. coli* Spot 42 small RNA, which plays a role in anti-sense mediated down-regulation of the third gene (galK) of the galactose operon [116,135], and whose expression is known to be affected by carbon source available in the media and is cAMP-CRP responsive [136]. The corresponding gene in *P. atrosepticum* does not change expression, rather levels are intermediate in both conditions, similar to the *E. coli* ortholog in the Kang experiments. The *rygA* gene is > 3-fold up-regulated in *D. dadantii*. The *P. atrosepticum* ortholog is not differentially expressed, and trends in the opposite direction. Targets of the two *E. coli* orthologs, neither of which have previously been implicated in anaerobiosis, include both transcriptionally up and down regulated genes many of which are involved with cell surface structures or functions. They also negatively regulate *fepA* and *fecA*, two genes associated with iron homeostasis, and fimbrial genes associated with adhesion and biofilm formation [130]. The *ryeB* RNA is up-regulated in *D. dadantii* and down-regulated in *P. atrosepticum*. In *E. coli*, RyeB interacts with RyeA, encoded on the opposite strand, to mediate RNAse III-dependent cleavage [127] and is known to be pH-responsive in O$_2$-limited conditions [128]. We detect *ryeA* as differentially expressed in *D. dadantii*, although not in *P. atrosepticum*, where it trends in the same direction as *ryeB*. *GlmY*, a small RNA implicated in amino-sugar metabolism, is detected as up-regulated in *D. dadantii* and unaffected in *P. atrosepticum*. Amino sugars are important precursors of the peptidoglycan and lipopolysaccharide components of the cell wall. The *rsmB* gene is highly expressed under both aerobic and anaerobic conditions in *D. dadantii* and *P. atrosepticum*. It is up-regulated in the absence of O$_2$ only in *D. dadantii*. This gene, like several others, was also not present on the Kang et al. arrays. *RsmB* has been linked to production of extracellular enzymes, quorum sensing and T3SS in *Dickeya* as well as in a related species [124-126]. This collection of lineage-specific expression patterns suggests that altering regulation of small RNAs may be a particularly labile mechanism of regulatory diversification.

**Conclusions**

We investigated the transcriptional response to O$_2$ under simple controlled laboratory conditions for two soft rot-associated phytopathogenic enterobacteria to begin to enumerate the regulatory and metabolic networks associated with a key environmental parameter that impacts the interaction of these organisms with plant hosts, and to explore the extent of regulatory divergence that occurs among enterobacteria. We analyzed data from *D. dadantii* and *P. atrosepticum* individually, and compared them to
each other, as well as the model organism *E. coli* K12, using predicted gene-by-gene orthology and by grouping related genes into subsystems. The latter approach provides insights into larger scale patterns of conserved and lineage-specific biological processes regulated by *O₂* availability.

The *O₂*-responsive stimulon for each organism is large, and includes genes conserved across the family enterobacteria, as well as lineage-specific and organism-specific genes that were likely acquired through lateral gene transfer events. Some conserved genes show a conserved response to *O₂*, but others vary across organisms in the magnitude or even direction of response. *D. dadantii* and *P. atrosepticum* are more closely related to each other than to *E. coli* K12, and overall, their gene expression profiles are more conserved in terms of total number of orthologous genes (including those not shared with *E. coli*) responding in a congruent way, and in the proportion of genes shared across all three organisms responding in a congruent way.

A subset of genes show a different trend, with the expression profile more similar between *E. coli* and *P. atrosepticum*, with *D. dadantii* behaving differently. We attribute many of these to the likely deficiency of ArcAB regulation in *D. dadantii* where the sensor kinase of this two-component regulatory system is a pseudogene; however, it is not possible from these experiments to rule out that ArcA may be partnered with a different signal transducer, opening up the possibility that existing binding sites for ArcA could be coopted to respond to an entirely different signal.

A relatively small number of conserved genes are >3-fold up-regulated or down-regulated in all three organisms. While these include some known components of the well characterized *E. coli* anaerobic stimulon, other important components are missing from this conserved core. Detailed investigation of the orthology relationships and the subsystem-based approach reveal a broader group of processes implicated in a net conserved response to *O₂*, but with variation across the organisms in the number of functionally redundant paralogs and/or non-paralogous isofunctional subsystems, which we refer to as changes in subsystem architecture. For example, hydrogenases are highly up-regulated under anaerobic conditions in all three organisms, but the genes involved show complex homology relationships, involving duplications, and/or deletions, as well as genome rearrangements, that obscure the common response. We detailed these types of relationships and the associated expression patterns for subsystems involved with regulation, metabolism and a variety of processes associated with interactions of the phytopathogens with plant hosts.

Our results indicate that the *O₂*-responsive gene network includes a variety of virulence and pathogenicity-relevant processes including secretion, response to environmental stress, metal homeostasis, and taxis. Further experiments aimed at investigating the specific role of *O₂* regulation of these biological processes may be fruitful. Several virulence-associated subsystems exhibit strikingly divergent behavior in the two phytopathogens, most notably the T6SS. More subtle differences, like down-regulation of the complete complement of pectate lyases in *D. dadantii*, which are largely unaffected in *P. atrosepticum*, may lead to insights into the differences in virulence of these two phytopathogens under *O₂*-limited conditions, but this requires exploration under a broader number of experimental conditions. The experimental conditions explored here are very limited, and the number of genes in the plant-pathogen anaerobic stimulons will only increase as additional variables (carbon sources, nitrate availability, intermediate *O₂* levels, time series of shifting *O₂* availability, etc.) are investigated.

The genes differentially expressed in one or both phytopathogens include known targets of regulators associated with quorum sensing, oxidative stress, iron homeostasis, nitrate/nitrite, and carbohydrate availability, as well as the established key regulators of anaerobiosis under the experimental conditions we chose, FNR and ArcAB. The underlying regulatory network behind the *O₂*-responsive stimulon we have described is complex, involving a larger number of regulators, and it clearly differs between the two phytopathogens. Further dissection of this network bioinformatically will certainly require simultaneous consideration of a large number of regulators. We do not attempt a comprehensive dissection in this paper. We observe that at least one known key regulator of virulence genes, PecM, is among the genes differentially affected between *D. dadantii* and *P. atrosepticum*.

Several small regulatory RNAs are also differentially expressed under aerobic and anaerobic conditions, including ones that are found in *E. coli*, but have not previously been characterized as *O₂*-responsive. These expression patterns should be experimentally validated using additional techniques, both because of their novelty, and because small genes may be particularly subject to measurement errors using arrays.

Many of the organism-specific and phytopathogen-specific genes in the anaerobic stimulon were likely acquired through lateral gene transfer events. These genes may have become a part of the stimulon in a variety of ways. A lateral transfer can include regulatory elements from the donor that also function in the recipient or a laterally acquired gene could be integrated into the recipient genome in a way that makes use of native *O₂*-responsive regulatory elements. Alternately, evolutionary events (point mutations, rearrangements, further lateral transfers in the same region) subsequent to acquisition
of a gene could render it O$_2$-responsive. We expect there to be examples of all of these, but further examination awaits comparison with additional representatives of each genus from ongoing genome projects that will permit more precise definition of the boundaries of laterally acquired elements and reconstruction of the ancestral regulatory states. Finally, these experiments addressed only a single representative of each species, and further investigation will be required to determine whether the expression patterns we observed here are typical of each species, and which aspects of the anaerobic stimolon vary within each species.

**Methods**

**Bacterial growth and RNA extraction**

We grew three replicates each of *Dickeya dadantii* 3937 and *Pectobacterium atrosepticum* SCR11043 in MOPS minimal medium (purchased from Teknova, Inc.) supplemented with 0.1% glucose at 30°C and 23°C respectively. Overnight cultures of each bacterium were diluted to an O.D.600 of 0.05 in fresh medium and cultivated. Overnight cultures of each bacterium were supplemented with 0.1% glucose at 30°C and 23°C respectively. Overnight cultures of each bacterium were diluted to an O.D.600 of 0.05 in fresh medium and cultured in a gas sparging system [137] apparatus described in Kang et al.) that permits precise control over the mixtures of O$_2$, N$_2$ and CO$_2$. Cultures were grown to early log phase under aerobic (70% N$_2$, 25% O$_2$ and 5% CO$_2$) and anaerobic (95% N$_2$ and 5% CO$_2$) conditions. 20 ml samples were collected in tubes containing 2 ml phenol-ethanol. RNA was extracted using the hot-phenol method [138]. Quality of RNA samples was assessed using the Agilent Bioanalyzer 2100 nanochip system (Agilent Technologies).

**Microarray design and hybridization**

334647 genome-specific probes for the *D. dadantii* and 344859 probes for the *P. atrosepticum* genomes were selected using chipD (target melting temperature 78°C, target probe length 40 to 70-mers, interval size 12) [139] and oligonucleotide arrays were synthesized by Nimblegen Inc. Procedures described in the Nimblegen Arrays User’s guide (http://www.nimblegen.com/products/lit/05434505001_NG_Expression_UGuide_v6p0.pdf) were followed for cDNA synthesis, labeling and hybridization. Arrays were scanned at 532 nm and signals were extracted using NimbleScan software (NimbleGen, Inc.).

**Analysis of gene expression data**

Signals from the 3 replicates of the hybridization experiments from each organism were normalized using RMA [129] implemented in the NimbleScan software and imported into a custom Microsoft Access database. Normalized signal intensities for each set of three replicates are in good agreement (R$^2$ ranging from 0.92-0.99). The median of signals from multiple probes (from coding and non-coding strand) for each individual gene was calculated using R, from which log$_2$ expression values were derived. To determine directional changes in gene expression, log$_2$ ratios were determined by calculating the difference between the log$_2$ median anaerobic signal and log$_2$ median aerobic signal.

To identify differentially expressed genes between aerobic and anaerobic growth, an empirical Bayesian analysis, EBarrays [18] was executed within the free statistical analysis software package R [140] and Bioconductor v2.1 [141]. The posterior probability for each pattern was calculated using a hierarchical log-normal expression model with the conditional false discovery rate (cFDR) at 0.01 to determine the appropriate threshold (cFDr(t)). The *E. coli* WT data set for aerobic and anaerobic conditions derived from Kang et al. was reanalyzed to identify differentially expressed genes as described above. The critical thresholds for the three data sets are as follows:

- *D. dadantii*: 0.8734342, *P. atrosepticum*: 0.9067187, *E. coli*: 0.9289846. Throughout the manuscript, we have used the term “differentially expressed” to denote genes that qualify our permissive criterion namely the probability for observed differential expression is higher than the critical threshold, for the dataset, when the conditional false discovery rate is set at 0.01 and the prefix “highly” is used to denote differentially expressed genes whose transcripts show more than a 3-fold change between the conditions (stringent criteria). Genes whose transcript abundance is higher under anaerobic conditions are referred to as “up-regulated” and those with lower transcript abundance under anaerobic conditions are referred to as “down-regulated”. In some instances, fold change values for poly-cistronic operons that are conserved across all three organisms are averaged across genes to simplify our analyses. Additional File 9, Additional File 10, and Additional File 11 contain the complete datasets for *D. dadantii*, *P. atrosepticum* and *E. coli*, respectively.

**Analysis of expression data for non protein coding RNA (small RNA)**

Our oligonucleotide arrays included probes tiled across the entire genomes for both *D. dadantii* and *P. atrosepticum*. Thus, we are also able to analyze the O$_2$-response for genes typically not included in gene expression arrays, like those that encode small RNAs (sRNAs), even if they were not present in the original genome annotations. OrthoMCL considers only protein-coding genes, and there are not comprehensive predictions of orthology for non-coding RNAs (ncRNAs) or tRNAs in the ASAP database (the sRNAbase [142] has predictions for 34 small RNAs in *P. atrosepticum*) so we do not include them in the cross-species analyses above. Instead, we used BLASTn to find orthologs in both phytopathogens on a case-by-case basis. Most small RNA genes discussed in
the results and discussion are missing or incorrectly annotated in at least one of the two phytopathogen GenBank genome sequences. We have corrected them in the ASAP database. These RNAs are short, and consequently, inferences about expression patterns are based on a relatively small number (typically around 10) of probes. We manually investigated probe behavior consistency in most cases, and found the expression patterns persuasive. All small RNA genes detected as differentially expressed in either organism are shown in Table 3.

Comparative analysis

Sequences and annotations for predicted protein-coding genes for D. dadantii 3937, P. atrosepticum SCR1043 and E. coli were obtained from the ASAP database [143] and clustered using OrthoMCL [19] using default parameters. OrthoMCL clustered genes from each of the three organisms into simple or complex ortholog groups depending on whether one or more than one ortholog is present for the gene in the organisms being compared as described using the following example. E. coli encodes paralogous genes for fumarase, fumA and fumB that are homologous to the gene identified as fumA in the phytopathogens. All of these four genes are clustered together in a single orthologous group (complex group) by OrthoMCL. The third E. coli fumarase isoenzyme, encoded by fumC has a simple 1-1-1 relationship with fumC of D. dadantii and P. atrosepticum and these three genes are clustered together as a different group (simple group). Strain-specific genes do not belong to any OrthoMCL group. Additional File 8 lists all protein-coding genes from all three organisms, OrthoMCL group identifiers, and counts of the number of members of the group from each organism along with a short form of the experimental data results. Operon structures and known and predicted regulator binding sites for E. coli were obtained from EcoCyc [144] unless otherwise noted.

Additional file 5: List of strain-specific genes in D. dadantii that are detected as differentially expressed using stringent criteria (cFDR = 0.01 and fold change > 3)

Additional file 6: List of strain-specific genes in P. atrosepticum that are detected as differentially expressed using stringent criteria (cFDR = 0.01 and fold change > 3)

Additional file 7: Fold change patterns for P. atrosepticum and D. dadantii genes implicated in virulence and for their orthologs in E. coli predicted by OrthoMCL. Anaerobic growth in the presence of glucose alters expression of virulence-associated genes in both P. atrosepticum and D. dadantii. This includes genes associated with cell wall degradation and the Out system (down-regulated in D. dadantii only), T6SS (down-regulated in D. dadantii and up-regulated in P. atrosepticum), iron uptake (down-regulated in both), methyl-accepting chemotaxis and regulators associated with virulence. Fold change patterns and genome order are as follows: E. coli, P. atrosepticum and D. dadantii (see key within the figure). Each orthologous group of genes is represented by three blocks colored by fold change (dark blue: down-regulated, fold change > 3; light blue: down-regulated, fold change < 3; bright yellow: up-regulated, fold change > 3; dirty yellow: up-regulated, fold change < 3; black: no change in expression, X: ortholog absent in that organism). Genes are represented by their symbols, or by ASAP feature IDs (especially for strain-unique genes) or by OrthoMCL group designations (‘G’ followed by number).

Additional file 8: Complete OrthoMCL analysis.

Additional file 9: Normalized signals, fold changes and significance for features in D. dadantii 3937.

Additional file 10: Normalized signals, fold changes and significance for features in P. atrosepticum SCR1043.

Additional file 11: Normalized signals, fold changes and significance for features in E. coli K12-MG1655.

Acknowledgements

JA is supported by the NHGRI training grant to the “Genomic Sciences Training Program” (ST32HG002760) and the NLM training grant to the “Computation and Informatics in Biology and Medicine Training Program” (NLM 5T15LM007359). Both LB and JA are also supported by the “Molecular Evolution of Microbial Pathogen Genomes”, NIH R01-GM62994, as were PL and JDG (P.I. NTP; Co-P.I.s JDG, PJK). LB was also supported in part by a Vilas Life Cycle Award to NTP. We would like to thank the Gene Expression Center at the University of Wisconsin-Madison for their support in providing technical support, facilities and equipment for use in our research.

Author details

1Biotechnology Center, University of Wisconsin-Madison, WI, USA.
2Department of Biomolecular Chemistry, University of Wisconsin-Madison, WI, USA.
3Department of Plant Pathology, University of Wisconsin-Madison, WI, USA.
4Department of Genetics, University of Wisconsin-Madison, WI, USA.

Authors’ contributions

All authors contributed to the overall concept and in the writing of the manuscript. LB and V8 performed the experiments designed by JA, LB, JDG and NP. LB and JA participated in statistical analysis. PL performed the OrthoMCL analysis. All authors read, contributed to revisions of and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 17 November 2011 Accepted: 22 March 2012 Published: 22 March 2012

References

1. Perombelon MCM. Potato diseases caused by soft rot erwinias: an overview of pathogenesis. Plant Pathol 2002, 51(1):1-12.
2. Lemattre P. Chimie horticole, Nouvelle éd. edn. Paris, J-B Baillère, 1972.
47. Ababou H, Pommier J, Benoit S, Giordano G, Mandrand-Berthelot MA. Expression and characterization of the *Escherichia coli* fdo locus and a possible physiological role for aerobic formate dehydrogenase. *J Bacteriol* 1995, 177(24):7141-7149.

48. Andrews BJ, Lehman JA, Turchi JJ. Kinetic analysis of the Ku-DNA binding activity reveals a redox-dependent alteration in protein structure that stimulates dissociation of the Ku-DNA complex. *J Biol Chem* 2006, 281(19):13566-13573.

49. Gort AS, Ferber DM, Imlay JA. The regulation and role of the periplasmic copper superoxide dismutase of *Escherichia coli*. *Mol Microbiol* 1999, 32(1):19-21.

50. Battistoni A, Poccallo F, Focarelli S, Ajello M, Donnarumma G, Greco R. The form of the DNA substrate required for stimulation of the Ku-DNA complex. *J Mol Biol* 2008, 377(3):879-888.

51. Retroskii K, Gottesman S. The role of the DNA substrate form for Ku-DNA dismutation. *Mol Microbiol* 2002, 46(1):147-156.

52. Albrektsson T, Bergman L, Figureau-Bossi N. Differential accumulation of *Salmonella* [Cu, Zn] superoxide dismutases SodCl and SodCII in intracellular bacteria: correlation with their relative contribution to pathogenicity. *Mol Microbiol* 2002, 41(1):237-247.

53. Piddington DL, Fang FC, Laessig T, Cooper AM, Orme IA, Buchmeier NA. Cu, Zn superoxide dismutase of *Mycobacterium tuberculosis* contributes to survival in activated macrophages that are generating an oxidative burst. *Immunity* 2001, 9(8):480-489.

54. Mitchell A, Romano GH, Groisman B, Yona A, Dekel E, Kupiec M, Dahan O. Adaptive prediction of environmental changes by *E. coli*. *PNAS* 2004, 101(20):7575-7580.

55. Casadevall A, Pirofski LA. The role of the copper/zinc superoxide dismutases SodCI and SodCII in intracellular growth arrest, stress and virulence. *J Bacteriol* 2001, 183(4):1205-1214.

56. Bell KS, Sebaihia M, Pritchard L, Holden MT, Hyman LJ, Holeva MC, Thomson NR, Bentley SD, Churcher LJ, Mungall K, et al. Genome sequence of the enterobacterial phytopathogen *Erwinia carotovora* subsp. *carotovora* and characterization of the type VI secretion system and its associated type III effectors. *FEMS Microbiol Lett* 2005, 250:1-10.

57. Bell KS, Sebaihia M, Pritchard L, Holden MT, Hyman LJ, Holeva MC, Thomson NR, Bentley SD, Churcher LJ, Mungall K, et al. Genomic sequence of the enterobacterial phytopathogen *Erwinia carotovora* subsp. *carotovora* and characterization of the type VI secretion system and its associated type III effectors. *FEMS Microbiol Lett* 2005, 250:1-10.

58. Boughnpsmora A, Matzneke BF, Botterl G, Reverchon S, Lesuisse E, Expert D, Franza T. Differential role of ferritins in iron metabolism and virulence of the plant-pathogenic bacterium *Erwinia chrysanthemi* 3937. *J Bacteriol* 2008, 190(5):1518-1528.

59. Gouin K, Touze T, Pajer Y, Jouan B, Blanco C. Mutations of ouaS alter the virulence of *Erwinia chrysanthemi*. *Mol Plant Microbe Interact* 2005, 18:150-157.

60. Hugouvieux-Cotte-Pattat N, Condernene G, Nasser W, Reverchon S. Regulation of pectinolysis in *Erwinia chrysanthemi*. *Annu Rev Microbiol* 1996, 50(1):213-237.

61. Shvedchik VE, Kester HOM, Benen JA, Visser J, Robert-Baudouy J, Hugouvieux-Cotte-Pattat N. Characterization of the exopolygalacturonate lyase PelX of *Erwinia chrysanthemi* 3937. *J Bacteriol* 1999, 181(5):1652-1663.

62. Doonev V, Daroza R, Kerenud A, Nishino S, Pirpi T, Kukkinen N, Pirhonen M. Microarray profiling of host-extract-induced genes and characterization of the type VI secretion cluster in the potato pathogen *Pectobacterium atrosepticum*. *Microbiology* 2008, 154(Pt 1):2378-2396.

63. Mattinen L, Somervuo P, Nykivuk J, Nissinen R, Kousonen P, Corhals G, Auvinen P, Attamapa M, Valkonen JP, Pirhonen M. Microarray profiling of host-extract-induced genes and characterization of the type VI secretion cluster in the potato pathogen *Pectobacterium atrosepticum*. *Microbiology* 2008, 154(Pt 1):2378-2396.

64. Mattinen L, Nissinen R, Riiyi T, Kukkinen N, Pirhonen M. Host-extract induced chitinase secretion in the secretome of the plant pathogenic bacterium *Pectobacterium atrosepticum*. *Proteomics* 2007, 7(19):3527-3537.

65. Kazemi-Pour N, Condernine G, Hugouvieux-Cotte-Pattat N. The secretome of the plant pathogenic bacterium *Erwinia chrysanthemi*. *Proteomics* 2004, 4(10):3177-3186.

66. Douet V, Expert D, Barra F, Py E. *Erwinia chrysanthemi* iron metabolism: the unexpected implication of the inner membrane protein within the type II secretion system. *J Bacteriol* 2009, 191(3):975-984.

67. Francetic O, Belin D, Badault C, Pugley AP. Expression of the endogenous type II secretion pathway in *Erwinia chrysanthemi* leads to chitinase secretion. *EMBO J* 2000, 19(24):6697-6703.

68. Joel N, Colthurst SJ, Pritchard L, Hedley PE, Ravensdale M, Humphris S, Burr T, Talke G, Burch MB, Birch PR, et al. Quorum sensing coordinates brute force and stealth modes of infection in the plant pathogen *Pectobacterium atrosepticum*. *PLoS Pathog* 2008, 4(6):e1000093.

69. Yang CH, Gavilanes-Ruiz M, Okinaka Y, Vedel R, Berthouy I, Boccara M, Chen JW, Perna NT, Keen NT. *rnp* genes of *Erwinia chrysanthemi* 3937 are important virulence factors. *Mol Plant Microbe Interact* 2002, 15(5):472-480.

70. Bell KS, Sebaihia M, Pritchard L, Holden MT, Hyman LJ, Holeva MC, Thomson NR, Bentley SD, Churcher LJ, Mungall K, et al. Genomic sequence of the enterobacterial phytopathogen *Erwinia carotovora* subsp. *atrosepticum* and characterization of virulence factors. *Proc Natl Acad Sci USA* 2004, 101(30):11105-11110.

71. Kim HS, Ma B, Perna NT, Charkowski AO. *Erwinia atrosepticum* 2002, 15(5):472-480.

72. Bell KS, Sebaihia M, Pritchard L, Holden MT, Hyman LJ, Holeva MC, Thomson NR, Bentley SD, Churcher LJ, Mungall K, et al. *Erwinia atrosepticum* type II secretion system is required for multicellular behavior. *J Bacteriol* 2005, 187(2):639-648.

73. Raja MO, Jam JH, Deng WL, Doyle JJ, Collmer A. HecA, a member of a class of adhesins produced by diverse pathogenic bacteria, contributes to the attachment, aggregation, epidermal cell killing, and virulence phenotypes of *Erwinia chrysanthemi* EC16 on *Nicotiana clevelandii* seedlings. *Proc Natl Acad Sci USA* 2002, 99(20):13142-13147.

74. Wijemt K, Alexander RS, Zulbin IL. Comparative genomic and protein sequence analyses of a complex system controlling bacterial chemotaxis. *Methods Enzymol* 2007, 422:1-33.

75. Borko S, Brian R, Rudd KE, Parkinson JS. A signal transducer for aerotaxis in *Escherichia coli*. *J Bacteriol* 1997, 179(12):4075-4079.

76. Kim HS, Ma B, Perna NT, Charkowski AO. *Erwinia atrosepticum* type II secretion system is required for multicellular behavior. *J Bacteriol* 2005, 187(2):639-648.

77. Raja MO, Jam JH, Deng WL, Doyle JJ, Collmer A. HecA, a member of a class of adhesins produced by diverse pathogenic bacteria, contributes to the attachment, aggregation, epidermal cell killing, and virulence phenotypes of *Erwinia chrysanthemi* EC16 on *Nicotiana clevelandii* seedlings. *Proc Natl Acad Sci USA* 2002, 99(20):13142-13147.

78. Wijemt K, Alexander RS, Zulbin IL. Comparative genomic and protein sequence analyses of a complex system controlling bacterial chemotaxis. *Methods Enzymol* 2007, 422:1-33.

79. Borko S, Brian R, Rudd KE, Parkinson JS. A signal transducer for aerotaxis in *Escherichia coli*. *J Bacteriol* 1997, 179(12):4075-4079.
carotovora in response to DNA-damage agents. Mol Microbiol 1994, 14(5):999-1010.

90. Nguyen HA, Kaneko J, Kanno Y: Temperature-dependent production of carotovorin Er and pectin lyase in phytopathogenic Erwinia carotovora subsp. carotovora JAR 1. Biosci Biotechnol Biochem 2002, 66(6):1444-47.

91. Reverchon S, Chantegrel B, Deshayes C, Dourche A, Cotte-Pattat N: New synthetic analogues of N-acyl homoserine lactones as agonists or antagonists of transcriptional regulators involved in bacterial quorum sensing. Bioorg Med Chem Lett 2002, 12(8):1153-1157.

92. Rodionov DA, Gelfand MS, Hugouvieux-Cotte-Pattat N: Comparative genomics of the KdpG regulon in Erwinia chrysanthemi 9373 and other gamma-proteobacteria. Microbiology 2004, 150(Pt 11):3571-3590.

93. Hugouvieux-Cotte-Pattat N, Robert-Baudouy J: Isolation of Erwinia chrysanthemi mutants altered in pectolytic enzyme production. Mol Microbiol 1989, 3(11):1587-1597.

94. Boysen A, Möller-Jensen J, Kalpiöllito B, Valentin-Hansen P, Overgaard M: Translational regulation of gene expression by an anarobically induced small non-coding RNA in Erichesichia coli. J Bacteriol 2010, 285(14):10900-10907.

95. Durand S, Storz G: Reprogramming of anaerobic metabolism by the FnrS small RNA. Mol Microbiol 2010, 75(5):1215-1231.

96. Mandin P, Gottesman S: Integrating anaerobic/aerobic sensing and the general stress response through the ArcS small RNA. EMBO J 2010, 29(18):3009-3017.

97. Gottesman S: Small RNAs shed some light. Cell 2004, 118(1):1-2.

98. Brown S, Fournier MJ: The 4.5 S RNA gene of Escherichia coli is essential for cell growth. J Mol Biol 1984, 178(3):533-550.

99. Wickstrom D, Wagner S, Baas L, Ytterberg AJ, Klepsch M, van Wijk KJ, Lutijn J, de Visser WJ: Consequences of depletion of the signal recognition particle in Escherichia coli. J Bacteriol 2011, 296(6):4598-4609.

100. Gopey Y, Luttmann D, Heroven AK, Reichenbach B, Dersch P, Gorbek B: Common and divergent features in transcriptional control of the homologous small RNAs Gm1y and Gm1z in Enterobacteriaceae. Nucleic Acids Res 2011, 39(4):1294-1309.

101. Urban JH, Vogel J: Two seemingly homologous noncoding RNAs act hierarchically to activate glmS mRNA translation. PLoS Biol 2006, 4(3):e464.

102. Urban JH, Vogel J: Translational control and target recognition by Escherichia coli small RNAs in vivo. Nucleic Acids Res 2007, 35(3):1018-1037.

103. Reichenbach B, Maes A, Kalamorz F, Hajnsdorf E, Gorke B: The PecM protein is a homoserine lactone. Virulence and the quorum-sensing signal, N-(3-oxohexanoyl)-L-homoserine lactone. 215 2000, 21(3):203.

104. Yang S, Peng G, Zhang Q, Yi X, Choi CJ, Reedy RM, Charkowski AO, Yang C: Dynamic regulation of GacA in type III secretion, pectinase expression, pellicle formation, and pathogenicity of Dickeya dadantii 3937. Appl Environ Microbiol 2011, 77(12):4231-4240.

105. Vögtle J, Bartels V, Tang TH, Churakov G, Slagter Jager JG, Huttenhofer A, Wagner EG: Bnorincs in Escherichia coli detects new sRNA species and indicates parallel transcriptional output in bacteria. Nucleic Acids Res 2003, 31(22):6435-6443.

106. Wagner EGH: Dynamic regulation of GacA in type III secretion, pectinase expression, pellicle formation, and pathogenicity of Dickeya dadantii 3937. Appl Environ Microbiol 2011, 77(12):4231-4240.

107. Praillet T, Reverchon S, Nasser W: Isolation of a novel RNA strain Ec71 negatively regulates production of RpoS and rsmB RNA, a global regulator of extracellular proteins, plant virulence and the quorum-sensing signal, N-(3-oxohexanoyl)-L-homoserine lactone. 215 2000, 21(3):203.

108. Yang S, Peng G, Zhang Q, Yi X, Choi CJ, Reedy RM, Charkowski AO, Yang C: Dynamic regulation of GacA in type III secretion, pectinase gene expression, pellicle formation, and pathogenicity of Dickeya dadantii 3937. Mol Microbiol 2008, 68(1):133-142.

109. Vogel J, Bartels V, Tang TH, Churakov G, Slagter Jager JG, Huttenhofer A, Wagner EG: Bnorincs in Escherichia coli detects new sRNA species and indicates parallel transcriptional output in bacteria. Nucleic Acids Res 2003, 31(22):6435-6443.

110. Wagner EGH: Dynamic regulation of GacA in type III secretion, pectinase expression, pellicle formation, and pathogenicity of Dickeya dadantii 3937. Appl Environ Microbiol 2011, 77(12):4231-4240.
130. Guillier M, Gottesman S: Remodelling of the *Escherichia coli* outer membrane by two small regulatory RNAs. *Mol Microbiol* 2006, 59(1):231-247.

131. Adhya S: Suboperonic regulatory signals. *Sci STKE* 2003, , 185: pe22.

132. Argaman L, Hershberg R, Vogel J, Reizer JN, Wagner EG, Margalit H, Altuvia S: Novel small RNA-encoding genes in the intergenic regions of *Escherichia coli*. *Curr Biol* 2001, 11(12):941-950.

133. Geissen R, Steuten B, Poken T, Wagner R E. *coli* 65 RNA: A universal transcriptional regulator within the centre of growth adaptation. *RNA Biol* 2010, 7(5):564-568.

134. Rivas E, Klein RJ, Jones TA, Eddy SR: Computational identification of noncoding RNAs in *E. coli* by comparative genomics. *Curr Biol* 2001, 11(17):1369-1373.

135. Moller T, Franch T, Udesen C, Gerdes K, Valentin-Hansen P: Spot 42 RNA mediates discoordinate expression of the *E. coli* galactose operon. *Genes Dev* 2002, 16(13):1696-1706.

136. Polayes DA, Rice PW, Dahlberg JE: DNA polymerase I activity in *Escherichia coli* is influenced by spot 42 RNA. *J Bacteriol* 1988, 170(5):2083-2088.

137. Sutton VR, Kiley PJ: Techniques for studying the oxygen-sensitive transcription factor FNR from *Escherichia coli*. Methods Enzymol 2003, 370:300-312.

138. Bernstein JA, Lin P-H, Cohen SN, Lin-Chao S: Global analysis of *Escherichia coli* RNA degradosome function using DNA microarrays. *PNAS* 2004, 101(9):2756-2763.

139. Dufour YS, Wesenberg GE, Tritt AJ, Gäsner JD, Perna NT, Mitchell JC, Donohue T J, chipD: a web tool to design oligonucleotide probes for high-density tiling arrays. *Nucleic Acids Res* 2010, 38(Suppl):W321-325.

140. Team RDC: *R*: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing 2011.

141. Keseler IM, Collado-Vides J, Gama-Castro S, Ingraham J, Paley S, Paulsen IT, Selengut JD, Shashkov SD, Singhal SR, et al: BioCyc: a comprehensive database resource for *Escherichia coli*. *Nucleic Acids Res* 2005, , 33 Database:D334-D337.