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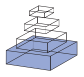

Burkholderia cenocepacia differential gene expression during host–pathogen interactions and adaptation to the host environment

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Members of the *Burkholderia cepacia* complex (Bcc) are important in medical, biotechnological, and agricultural disciplines. These bacteria naturally occur in soil and water environments and have adapted to survive in association with plants and animals including humans. All Bcc species are opportunistic pathogens including *Burkholderia cenocepacia* that causes infections in cystic fibrosis and chronic granulomatous disease patients. The adaptation of *B. cenocepacia* to the host environment was assessed in a rat chronic respiratory infection model and compared to that of high cell-density *in vitro* grown cultures using transcriptomics. The distribution of genes differentially expressed on chromosomes 1, 2, and 3 was relatively proportional to the size of each genomic element, whereas the proportion of plasmid-encoded genes differentially expressed was much higher relative to its size and most genes were induced *in vivo*. The majority of genes encoding known virulence factors, components of types II and III secretion systems and chromosome 2-encoded type IV secretion system were similarly expressed between *in vitro* and *in vivo* environments. Lower expression *in vivo* was detected for genes encoding *N*-acyl-homoserine lactone synthase CepI, orphan LuxR homolog CepR2, zinc metalloproteases ZmpA and ZmpB, LysR-type transcriptional regulator ShvR, nematocidal protein AidA, and genes associated with flagellar motility, Flp type pilus formation, and type VI secretion. Plasmid-encoded type IV secretion genes were markedly induced *in vivo*. Additional genes induced *in vivo* included genes predicted to be involved in osmotic stress adaptation or intracellular survival, metal ion, and nutrient transport, as well as those encoding outer membrane proteins. Genes identified in this study are potentially important for virulence during host–pathogen interactions and may be associated with survival and adaptation to the host environment during chronic lung infections.

Keywords: *Burkholderia cenocepacia*, *Burkholderia cepacia* complex, microarray, lung infection, rat chronic respiratory infection model, *in vitro*, *in vivo*

INTRODUCTION

Members of the *Burkholderia cepacia* complex (Bcc) are commonly found in soil and aquatic environments (LiPuma, 2010; Loutet and Valvano, 2010). Seventeen Bcc species have been identified, all of which have the potential to be opportunistic pathogens, although *Burkholderia cenocepacia* is the most clinically significant. *B. cenocepacia* causes lung infections resulting in significantly decreased survival rates in cystic fibrosis and chronic granulomatous disease patients (Mahenthiralingam et al., 2005). The organism is intrinsically multidrug resistant and can persist in the lungs of CF patients for many years (Mahenthiralingam et al., 2008). In some patients, infection with *B. cenocepacia* can progress to what is termed "cepacia syndrome." Cepacia syndrome is associated with a rapid deterioration in lung function associated with necrotizing pneumonia, bacteremia and sepsis that can result in death (Isles et al., 1984).

Many virulence factors have been identified in *B. cenocepacia* including extracellular enzymes, toxins, secretions systems, iron acquisition systems, cell–cell communication (quorum sensing, QS) systems, regulatory proteins as well as genes contributing to motility, biofilm formation, adhesion, cell invasion, intracellular survival, and bacterial protection from host factors (for review see Loutet and Valvano, 2010). Several infection models have been employed to identify and characterize the contribution of numerous genes to pathogenesis (Uehlinger et al., 2009). *B. cenocepacia* exhibits virulence against *Caenorhabditis elegans* (Kothe et al., 2003), *Galleria mellonella* (Seed and Dennis, 2008), *Acanthamoeba* (Marolda et al., 1999), *Dictyostelium discoideum* (Aubert et al., 2008), *Danio rerio* (Vergunst et al., 2010), *Drosophila melanogaster* (Castonguay-Vanier et al., 2010), and alfalfa seedlings (Bernier et al., 2003). Chronic respiratory infection models have been developed in mice and rats to investigate pathogenesis of Bcc species. The rat chronic respiratory infection model described by Cash et al. (1979) involves transtracheal delivery of agar-embedded bacteria directly into the lung allowing for bacterial persistence and pathology to be measured.

This chronic infection model has been used to identify Bcc species and bacterial strains that persisted or caused lung pathology from less virulent strains such as mutants in ornibactin biosynthesis, uptake and utilization, zinc metalloproteases, and genes encoding other enzymes, transcriptional regulators, and lipopolysaccharide (Sokol et al., 1999, 2000; Bernier et al., 2003, 2008; Corbett et al., 2003; Baldwin et al., 2004; Bernier and Sokol, 2005; Kooi et al., 2006; Loutet et al., 2006; Flannagan et al., 2007). These studies have revealed the importance of individual genes or systems to virulence but have not assessed bacterial gene expression during infection.

Transcriptional profiling using custom *B. cenocepacia* microarrays and RNA sequencing technology have enabled *in vitro* gene expression studies to be performed at a genome level. Transcriptional profiling has been used to examine gene expression in different environmental conditions such as those mimicking CF sputum or soil, or in response to antimicrobials (Drevinek et al., 2008; Yoder-Himes et al., 2009, 2010; Peeters et al., 2010; Bazzini et al., 2011; Coenye et al., 2011; Sass et al., 2011). In addition to further characterizing genes previously known to be important in virulence, these studies have also identified many genes with potential importance in virulence. Our current understanding of *B. cenocepacia* physiology, pathogenesis, and survival is incomplete since the *B. cenocepacia* genome, which is over 8 Mb, contains genes encoding many uncharacterized proteins. Identifying such proteins and determining their functional significance will improve our abilities to target such proteins for therapeutic purposes. To date, no studies have profiled *B. cenocepacia* gene expression at the whole genome level directly from infected cells/tissues or during infection of a susceptible host. To further understand *B. cenocepacia* adaptation to the host environment, we have used microarrays to examine the *B. cenocepacia* gene expression signature in the rat chronic respiratory infection model and compared this to high cell-density laboratory-grown cultures.

MATERIALS AND METHODS

BACTERIAL STRAINS AND GROWTH CONDITIONS FOR *IN VITRO* SAMPLES

Burkholderia cenocepacia K56-2 is a CF isolate that belongs to the ET12 lineage (RAPD type 2) and is clonally related to the sequenced strain J2315 (Mahenthiralingam et al., 2000; Baldwin et al., 2004; Holden et al., 2009). To generate *in vitro* samples, K56-2 cultures were grown at 37˚C, in 10 ml Miller's Luria broth (LB; Invitrogen, Burlington, ON, Canada) with shaking in 125 ml Erlenmeyer flasks to stationary phase (16 h) as previously described (O'Grady et al., 2009). Bacterial growth was assessed by determining the optical density (OD) at 600 nm.

ANIMAL STUDIES

Animal infections were performed using the rat agar bead respiratory infection model (Cash et al., 1979). Adult male Sprague-Dawley rats (150–180 g; Charles River, QC, Canada) were inoculated transtracheally with approximately 10^7 CFU of K56-2. At 3 days postinfection, infected lungs were aseptically removed, stored at 4˚C overnight in 15 ml of RNA *later* (Ambion, Streetsville, ON, Canada), and subsequently maintained at −70˚C to prevent RNA degradation. Animal experiments were conducted according to the guidelines of the Canadian Council of Animal Care for the care and use of experimental animals under protocol M08089 approved by the University of Calgary Animal Care Committee.

RNA MANIPULATIONS

Total RNA from *in vitro* samples was prepared as previously described (O'Grady et al., 2009) using a RiboPure bacterial RNA isolation kit according to manufacturer's instructions (Ambion). For *in vivo* samples, total RNA from infected lungs was isolated using Tri Reagent (Invitrogen) as recommended by the manufacturer. Total RNA samples were enriched for bacterial RNA using a Microb*Enrich* kit (Ambion) and purified using a MegaClear kit (Ambion). Enriched and purified bacterial RNA was depleted of 16S and 23S rRNAs using a Microb*Express* kit (Ambion) to isolate mRNA according to manufacturer's instructions to provide enhanced sensitivity for microarray experiments. DNase treatment was performed on all RNA samples using DNA-Free (Ambion), and samples were confirmed by PCR using *Taq* polymerase (Invitrogen) to be free of DNA prior to cDNA synthesis.

MICROARRAY ANALYSIS

In vitro-derived total RNA and *in vivo*-derived mRNA samples were indirectly labeled with the CyScribe Post-Labelling Kit (GE Healthcare) and cDNA synthesis performed as described by Sass et al. (2011) with the following modifications. Three independent RNA samples were used for *in vitro* samples and two mRNA samples (each consisting of an mRNA pool isolated from two infected rats to reduce variability between animals) were used for *in vivo* samples. Approximately 10 μg total RNA was labeled for each *in vitro* sample and 8 μg mRNA was labeled for each *in vivo* sample. The reference pool for microarray experiments consisted of *B. cenocepacia* J2315 genomic DNA isolated and labeled as described (Sass et al., 2011). The *B. cenocepacia* J2315 custom microarray, with each probe printed four times using the Agilent Sure Print 4 × 44 microarray platform, was used (Drevinek et al., 2008; Sass et al., 2011). Approximately 700–1000 ng labeled cDNA from the *in vitro* and *in vivo* samples and 55 ng labeled control genomic DNA was used per microarray. Hybridization, washing, and scanning were performed as described using the Two Color Microarray Based Gene Expression Analysis Protocol (Agilent) and the data analyzed using GeneSpring GX version 7.3.1. All labeling, hybridization, and scanning were performed by the Mahenthiralingam Laboratory, Cardiff University, Wales. Initial data were preprocessed by employing the enhanced Agilent FE import method. Probes specific to J2315 were filtered on a 1.5-fold change in expression between conditions to identify clusters of differentially regulated genes related to specific functions or potentially organized in operons. To eliminate potential differences in RNA between samples, data were normalized to control samples and mean $\log_2$ ratios (*in vivo*/*in vitro*) calculated from replicates were used and reported as expression ratios. Mean $\log_2$ ratios were also filtered on twofold changes in expression between *in vivo* and *in vitro* conditions to identify more stringently differentially regulated genes. The *in vitro*- or *in vivo*-derived K56-2

cDNA produced a signal that was detected by at least 94% of the probes on the microarray. Operon prediction and gene annotation or predicted protein function were retrieved from the *B. cenocepacia* J2315 genome at http://www.burkholderia.com (Winsor et al., 2008) or http://www.microbesonline.org (Dehal et al., 2009). The entire microarray data set has been deposited in the Array Express database http://www.ebi.ac.uk/arrayexpress under accession number E-MEXP-3367.

QUANTITATIVE RT-PCR

RNA for quantitative RT-PCR (qRT-PCR) was derived independently of that used for microarray analysis. Briefly, total RNA was isolated from three independent *in vitro* cultures prepared as described above. In a separate animal experiment to that used to prepare the microarray samples, enriched and purified total RNA was isolated as described above from three infected rats yielding three independent *in vivo* samples. Oligonucleotide primers (**Table 1**) were designed with Primer3 (Rozen and Skaletsky, 2000) and were synthesized by the University of Calgary Core DNA Services (Calgary, AB, Canada). BCAL0421 (*gyrB*) encoding DNA gyrase subunit B, previously used as a housekeeping gene in the Bcc multilocus sequence typing scheme (Baldwin et al., 2005) was used as a control as described previously (Peeters et al., 2010). Expression of *gyrB* was not significantly altered according to microarray analysis (data not shown). RT-PCR was performed using an iScript Select cDNA synthesis kit (Bio-Rad, Mississauga, ON, Canada). Quantification and melting curve analyses were performed with SsoFast Evagreen supermix with low ROX on an iCycler (Bio-Rad) according to manufacturer's instructions. For each of the three *in vitro* and *in vivo* cDNA samples, qRT-PCRs were performed in triplicate, normalized to the control gene, *gyrB*. Data were calculated as previously described (Schmittgen and Livak, 2008) and represented as fold change of the *in vivo* samples relative to the *in vitro* samples.

RESULTS

GENES ON ALL GENOMIC ELEMENTS ARE INDUCED IN RESPONSE TO THE HOST ENVIRONMENT

Global gene expression profiles were generated using microarrays from *B. cenocepacia* cultures recovered from rat lungs 3 days postinfection using a chronic respiratory infection model and compared to those of *B. cenocepacia* cultures grown to high cell-density *in vitro*. Using a fold change cut off of $\geq$1.5, we identified 366 genes that were induced *in vitro* and 304 genes that were induced *in vivo* (**Table 2**). The *B. cenocepacia* J2315 genome is comprised of four genetic elements: chromosome 1, 3.87 Mb; chromosome 2, 3.22 Mb; chromosome 3, 0.88 Mb; and a plasmid, 0.09 Mb (Holden et al., 2009). Differential expression was observed for genes present on the three chromosomes as well as the plasmid. The number of genes induced *in vitro* or induced *in vivo* on each genomic element and the percentage of the total number of genes induced *in vitro* or *in vivo* located on each genomic element is shown in **Table 2**. For *in vitro* induced genes, the distribution of changes across the genome was relatively proportional to the size of each genomic element, i.e., a decreasing percentage of genes showed altered expression from chromosomes 1 through 3 and to the plasmid. Interestingly, more than 20% of genes induced *in vivo* were plasmid genes indicating this group of genes was highly overrepresented (**Table 2**). Consistent with this observation, for chromosomes 1 through 3, the percentage of genes on each replicon induced *in vivo* was similar and ranged from 2.9 to 4.8%, in marked contrast to the plasmid where 66% of plasmid-encoded genes were induced *in vivo* (**Table 2**).

A MAJORITY OF CHARACTERIZED VIRULENCE GENES ARE SIMILARLY EXPRESSED BETWEEN *IN VITRO* AND *IN VIVO* ENVIRONMENTS

At least 28 genes have been characterized in *B. cenocepacia* that are known to be important for virulence and belong to functional groups including stress resistance, extracellular enzymes or

Table 1 | Oligonucleotide primers used in this study for qRT-PCR.

Primer	Sequence (5′–3′)	Product size (bp)	Reference
L0114fliCRTfor1	GCGTGTCGATGATTCAAACGGCAT	159	O'Grady et al. (2009)
L0114fliCRTrev1	TCACTTCCTGGATCTGCTGCGAAA		
L0343hcpRTfor1	ACGTTCTCGCTGAAGTACGC	120	This study
L0343hcpRTrev1	CGCGTAGGTCTTGTCGTTCT		
L1525qRTfor1	AGCAATCATCAAGCGTTTCC	87	This study
L1525qRTrev1	AGAGCGACTGCGATAAGTCC		
M2194mmsAqRTfor1	ATGACGGTCTACACGCATGA	164	This study
M2194mmsAqRTrev1	TCGATCTCGCTCTGGAACTT		
M2702prpCqRTfor1	GAAATCCAGAGCCGCTACAG	83	This study
M2702prpCqRTrev1	CCGATCACCACTTCCTTGTT		
pBCA025traFqRTfor1	TCGACCTTTGCTGATACGTG	196	This study
pBCA025traFqRTrev1	GGCAGTAAGGGCAGTCAGAG		
pBCA045traKqRTfor1	CGAGCCATCAAGAAGGTTGT	157	This study
pBCA045traKqRTrev1	ACTGGTAGGTAGCGCCTTGA		
pBCA053qRTfor1	GCAAAGGCCGACACATCTAT	90	This study
pBCA053qRTrev1	TCTACGGGATACCGAACAGC		
L0421gyrBqRTfor1	GTTCCACTGCATCGCGACTT	109	Peeters et al. (2010)
L0421gyrBqRTrev1	GGGCTTCGTCGAATTCATCA		

Table 2 | Microarray analysis of *B. cenocepacia* genes induced *in vitro* or induced *in vivo*.

	Genomic element				Total
	Chr 1[a]	Chr 2	Chr 3	Plasmid	
Number of genes induced *in vitro*	182[b]	139	44	1	366
Number of genes induced *in vivo*	104	102	36	62	304
Percentage of total genes induced *in vitro* (%)	49.7[c]	38.0	12.0	0.3	100
Percentage of total genes induced *in vivo* (%)	34.2	33.6	11.8	20.4	100
Percentage of genes on each replicon induced *in vitro* (%)	5.1[c]	4.9	6.0	1.1	5.1[d]
Percentage of genes on each replicon induced *in vivo* (%)	2.9	3.6	4.8	66.0	4.2

[a]Chr, chromosomes 1, 2, or 3 of B. cenocepacia J2315.

[b]Number or [c]percentage of B. cenocepacia genes or [d]percentage of a total of 7210 B. cenocepacia genes exhibiting changes (≥ 1.5-fold) in expression in RNA recovered from rat lungs (in vivo) relative to RNA isolated from in vitro grown cultures as determined by microarray analysis.

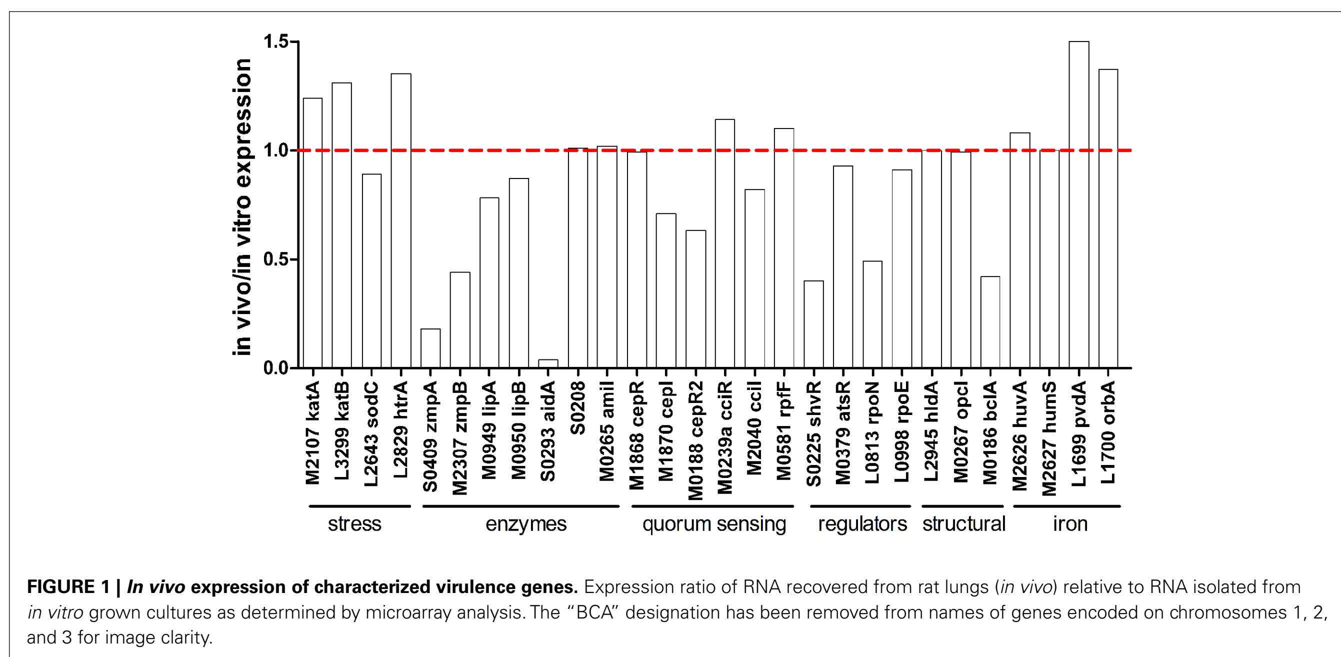

FIGURE 1 | *In vivo* expression of characterized virulence genes. Expression ratio of RNA recovered from rat lungs (*in vivo*) relative to RNA isolated from *in vitro* grown cultures as determined by microarray analysis. The "BCA" designation has been removed from names of genes encoded on chromosomes 1, 2, and 3 for image clarity.

secreted toxins, QS, transcriptional regulation, as well as genes involved in heme uptake, iron acquisition, and the synthesis of structural components such as lipopolysaccharide, porins, and lectins (Loutet and Valvano, 2010). Analysis of these virulence genes showed that expression of the majority of these genes was similar between *in vitro* and *in vivo* conditions (**Figure 1**). The expression of *cepI*, encoding an *N*-acyl-homoserine lactone (AHL) synthase, was somewhat lower *in vivo* and this observation was consistent with lower expression of CepIR-regulated genes including those encoding extracellular zinc metalloproteases ZmpA and ZmpB, the orphan LuxR homolog CepR2 and the LysR-type transcriptional regulator ShvR (**Figure 1**). Two other genes known to be influenced by CepIR such as the major catalase/peroxidase encoded by *katB* and an acyl-CoA dehydrogenase encoded by BCAS0208 were similarly expressed in the *in vitro* and *in vivo* environments (**Figure 1**). The BCAS0208 mutant caused less lung pathology than wild type in the rat chronic respiratory infection model (Subramoni et al., 2011).

Limited iron availability in mammals is circumvented by infectious pathogens by the production of iron binding and transport complexes such as heme binding proteins and siderophores. Although genes involved in heme transport (*huvA* and *hmuS*) were not differentially expressed between *in vivo* and *in vitro* environments (**Figure 1**), *huvA* mutants exhibited survival defects in the rat chronic respiratory infection model (Hunt et al., 2004). Genes involved in ornibactin biosynthesis and transport were also expressed at similar levels in both environments, although ornibactin mediated iron uptake is required for persistence in the rat chronic respiratory infection model (Visser et al., 2004). Among the characterized virulence genes, the lowest *in vivo* expression ratio (0.04) was observed for BCAS0293 (*aidA*; **Figure 1**). The *aidA* gene encodes a protein that significantly contributes to virulence against *C. elegans* (Huber et al., 2004), but an *aidA* mutation had no effect on virulence in the rat chronic respiratory infection model (Uehlinger et al., 2009).

SECRETION SYSTEMS ARE SELECTIVELY REGULATED BETWEEN *IN VITRO* AND *IN VIVO* ENVIRONMENTS

Burkholderia cenocepacia has one type II, type III, and type VI protein secretion systems (T2SS, T3SS, and T6SS, respectively) that contribute to pathogenesis, and two type IV secretion systems (T4SS), one of which has been shown to be important in virulence. Expression of genes encoding components of each of these systems varied between *in vitro* and *in vivo* environments.

The T2SS is composed at least 12 ORFs on three *gsp* operons and is involved in secretion of extracellular zinc metalloproteases ZmpA, ZmpB, and other extracellular proteins that have enzymatic activity such as phospholipase C, hemolysin, lipase, and polygalacturonase (Fehlner-Gardiner et al., 2002; Kothe et al., 2003; Gingues et al., 2005; Somvanshi et al., 2010). Expression of the three *gsp* operons encoding the T2SS was similar between *in vitro* and *in vivo* conditions (**Figure 2A**). Apart from the lower expression of *zmpA* and *zmpB in vivo* (**Figure 1**), expression of other genes encoding enzymes secreted by the T2SS described above was not different between *in vitro* and *in vivo* conditions (data not shown). The *B. cenocepacia* T3SS genes are organized in two operons on chromosome 2 thought to be responsible for secretion of effector proteins that have yet to be identified (Tomich et al., 2003; Glendinning et al., 2004). Mutation of *bcscN*, encoding an ATP-binding protein, reduced bacterial survival, and lung inflammation in a mouse agar bead infection model (Tomich et al., 2003). In our study, the mean expression ratio of genes in the *bcscQ* and *bcscV* operons was 1.03 and 0.99, respectively, in the *in vivo* compared to *in vitro* conditions (**Figure 2B**) indicating that there was no difference in expression.

Two gene clusters located on chromosome 2 and the plasmid have been identified to encode components of T4SS. Interestingly, the plasmid-encoded T4SS was induced *in vivo*. The bc-VirB/D4 T4SS on chromosome 2 shares homology with the *Agrobacterium tumefaciens* T4SS and is involved in plasmid mobilization

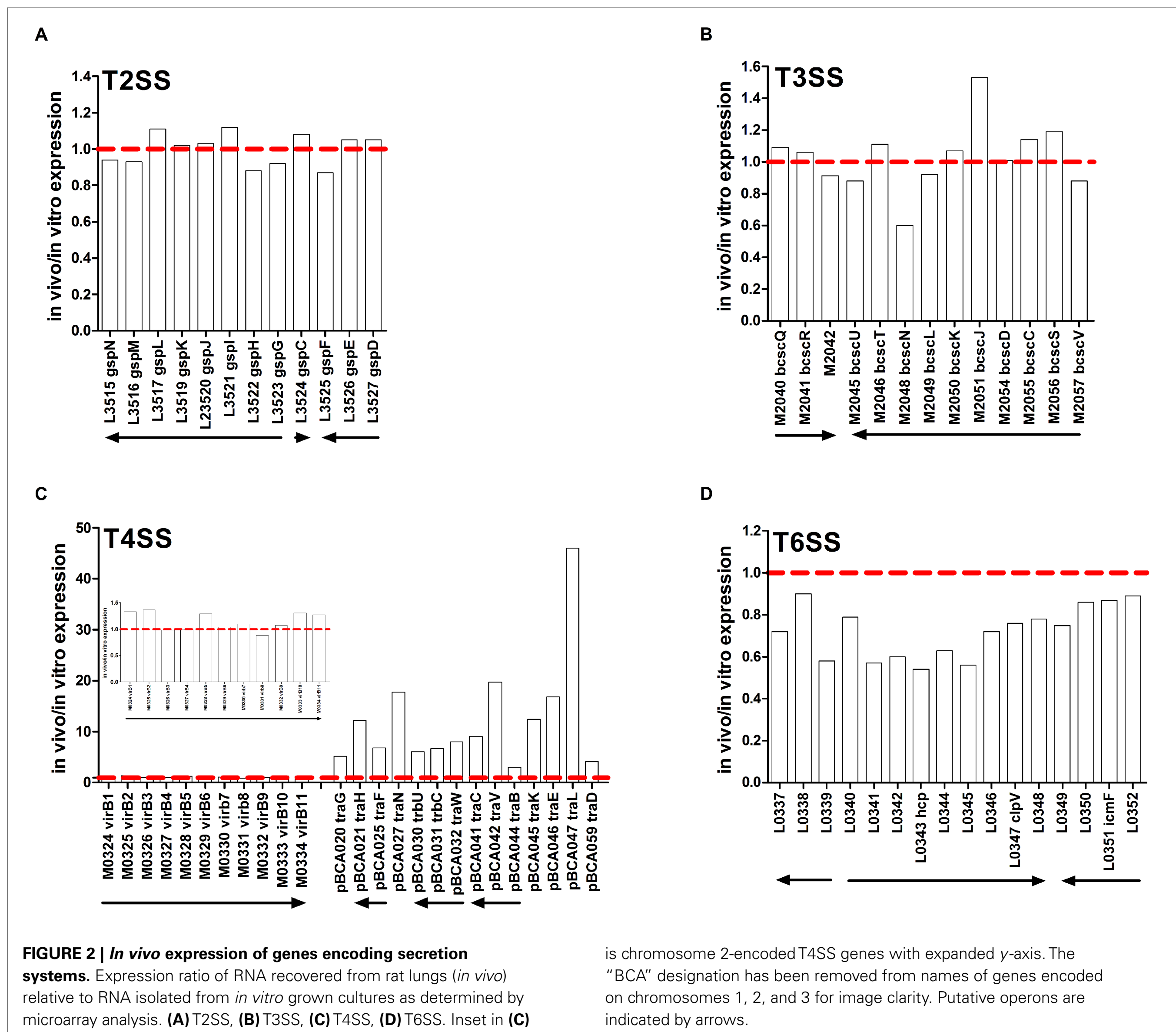

FIGURE 2 | *In vivo* expression of genes encoding secretion systems. Expression ratio of RNA recovered from rat lungs (*in vivo*) relative to RNA isolated from *in vitro* grown cultures as determined by microarray analysis. **(A)** T2SS, **(B)** T3SS, **(C)** T4SS, **(D)** T6SS. Inset in **(C)** is chromosome 2-encoded T4SS genes with expanded *y*-axis. The "BCA" designation has been removed from names of genes encoded on chromosomes 1, 2, and 3 for image clarity. Putative operons are indicated by arrows.

(Engledow et al., 2004). The second T4SS gene cluster exists on a 92.7-kb plasmid that is found in relatively few *B. cenocepacia* strains including J2315 and K56-2 (Engledow et al., 2004) but not AU1054 or MCO-3 (Winsor et al., 2008). This plasmid-encoded T4SS contributes to the plant tissue watersoaking (ptw) phenotype and disease symptoms in onion tissue (Engledow et al., 2004) and increased survival of *B. cenocepacia* in macrophages and airway epithelial cells (Sajjan et al., 2008). Expression of genes on the chromosome 2-encoded T4SS were similar in the *in vitro* and *in vivo* conditions (**Figure 2C**). In contrast, several genes that are part of the plasmid-encoded T4SS were markedly induced *in vivo* at levels ranging from 3- to 46.1-fold (**Figure 2C**). Higher *in vivo* expression of pBCA025 encoding the putative conjugative transfer protein TraF and pBCA045 encoding the putative exported protein TraK was confirmed using qRT-PCR (**Table 3**). These data indicated differential regulation of chromosome 2- and plasmid-encoded T4SS between *in vitro* and *in vivo* conditions.

Table 3 | Microarray and qRT-PCR analysis of selected genes showing differential expression from *in vivo* compared to *in vitro* grown cultures.

Gene	Annotation or predicted function[a]	Fold change[b]	
		microarray	qRT-PCR
BCAL0114	fliC, type II flagellin protein	−8.29	−28.00
BCAL0343	Hcp, hemolysin-coregulated protein	−1.86	−7.82
BCAL1525	Flp type pilus subunit	−11.75	−12.95
BCAM2194	mmsA, methylmalonate-semialdehyde dehydrogenase	2.26	1.74
BCAM2702	prpC, 2-methylcitrate synthase	5.88	8.29
pBCA025	traF, putative conjugative transfer protein	7.10	344.86
pBCA045	traK, putative exported protein	12.43	33.26
pBCA053	Putative extracellular solute-binding protein	480.70	10.44

[a] *Derived from B. cenocepacia J2315 (Holden et al., 2009) at http://www.burkholderia.com (Winsor et al., 2008) or http://www.microbesonline.org (Dehal et al., 2009).*

[b] *Fold change of RNA recovered from rat lungs (in vivo) relative to RNA isolated from in vitro grown cultures as determined by microarray or qRT-PCR analysis.*

The *B. cenocepacia* T6SS comprises 16 genes organized in three adjacent operons on chromosome 1. The T6SS contributes to survival of *B. cenocepacia* in the rat chronic respiratory infection model (Hunt et al., 2004) and influences infection of macrophages (Aubert et al., 2008). Expression of BCAL0339 and BCAL0346 was lower in *B. cenocepacia* growing in medium supplemented with CF sputum compared to control cultures (Drevinek et al., 2008). In our study, expression of six T6SS genes was lower *in vivo* compared to *in vitro* conditions. The BCAL0340–0348 operon exhibited the lowest expression *in vivo* (0.66) compared to the other two T6SS operons (**Figure 2D**). The BCAL0340 operon includes genes encoding the ClpV-like chaperone (BCAL0347) and the hemolysin-coregulated protein (Hcp) (BCAL0343; Aubert et al., 2008). The ClpV-like chaperone is required for secretion of Hcp in *Pseudomonas aeruginosa* (Mougous et al., 2006). The *hcp* gene showed the lowest *in vivo* expression (0.54) of any T6SS gene and the low *hcp* expression *in vivo* was confirmed using qRT-PCR (**Figure 2D**; **Table 3**).

MOTILITY AND Flp TYPE PILUS-ENCODING GENES ARE INDUCED *IN VITRO*

Bacterial motility, attachment, and invasion via flagellar- and pilus-encoding genes are known to be important in virulence (Tomich et al., 2002; Urban et al., 2004). Expression of 24 flagellar-associated genes from eight different operons distributed across chromosome 1 was lower *in vivo*, with the lowest *in vivo/in vitro*

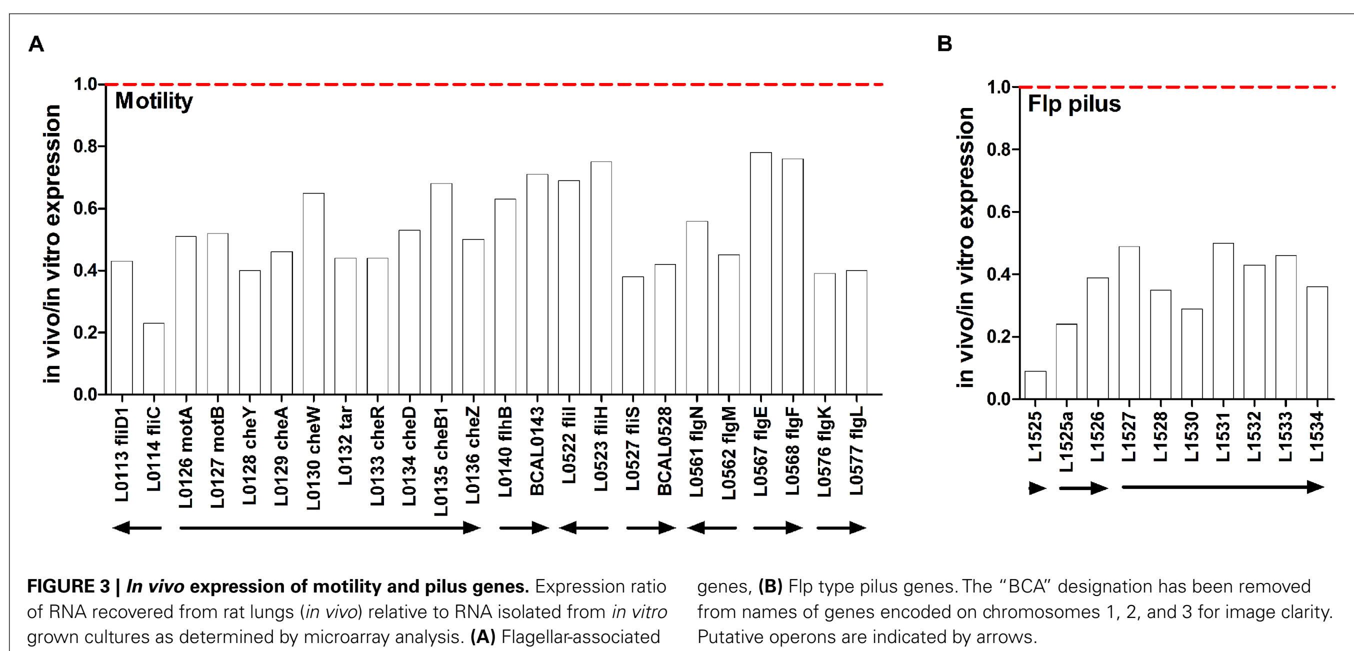

FIGURE 3 | *In vivo* expression of motility and pilus genes. Expression ratio of RNA recovered from rat lungs (*in vivo*) relative to RNA isolated from *in vitro* grown cultures as determined by microarray analysis. **(A)** Flagellar-associated genes, **(B)** Flp type pilus genes. The "BCA" designation has been removed from names of genes encoded on chromosomes 1, 2, and 3 for image clarity. Putative operons are indicated by arrows.

expression ratio (0.23) observed for *fliC*, encoding type II flagellin (**Figure 3A**). Lower expresssion of *fliC in vivo* compared to *in vitro* conditions was independently confirmed using qRT-PCR (**Table 3**).

The genomic locus from BCAL1520–1537 encodes components of a subclass of type IVb prepilins, called a Flp type pilus, that is similar to the *flp–tad–rcp* locus that is involved in adherence and biofilm formation in *Actinobacillus actinomycetemcomitans* (Kachlany et al., 2001; Inoue et al., 2003) and aggregation and biofilm formation in *P. aeruginosa* (de Bentzmann et al., 2006). Ten genes encoding components of the chromosome 1-encoded Flp type pilus had lower *in vivo* expression. The lowest expression was observed for BCAL1525 encoding a Flp type pilus subunit and this trend was confirmed using qRT-PCR (**Figure 3B**; **Table 3**).

IDENTIFICATION OF GENES POTENTIALLY IMPORTANT IN THE HOST ENVIRONMENT

Approximately 300 genes were identified with at least a 1.5-fold change increase in expression *in vivo* compared to *in vitro* grown cultures (**Table A1** in Appendix). Selected genes and their fold change differences are shown in **Table 4**. Many of these genes have not been previously characterized in *B. cenocepacia.* The most common putative functions of these *in vivo* induced genes were related to adaptation to stress or a host environment, metabolism, or nutrient acquisition (**Table 4**).

NOVEL GENES INDUCED *IN VIVO*

A four gene operon (BCAM2703–2700) containing genes involved in the methylcitrate cycle, required for propionyl-CoA metabolism, and fatty-acid utilization, were markedly induced *in vivo* (**Table 4**). Induced *in vivo* expression of BCAM2702 (*prpC*) encoding 2-methylcitrate synthase was confirmed using qRT-PCR (**Table 3**). Genes involved in the methylcitrate and glyoxylate cycles are required for virulence in *Mycobacterium tuberculosis*, which relies more on fatty acids than carbohydrates during infection (Munoz-Elias and McKinney, 2005). Genes involved in the methylcitrate cycle are upregulated in *M. tuberculosis* isolated from murine macrophages (Schnappinger et al., 2003) and are important for growth in macrophages but not for intracellular survival (Munoz-Elias et al., 2006). It is unknown whether the methylcitrate cycle plays a role in *B. cenocepacia* intracellular survival in macrophages. An uncharacterized seven gene operon (BCAM2196–BCAM2191) containing genes putatively involved in lipid metabolism was also induced *in vivo* (**Table 4**), suggesting that fatty-acid metabolism or utilization may be important in *B. cenocepacia* lung infections. Using qRT-PCR we confirmed expression of BCAM2194 (*mmsA*) encoding methylmalonate-semialdehyde dehydrogenase was induced *in vivo* (**Table 3**). A four gene operon (BCAL1212–1215) induced *in vivo* encodes genes for a 2-oxo acid dehydrogenase complex (**Table 4**). The dihydrolipoamide dehydrogenase gene component of a similar complex was shown to be important for persistence and virulence in *Streptococcus pneumoniae* infection models likely due to having a role in capsule synthesis rather than metabolism of 2-oxo acids (Smith et al., 2002).

BCAM0415 encodes a betaine aldehyde dehydrogenase (BADH; **Table 4**). In *P. aeruginosa*, BADH has been shown to

Table 4 | Selected genes induced during chronic lung infection.

Gene	Annotation or predicted function[a]	Fold change[b]
OSMOTIC STRESS AND ADAPTATION		
BCAL1103	Putative OsmB-like lipoprotein	2.1
BCAL2044	LdcA LD-carboxypeptidase A	1.5
BCAL2558	Pyridine nucleotide-disulfide oxidoreductase	2.1
BCAL3297	DPS-family DNA-binding ferritin like protein	1.7
BCAL3310	YceI family protein, osmotic, and acid stress adaptation	1.7
BCAL3311	YceI family protein, osmotic, and acid stress adaptation	1.6
BCAL3314	PqiA paraquat inducible protein A	2.4
BCAL3362	Putative oxidoreductase	1.8
BCAM0027	PadR family regulatory protein, phenolic acid induced stress response	1.5
BCAM0414	Conserved hypothetical protein	2.0
BCAM0415	Putative betaine aldehyde dehydrogenase	1.5
BCAM2700	prpF, putative membrane protein	1.8
BCAM2701	acnA, aconitate hydratase 1	2.7
BCAM2702	prpC, 2-methylcitrate synthase	5.9
BCAM2703	prpB, probable methylisocitrate lyase	2.8
METAL ION TRANSPORT OR METABOLISM		
BCAL0269	Oxidoreductase, molybdopterin-binding domain	1.6
BCAL0366	Nitroreductase family protein, metal ion oxidation	1.6
BCAL0580	Putative chromate transport protein	1.6
BCAL1789	ExbB, iron-transport protein	1.7
BCAL2485	Putative iron–sulfur cluster-binding electron	2.1
BCAL2486	Putative iron–sulfur oxidoreductase	2.1
BCAM0447	Putative exported multicopper oxidase	13.0
BCAM1187	TonB-dependent siderophore receptor	1.7
BCAM1527	Putative cation efflux protein	1.8
BCAM2007	TonB-dependent siderophore receptor	1.6
BCAS0028	Succinylglutamate desuccinylase/aspartoacylase	2.8
BCAS0449	Nickle ion binding-protein-dependent transport	1.6
CARBOHYDRATE TRANSPORT AND METABOLISM		
BCAL0804	*N*-acetylglucosamine transferase	1.5
BCAL1657	Putative ribose transport system	1.8
BCAL1658	Putative ribose ABC transporter ATP-binding	1.5
BCAL1754	Major facilitator superfamily protein, carbohydrate transport	3.5
BCAL2040	Polysaccharide deacetylase, carbohydrate transport	1.5
BCAL3038	ABC transporter ATP-binding component, carbohydrate ABC transporter	1.6
BCAL3039	ABC transporter, membrane permease	1.5
BCAL3040	ABC transporter, membrane permease	1.7

(Continued)

Table 4 | Continued

Gene	Annotation or predicted function[a]	Fold change[b]
BCAL3041	MalE, maltose-binding protein	2.1
BCAL3364	Putative gluconokinase	1.7
BCAM0094	Xylulose kinase	1.7
BCAM1330	Cellulose polysaccharide export protein	1.7
BCAM1333	Cellulose exopolysaccharide acyltransferase	1.6
BCAM1390	Putative aldolase	3.0
BCAM2260	Major facilitator superfamily protein	1.6
BCAS0230	Putative sugar ABC transporter ATP-binding	1.6
AMINO ACID TRANSPORT AND METABOLISM		
BCAL0446	Putative aminotransferase	2.9
BCAL1212	bkdA1, 2-oxoisovalerate dehydrogenase alpha subunit	3.0
BCAL1213	bkdA2, 2-oxoisovalerate dehydrogenase beta subunit	2.9
BCAL1214	bhdB, lipoamide acyltransferase	3.7
BCAL1215	lpdV, dihydrolipoamide dehydrogenase	2.2
BCAL1749	Putative CoA-transferase	2.4
BCAL1750	Conserved hypothetical protein, pyruvate decarboxylase	2.4
BCAL1751	Glyoxalase/bleomycin resistance, amino acid transport	1.7
BCAM0047	Lysine exporter – LysE/YggA	2.6
BCAM0178	ABC transporter periplasmic solute-binding protein	2.7
BCAM0368	Putative branched-chain amino acid transport	1.5
BCAM0459	Cysteine desulfurase	3.6
BCAM0983	leuC1, 3-isopropylmalate dehydratase large subunit	2.9
BCAM0983A	Putative entericidin B-like bacteriolytic toxin	2.0
BCAM0984	leuD1, 3-isopropylmalate dehydratase small subunit	2.1
BCAM1150	3-Hydroxyisobutyrate dehydrogenase	1.6
BCAM1151	Methylmalonate-semialdehyde dehydrogenase	2.4
BCAM1427	LysE family transporter	3.7
BCAM1487	Putative ABC transporter, substrate-binding	3.1
BCAM1488	Putative proline racemase	1.9
BCAM2095	Putative HTH transcriptional regulator	1.6
BCAM2096	puuB gamma-glutamylputrescine oxidoreductase	1.9
BCAM2191	Enoyl-CoA hydratase/isomerase family	1.9
BCAM2192	Enoyl-CoA hydratase/isomerase family protein	2.4
BCAM2193	mmsB, 3-hydroxyisobutyrate dehydrogenase	2.4
BCAM2194	mmsA, methylmalonate-semialdehyde dehydrogenase	2.3
BCAM2195	Putative AMP-binding enzyme	2.5
BCAM2196	Putative acyl-CoA dehydrogenase	2.1
BCAM2237	Putative 2,2-dialkylglycine decarboxylase	2.4
BCAS0397	Metallo peptidase, subfamily M20D	2.0
BCAS0443	Putative binding-protein-dependent transport	5.3
BCAS0574	Amino acid ABC transporter ATP-binding protein	3.7
BCAS0575	Putative binding-protein-dependent transport	2.0
BCAS0577	Periplasmic solute-binding protein	1.5
MEMBRANE PROTEINS		
BCAL0403	Putative outer membrane-bound lytic murein	1.5
BCAL0624	Putative OmpC, outer membrane porin protein precursor	1.6
BCAL1678	Putative outer membrane usher protein precursor, fimD pilin biogenesis	2.4
BCAL2083	YaeT, Outer membrane protein assembly factor	1.5
BCAL2191	Putative 17 kDa membrane protein surface antigen	3.1
BCAL2468	Putative membrane protein	1.9
BCAL2482	Putative OmpC outer membrane protein	3.1
BCAL2505	Putative membrane protein	1.5
BCAL2552	Putative membrane protein	1.5
BCAL2553	Putative membrane protein	1.8
BCAL3033	Probable outer membrane lipoprotein carrier	1.5
BCAL3203	Putative periplasmic TolB protein	1.6
BCAL3204	Putative OmpA family lipoprotein/PAL	1.7
BCAL3205	YbgF, Tol-PAL system protein	1.6
BCAL3473	Putative OmpC-like outer membrane porin	1.9
BCAM0926	Multidrug efflux system transporter protein	5.9
BCAM1207	ABC transporter ATP-binding membrane protein	1.5
BCAM1341	Acyltransferase like protein	3.2
BCAM1425	Putative membrane protein	2.9
BCAM1563	ABC transporter ATP-binding membrane protein	1.7
BCAM1946	Putative quinoxaline efflux system transporter	1.6
BCAM1957	ABC transporter ATP-binding protein	1.6
BCAM2647	Putative membrane protein	1.7
BCAM2648	NAD dependent epimerase/dehydratase family, outer membrane biogenesis	1.6
BCAS0308	Putative flp type pilus assembly protein, TadG-like pilus	2.4
BCAS0463	Putative membrane protein	1.6
pBCA010	Putative membrane protein	3.2

(Continued)

Table 4 | Continued

Gene	Annotation or predicted function[a]	Fold change[b]
pBCA014	Putative membrane protein	3.3
pBCA019	Putative membrane protein	2.4
pBCA026	Putative membrane protein	10.6
pBCA029	Putative membrane protein	8.6
pBCA034	Putative membrane protein	6.0
pBCA036	Putative membrane protein	13.8
pBCA037	Putative membrane protein	7.3
pBCA048	Putative membrane protein	55.6
EXPORTED PROTEINS		
BCAL0305	Putative exported protein	2.2
BCAL0623	Putative exported protein	1.7
BCAL1279	Putative exported protein	1.6
BCAL1499	Putative exported protein	1.8
BCAL1539	Putative exported protein	2.3
BCAL1798	Putative exported protein	1.9
BCAL1961	Putative exported protein	1.9
BCAL2187	Putative exported protein	1.6
BCAL2607	Putative exported protein	2.7
BCAL2615	Putative exported outer membrane porin protein	2.2
BCAL2911	Proline-rich exported protein	1.6
BCAL2956	Putative exported protein	1.5
BCAL3024	Putative exported protein	1.6
BCAL3490	Putative exported protein	2.0
BCAL3492	Putative exported protein	1.6
BCAM0676	Putative exported protein	1.8
BCAM1726	Putative exported protein	2.0
BCAM1742	Putative exported protein	1.9
BCAM1964	Putative exported protein	1.6
BCAM2073	Putative exported protein	3.0
pBCA013	Putative exported protein	6.3
REGULATORY PROTEINS		
BCAL2488	LysR family regulatory protein	2.0
BCAL2529	LysR family regulatory protein	1.5
BCAL3486	ecfM, RNA polymerase sigma factor, sigma-70	1.8
BCAM0422	LuxR superfamily regulatory protein	1.9
BCAM0595	LysR family regulatory protein	2.6
BCAM2025	Sigma-54 interacting regulatory protein	1.9
BCAM2162	MarR family regulatory protein	2.0
BCAS0436	AraC family regulatory protein	1.7
pBCA035	GntR family regulatory protein	18.9

[a]*Derived from B. cenocepacia J2315 (Holden et al., 2009) at http://www.burkholderia.com (Winsor et al., 2008) or http://www.microbesonline.org (Dehal et al., 2009).*

[b]*Fold change of RNA recovered from rat lungs (in vivo) relative to RNA isolated from in vitro grown cultures as determined by microarray analysis.*

be induced by choline and choline precursors (Velasco-Garcia et al., 2006a) which are abundant in infected lung tissues (Wright and Clements, 1987). In addition to playing a role in assimilating carbon and nitrogen from choline, BADH produces glycine betaine which can protect bacteria from high osmolarity stress and oxidative stress in infected tissues. BADH has been proposed as a therapeutic target for *P. aeruginosa* since inactivation of this enzyme leads to intracellular accumulation of betaine aldehyde, which is toxic, and the inability to grow in medium with choline (Velasco-Garcia et al., 2006b; Zaldivar-Machorro et al., 2011). Homologs of other genes induced by osmotic stress in bacteria were also identified as being induced *in vivo* (**Table 4**). BCAL1103, encodes an OsmB-like protein. OsmB is induced by osmotic stress and stationary phase growth conditions in *E. coli* (Jung et al., 1990; Boulanger et al., 2005). BCAL3310 and BCAL3311 are predicted to be co-transcribed YceI family proteins, homologs of which have been shown to be induced in response to osmotic stress in *E. coli* (Weber et al., 2006) and acid stress in *Helicobacter pylori* (Sisinni et al., 2010). BCAL2558, a putative pyridine nucleotide-disulfide oxidoreductase with some similarity to TrxB (thioredoxin reductase) homologs, was induced twofold *in vivo.* TrxB genes are involved in cellular redox processes and defense against oxidative stress and are important in intracellular survival in some pathogens (Bjur et al., 2006; Potter et al., 2009). BCAL3314 encodes a homolog of PqiA-like proteins, which are induced by paraquat and other superoxide generators in *E. coli* (Koh and Roe, 1995). BCAL3297 encodes a DPS-family DNA-binding ferritin. Homologs of these proteins are involved in resistance as well as iron sequestration (Calhoun and Kwon, 2011).

Although many of the *in vivo* induced outer membrane protein encoding genes are uncharacterized, a few have homology to proteins with predicted functions. BCAL3203, L3204, and L3205 form part of the Tol-PAL system membrane complex that is required for membrane integrity and has been implicated in the pathogenesis of several Gram-negative bacteria (Bowe et al., 1998; Godlewska et al., 2009; Paterson et al., 2009). TolB (BCAL3203) is a periplasmic protein involved in biopolymer transport. BCAL3205 is a YbgF homolog which is the last gene of the Tol-PAL complex and interacts with TolA (Krachler et al., 2010). BCAL3204 has been annotated as OmpA/PAL. PAL has been shown to contribute to virulence in several Gram-negative bacteria and in *E. coli* has been shown to be released into the bloodstream contributing to septic shock (Hellman et al., 2002; Liang et al., 2005). A 17 kDa OmpA-like protein has recently been shown to be an immunodominant antigen following intranasal immunization with a *B. cenocepacia* outer membrane protein preparation in mice (Makidon et al., 2010). Although the immunoreactive protein reported to be an OmpA-like protein was not conclusively identified, the partial amino acid sequence determined from a peptide of this molecular mass isolated from SDS-polyacrylamide gels, has 95.8% identity to BCAL3204. There are at least six other OmpA-like proteins in *B. cenocepacia* with varying degrees of sequence identify; however, PAL has been shown to highly immunogenic in other bacteria (Godlewska et al., 2009). Therefore it is possible that the immunodominant antigen identified by Makidon et al. (2010) is PAL. BCAL2191, which was increased threefold *in vivo* (**Table 4**) is predicted to be an outer membrane lipoprotein with similarity to 17 kDa surface antigens in other species and therefore it is also possible that this protein contributed to the observed reaction with antiserum on Western blots in the study by Makidon et al. (2010). Several other proteins involved in biogenesis of membrane and other cell surface components were also identified (**Table 4**) including BCAL2083, a YaeT homolog, which in *E. coli* is an essential gene required for outer membrane assembly

(Werner and Misra, 2005). BCAL2482 is a putative outer membrane porin (OmpC) and is in the same predicted operon as BCAL2486 and BCAL2485, which are iron–sulfur oxidoreductase and iron–sulfur electron transport proteins, respectively. All three genes are induced at least twofold *in vivo* (**Table 4**).

Although ornibactin biosynthesis and uptake genes were expressed at similar levels in the *in vitro* and *in vivo* conditions used in this study, a number of other genes potentially involved in metal ion transport and metabolism were identified as being induced *in vivo* (**Table 4**). These included *exbB*, genes coding for iron–sulfur proteins and receptors for unknown siderophores. One of the most highly induced genes *in vivo* was BCAM0447 which encodes a putative multicopper oxidase (MCO). MCO genes are found in a number of genomes but have only recently been characterized. The MCO protein of *P. aeruginosa* has been shown to be involved in the oxidation of ferrous to ferric iron and may be important in iron acquisition (Huston et al., 2002). MCO homologs are also involved in copper resistance and dissemination in mice in *S. typhimurium* (Achard et al., 2010) and copper tolerance in *Campylobacter jejuni* (Hall et al., 2008).

Genes encoding proteins of unknown function induced *in vivo* are shown in **Table 4** and **Table A1** in Appendix. Many of the expressed genes encode outer membrane proteins (11) and exported proteins (24) that could contribute to cell surface alterations or virulence. Genes encoding six hypothetical proteins were unique to *B. cenocepacia* (**Table A1** in Appendix), whereas, 23 genes encoding hypothetical proteins were conserved in one or more members of the Bcc, of which 11 were also conserved in *Burkholderia pseudomallei* (**Table A1** in Appendix). It is possible that these proteins are involved in adaptation, survival, or virulence in lung infections although further studies are required to determine their potential importance.

PLASMID-ASSOCIATED GENES

Interestingly, the most highly induced genes *in vivo* were located on the plasmid where the vast majority of the genes were expressed at much higher levels *in vivo* than *in vitro* (**Figure 4**). Of the plasmid genes annotated in the J2315 sequence (Winsor et al., 2008), 62 genes had higher expression in the lung infection model. Only one gene, pBCA055, had higher expression levels *in vitro*, and the following genes had similar expression: pBCA003–007, 061, 063, 064, 066–075, 078–081, 083–086, 091–094.

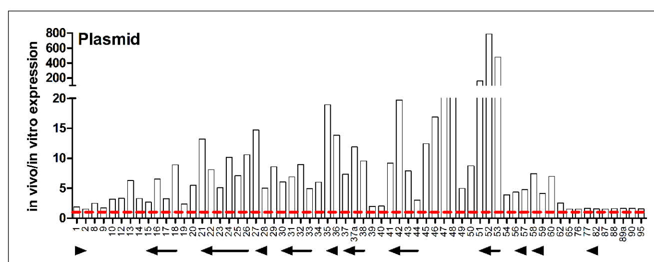

FIGURE 4 | *In vivo* expression of plasmid-encoded genes. Expression ratio of RNA recovered from rat lungs (*in vivo*) relative to RNA isolated from *in vitro* grown cultures as determined by microarray analysis. The "pBCA" designation has been removed from names of plasmid-encoded genes for image clarity. Putative operons are indicated by arrows.

Many of the highly induced genes are part of the plasmid-encoded T4SS, which has been shown to play a role in both plant pathogenesis and survival in eukaryotic cells (Engledow et al., 2004; Sajjan et al., 2008). Expression ratios of genes known or predicted to be a part of the T4SS are shown in **Figure 2C** and described above. The presence of the plasmid-encoded T4SS in the *B. cenocepacia* ET12 lineage strains J2315 and K56-2 but not AU1054 or MCO-3 that entirely lack a plasmid is an interesting characteristic. Gene expression of pBCA054 encoding a LuxR family regulatory protein was higher *in vivo*. Interestingly, the most closely related pBCA054 orthologs are found in *B. pseudomallei* and *Burkholderia mallei*, rather than in other members of the Bcc. pBCA001–002 are *parAB*-like homologs that are putatively involved in chromosome partitioning. pBCA017 is similar to the zeta toxin family of toxin–antitoxin complexes which are involved in programmed cell death to prevent proliferation of plasmid free cells (Gerdes et al., 2005). In addition to plasmid maintenance, toxin–antitoxin pairs can also be involved in responding to nutrient stress. Zeta toxins have recently been shown to target peptidoglycan synthesis triggering autolysis (Mutschler et al., 2011). Zeta toxins are typically paired with epsilon antitoxins; however, there does not appear to be an epsilon homolog adjacent to pBCA017. In some cases, a chromosomal antitoxin can neutralize the plasmid toxin, but in this case toxin expression would not favor plasmid maintenance (Van Melderen and Saavedra De Bast, 2009). Alternatively the toxin can be integrated into other regulatory networks or serve to reduce the overall population to increase nutrient availability for the survivors. Three genes forming an operon (pBCA053–051) exhibited the highest induction of any group of genes *in vivo* (**Figure 4**). pBCA053 encodes a extracellular solute-binding protein involved in dicarboxylate transporter carbohydrate metabolism and we confirmed higher *in vivo* expression of this gene using qRT-PCR (**Table 3**). The second and third genes in the operon encode an exported protein and a protein with homology to LamB/YcsF family proteins, respectively. In addition to the hypothetical proteins noted above, four putative exported proteins, nine putative membrane proteins, 12 conserved hypothetical proteins and 10 hypothetical proteins encoded on the plasmid were induced *in vivo* (**Table A1** in Appendix). Few genes on this plasmid have been studied in detail opening the possibility for identifying proteins with potentially novel functions.

DISCUSSION

In this study, we have identified the gene expression signature of *B. cenocepacia* during lung infections. To the best of our knowledge, this is the first study to apply transcriptomics for any member of the Bcc to study gene expression during infection of a susceptible host. Differential gene expression was observed for characterized virulence genes as well as potential novel virulence genes between *in vitro* and *in vivo* environments.

Altered *in vivo* gene expression was observed for genes encoding enzymes, regulators, structural appendages as well as those contributing to ornibactin biosynthesis, and quorum sensing systems. Lower *in vivo* expression was observed for AHL-dependent QS controlled genes that are directly (e.g., *aidA*) and indirectly (e.g., *shvR*) regulated at the transcriptional level by CepR (Weingart et al., 2005; O'Grady et al., 2011). These observations suggest that

more favorable conditions exist for CepIR-dependent regulation of selected genes in high cell-density ($\sim 10^9$) laboratory-grown cultures compared to the lower cell-density ($\sim 10^5$) in the lung infections, although it is possible that higher expression of QS regulated genes occurs in selected locations in the lungs where bacteria are present in high cell-density biofilms. Since *cepI* and CepR-regulated genes including *zmpA*, *zmpB*, and *shvR* have been shown to be important for virulence in the rat chronic respiratory infection model (Corbett et al., 2003; Sokol et al., 2003; Kooi et al., 2006; Bernier et al., 2008), it is clear that these genes are expressed at sufficient levels to play a role in infection. The majority of characterized virulence genes were similarly expressed in the *in vivo* and *in vivo* conditions. This suggests that expression of these genes is just as important in high cell-density cultures and during lung infections. The contribution of these individual genes has been characterized in one or more infection models highlighting their importance in *B. cenocepacia* pathogenesis. Similar expression of characterized virulence genes during growth *in vivo* in hamsters and *in vitro* has previously been observed for *B. pseudomallei* (Tuanyok et al., 2006).

Increased expression of some genes belonging to the T3SS was observed in the closely related pathogens *B. mallei* and *B. pseudomallei* during infection of mice and hamsters, respectively (Kim et al., 2005; Tuanyok et al., 2006). In the present study, expression of T2SS and T3SS genes was similar between *in vitro* and *in vivo* environments. Genes in these secretion systems appear to be expressed at moderate levels in both *in vitro* and *in vivo* environments. We previously showed expression of the T2SS genes *gspC* and *gspG* was influenced by growth medium composition (O'Grady et al., 2011). A previous study was not able to identify growth conditions that altered expression of T3SS genes suggesting these genes are constitutively expressed (Engledow et al., 2004). The *in vivo* growth conditions provided a stimulus for expression of genes in the plasmid-encoded T4SS but did not affect expression of the T4SS genes on chromosome 2. A mutation in the chromosome 2-encoded T4SS was shown not to contribute to bacterial persistence or histopathology in the rat chronic respiratory infection model (Bernier and Sokol, 2005). To date, no studies have observed such a dramatic increase in expression of plasmid-encoded T4SS genes suggesting that specific environmental signal(s) in the lung environment enabled increased expression of these genes to be detected. It was shown that the plasmid-encoded T4SS contributed to onion tissue maceration through secretion of one or more effectors (Engledow et al., 2004). Whether this plasmid-encoded T4SS or its effectors have a role in mammalian cell/tissue damage has yet to be determined. We observed some T6SS genes had lower *in vivo* expression, in particular those genes on the BCAL0340 operon that includes a gene encoding the secreted effector Hcp. Previous work identified a transposon insertion in each of the three operons of the T6SS locus affected survival of *B. cenocepacia* in the rat chronic respiratory infection model (Hunt et al., 2004).

Using a mouse agar bead infection model, a flagellin mutant failed to cause mortality compared to wild type (Urban et al., 2004). It was also shown that motility mutants were less able to invade epithelial cells (Tomich et al., 2002). Recent work showed expression of flagellar- and chemotaxis-associated genes and motility was reduced in *B. cenocepacia* strains of the ET12 lineage that were isolated from CF patients (Sass et al., 2011). However, a previous study showed transcription of flagellar-associated genes was increased in *B. cenocepacia* J2315 cultured in medium supplemented with CF sputum (Drevinek et al., 2008). Conflicting data regarding expression of flagellar-associated genes in these two studies likely reflect the experimental conditions employed where increased expression of flagellar-associated genes was detected in rapidly growing cultures (Drevinek et al., 2008). The phenotypic characteristics of the *B. cenocepacia* non-motile CF isolates are similar to *P. aeruginosa* clinical isolates which often acquire loss-of-function mutations associated with motility during chronic lung infection (Mahenthiralingam et al., 1994). It has also been shown that *P. aeruginosa* exhibited decreased transcription of flagellar-associated genes when cultured in CF sputum (Wolfgang et al., 2004). In our study, we detected lower *in vivo* expression of genes involved in motility and Flp type pilus formation. This result was likely due to differences in culture conditions between *in vitro* and *in vivo* environments. The agar bead infection model bypasses the colonization step during infection (Cash et al., 1979). Our data suggest expression of these genes is not required in an established infection taking place in the lower respiratory tract. Therefore, decreased expression of these genes was expected since expression of these genes is an energy-expensive process and is more likely associated with rapidly growing cultures than cultures recovered from chronic lung infection.

We identified numerous genes that were induced during lung infections. Many of these genes encode proteins with functions related to metabolism, physiology, or adaptation to a stressful environment. While homologs of some of these proteins have been studied in other pathogens, these proteins have not been specifically studied in *B. cenocepacia.* Several *B. cenocepacia* ET12 lineage strains contain at least a 45-kb fragment of the plasmid found in K56-2 and J2315 (Engledow et al., 2004) while strains AU1054 and MCO-3 lack a plasmid (Winsor et al., 2008). While plasmid-minus derivatives of *B. cenocepacia* J2315 or K56-2 have not been reported, it would be interesting to determine what influence absence of the plasmid may have on infection considering the vast majority of plasmid-encoded genes were induced *in vivo.* Further confirmatory experiments are required to substantiate trends for additional genes that exhibited altered expression in the *in vivo* environmental conditions. Revealing the changes in gene expression that occur in bacterial cells during infection is a first step in understanding the response of bacterial cells to the host environment. Increased expression of genes during infection suggests these genes promote bacterial survival and adaptation in the lungs and potentially influence virulence. The identification of potential novel virulence genes among these *in vivo* induced genes provides an opportunity to characterize these genes in more detail in future studies. Determining what growth conditions alter the expression of these genes and how they are regulated in *B. cenocepacia* will shed light on their expression pattern. Increased expression of genes during lung infection could be due to a change in environmental cues that enable transcriptional activation by a positive regulator(s) or derepression by a negative regulator(s). For potentially novel virulence genes, it will be important to construct mutations and examine their influence on virulence-related

phenotypes and pathogenesis in one or more infection models. This study provides an insight into *B. cenocepacia* gene expression *in vivo* and may provide opportunities to devise strategies to reduce or control *B. cenocepacia* lung infections.

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APPENDIX

Table A1 | *Burkholderia cenocepacia* genes induced during chronic lung infection.

Gene	Annotation or predicted function[a]	Fold change[b]
BCAL0123	Putative glycosyltransferase	2.17
BCAL0194	Putative oxidoreductase	1.62
BCAL0206A	Putative outer membrane protein	2.36
BCAL0226	DNA-directed RNA polymerase beta chain	1.73
BCAL0227	DNA-directed RNA polymerase beta′ chain	1.56
BCAL0269	Putative oxidoreductase	1.59
BCAL0278	Putative type IV pilus secretin	1.58
BCAL0290	Glutamate synthase small subunit	1.78
BCAL0292	2′,5′ RNA ligase family protein	1.66
BCAL0305	Putative exported protein	2.21
BCAL0366	Nitroreductase family protein	1.60
BCAL0403	Putative outer membrane-bound lytic murein	1.54
BCAL0446	Putative aminotransferase	2.86
BCAL0580	Putative chromate transport protein	1.62
BCAL0623	Putative exported protein	1.72
BCAL0624	Putative outer membrane porin protein precursor	1.62
BCAL0658	Allophanate hydrolase subunit 2	1.56
BCAL0668	Serine peptidase, family S9 unassigned	1.52
BCAL0704	d-alanyl-d-alanine carboxypeptidase	1.64
BCAL0804	Putative membrane protein	1.53
BCAL1103	Putative OsmB-like lipoprotein	2.13
BCAL1121	Hypothetical protein	1.65
BCAL1212	2-Oxoisovalerate dehydrogenase alpha subunit	3.02
BCAL1213	2-Oxoisovalerate dehydrogenase beta subunit	2.91
BCAL1214	Lipoamide acyltransferase component of	3.68
BCAL1215	Dihydrolipoamide dehydrogenase	2.22
BCAL1226	Major facilitator superfamily protein	1.52
BCAL1279	Putative exported protein	1.64
BCAL1468	Putative electron transport protein	1.51
BCAL1499	Putative exported protein	1.79
BCAL1539	Putative exported protein	2.30
BCAL1657	Putative ribose transport system	1.77
BCAL1658	Putative ribose ABC transporter ATP-binding	1.56
BCAL1671	Metallo peptidase, subfamily M23B	1.61
BCAL1678	Putative outer membrane usher protein precursor	2.40
BCAL1699	Putative l-ornithine 5-monooxygenase	1.61
BCAL1715	Conserved hypothetical protein	1.53[c]
BCAL1749	Putative CoA-transferase	2.39
BCAL1750	Conserved hypothetical protein	2.39[d]
BCAL1751	Glyoxalase/bleomycin resistance	1.70
BCAL1754	Major facilitator superfamily protein	3.50
BCAL1783_J_0	TonB-dependent receptor (pseudogene)	1.59
BCAL1789	Putative iron-transport protein	1.73
BCAL1798	Putative exported protein	1.95
BCAL1961	Putative exported protein	1.94
BCAL1980	Putative acyl-CoA synthetase	1.54
BCAL1992	Putative acyl-CoA thioesterase precursor	2.00
BCAL2037	Putative ureidoglycolate hydrolase	1.61
BCAL2038	Putative allantoicase	1.53
BCAL2039	Putative uricase	1.72
BCAL2040	Polysaccharide deacetylase	1.54
BCAL2044	Muramoyltetrapeptide carboxypeptidase	1.51
BCAL2083	Outer membrane protein assembly factor YaeT	1.53
BCAL2155	Putative serine acetyltransferase	1.61
BCAL2179	Enolase	1.51
BCAL2187	Putative exported protein	1.56
BCAL2191	Putative membrane protein	3.09
BCAL2272	Conserved hypothetical protein	1.57[d]
BCAL2357	Ketol-acid reductoisomerase	1.59
BCAL2467	Putative lipoprotein	2.10
BCAL2468	Putative membrane protein	1.91
BCAL2475a	Conserved hypothetical protein	1.63[d]
BCAL2476	Hypothetical protein	1.73
BCAL2482	Putative outer membrane protein	3.15
BCAL2485	Putative iron–sulfur cluster-binding electron	2.12
BCAL2486	Putative iron–sulfur oxidoreductase	2.11
BCAL2488	Lysr family regulatory protein	2.07
BCAL2500	Hypothetical protein	1.67
BCAL2505	Putative membrane protein	1.55
BCAL2507	Conserved hypothetical protein	1.93[c]
BCAL2516	Hypothetical protein	1.70
BCAL2529	Putative transcriptional regulator	1.53
BCAL2541	Putative hydrolase	1.53
BCAL2552	Putative membrane protein	1.53
BCAL2553	Putative membrane protein	1.85
BCAL2558	Putative thioredoxin/FAD-dependent pyridine	2.09
BCAL2588	Putative transposase (fragment)	1.81
BCAL2607	Putative exported protein	2.70
BCAL2615	Putative exported outer membrane porin protein	2.16
BCAL2777	Putative *N*-acetylmuramoyl-l-alanine amidase	1.58
BCAL2819	Putative permease protein	1.61
BCAL2911	Proline-rich exported protein	1.58
BCAL2956	Putative exported protein	1.52
BCAL3024	Putative exported protein	1.57
BCAL3033	Probable outer membrane lipoproteins carrier	1.53

(Continued)

Table A1 | Continued

Gene	Annotation or predicted function[a]	Fold change[b]
BCAL3038	ABC transporter ATP-binding component	1.61
BCAL3039	ABC transporter, membrane permease	1.54
BCAL3040	ABC transporter, membrane permease	1.71
BCAL3041	Maltose-binding protein	2.09
BCAL3163	Putative nucleotidyltransferase	1.68
BCAL3203	Putative periplasmic TolB protein	1.64
BCAL3204	Putative OmpA family lipoprotein	1.68
BCAL3205	Putative exported protein	1.62
BCAL3289	Putative glycolate oxidase subunit GlcE	1.64
BCAL3297	Putative ferritin DPS-family DNA-binding	1.67
BCAL3310	Putative exported protein	1.74
BCAL3311	Putative exported protein	1.60
BCAL3314	Putative membrane protein	2.43
BCAL3362	Putative oxidoreductase	1.77
BCAL3364	Putative gluconokinase	1.66
BCAL3473	Putative outer membrane porin	1.87
BCAL3486	Putative RNA polymerase sigma factor, sigma-70	1.84
BCAL3490	Putative exported protein	1.96
BCAL3492	Putative exported protein	1.63
BCAM0027	PadR family regulatory protein	1.51
BCAM0042	Putative aldo/keto reductase	1.75
BCAM0047	Putative transporter – LysE family	2.57
BCAM0094	Xylulose kinase	1.67
BCAM0126	Putative AMP-binding enzyme	1.65
BCAM0166	NADH dehydrogenase	1.66
BCAM0178	Putative periplasmic solute-binding protein	2.74
BCAM0195	Putative non-ribosomal peptide synthetase	1.53
BCAM0207	Putative tyrosine-protein kinase	1.61
BCAM0235	Putative sodium bile acid symporter family	1.51
BCAM0271	Conserved hypothetical protein	1.66[d]
BCAM0273	Conserved hypothetical protein	2.08[d]
BCAM0274a	Hypothetical protein	1.95
BCAM0275	Conserved hypothetical protein	1.60[d]
BCAM0275a	Conserved hypothetical protein	1.70[d]
BCAM0277	Conserved hypothetical protein	1.72[c]
BCAM0303	ABC transporter ATP-binding membrane protein	1.62
BCAM0368	Putative branched-chain amino acid transport	1.52
BCAM0414	Conserved hypothetical protein	2.01[d]
BCAM0415	Putative betaine aldehyde dehydrogenase	1.53
BCAM0422	LuxR superfamily regulatory protein	1.89
BCAM0447	Putative exported multicopper oxidase	13.01
BCAM0459	Cysteine desulfurase	3.60
BCAM0478	Glucosamine – fructose-6-phosphate	1.52
BCAM0502	Conserved hypothetical protein	1.78[c]
BCAM0595	LysR family regulatory protein	2.56
BCAM0630	Putative dehydrogenase	1.73
BCAM0676	Putative exported protein	1.84
BCAM0880	Putative methyltransferase	7.10
BCAM0895	Conserved hypothetical protein	1.55[c]
BCAM0926	Multidrug efflux system transporter protein	5.89
BCAM0944	Putative lipoprotein	1.58
BCAM0983	3-Isopropylmalate dehydratase large subunit	2.87
BCAM0983A	Putative entericidin B-like bacteriolytic toxin	2.01
BCAM0984	3-Isopropylmalate dehydratase small subunit	2.08
BCAM0985	3-Isopropylmalate dehydrogenase	1.55
BCAM1016	Putative ribonuclease	1.81
BCAM1053	Putative reverse transcriptase – Group II	1.72
BCAM1150	3-Hydroxyisobutyrate dehydrogenase	1.64
BCAM1151	Methylmalonate-semialdehyde dehydrogenase	2.40
BCAM1171	Major facilitator superfamily protein	1.55
BCAM1187	TonB-dependent siderophore receptor	1.71
BCAM1207	ABC transporter ATP-binding membrane protein	1.52
BCAM1263	Putative malate/l-lactate dehydrogenase	1.79
BCAM1279	Conserved hypothetical protein	1.54[d]
BCAM1313	Putative amidase accessory protein	1.60
BCAM1315	Aliphatic amidase (acylamide amidohydrolase)	1.55
BCAM1330	Putative polysaccharide export protein	1.73
BCAM1333	Putative exopolysaccharide acyltransferase	1.56
BCAM1341	Conserved hypothetical protein	3.22[c]
BCAM1374	Conserved hypothetical protein	1.87[c]
BCAM1390	Putative aldolase	3.00
BCAM1425	Putative membrane protein	2.88
BCAM1427	LysE family transporter	3.76
BCAM1487	Putative ABC transporter, substrate-binding	3.14
BCAM1488	Putative proline racemase	1.90
BCAM1527	Putative cation efflux protein	1.82
BCAM1563	ABC transporter ATP-binding membrane protein	1.70
BCAM1679	Putative lysylphosphatidylglycerol synthetase	1.62
BCAM1726	Putative exported protein	2.01
BCAM1742	Putative exported protein	1.87
BCAM1775	Putative transglycosylase associated protein	1.76

(Continued)

Table A1 | Continued

Gene	Annotation or predicted function[a]	Fold change[b]
BCAM1823	Putative methyltransferase	1.52
BCAM1901	Hypothetical phage protein	1.65
BCAM1904	Hypothetical phage protein	1.58
BCAM1911	Hypothetical phage protein	1.65
BCAM1946	Putative quinoxaline efflux system transporter	1.61
BCAM1957	ABC transporter ATP-binding protein	1.56
BCAM1964	Putative exported protein	1.57
BCAM2007	TonB-dependent siderophore receptor	1.58
BCAM2025	Sigma-54 interacting regulatory protein	1.87
BCAM2051	Type III secretion system protein	1.73
BCAM2073	Putative exported protein	2.98
BCAM2095	Putative HTH transcriptional regulator	1.57
BCAM2096	Putative gamma-glutamylputrescine	1.87
BCAM2119	Carboxylesterase	1.81
BCAM2162	MarR family regulatory protein	1.99
BCAM2191	Enoyl-CoA hydratase/isomerase family	1.94
BCAM2192	Enoyl-CoA hydratase/isomerase family protein	2.37
BCAM2193	Putative 3-hydroxyisobutyrate dehydrogenase	2.39
BCAM2194	Methylmalonate-semialdehyde dehydrogenase	2.26
BCAM2195	Putative AMP-binding enzyme	2.51
BCAM2196	Putative acyl-CoA dehydrogenase	2.10
BCAM2237	Putative 2,2-dialkylglycine decarboxylase	2.41
BCAM2260	Major facilitator superfamily protein	1.61
BCAM2338	Putative glycosyltransferase	1.53
BCAM2356	Conserved hypothetical protein	1.63[d]
BCAM2453	Putative redoxin protein	1.69
BCAM2479	Putative transporter – LysE family	1.54
BCAM2488	Putative phosphoglycerate/bisphosphoglycerate	1.56
BCAM2504	Conserved hypothetical protein	1.84[c]
BCAM2542	Fenitrothion hydrolase protein FedA	1.57
BCAM2618	Putative periplasmic	1.64
BCAM2623	Conserved hypothetical protein	2.05[c]
BCAM2647	Putative membrane protein	1.71
BCAM2648	NAD dependent epimerase/dehydratase family	1.61
BCAM2685	Conserved hypothetical protein	2.11[c]
BCAM2700	Putative membrane protein	1.81
BCAM2701	Aconitate hydratase 1	2.66
BCAM2702	2-Methylcitrate synthase	5.88
BCAM2703	Probable methylisocitrate lyase	2.78
BCAM2730	Putative tripeptide permease	1.54
BCAS0028	Succinylglutamate desuccinylase/aspartoacylase	2.80
BCAS0043	Putative l-lysine 6-monooxygenase	3.11
BCAS0050	Putative amidohydrolase	1.53
BCAS0053	FMN reductase	2.34
BCAS0097	Putative cobalamin synthesis protein	1.66
BCAS0100	Putative ribokinase	1.52
BCAS0230	Putative sugar ABC transporter ATP-binding	1.58
BCAS0251	Putative lipoprotein	1.61
BCAS0260	Conserved hypothetical protein	2.29[d]
BCAS0278	Tartrate dehydrogenase	1.66
BCAS0308	Putative flp type pilus assembly protein	2.44
BCAS0362	Putative ketopantoate reductase	1.58
BCAS0397	Metallo peptidase, subfamily M20D	2.01
BCAS0436	AraC family regulatory protein	1.66
BCAS0443	Putative binding-protein-dependent transport	5.32
BCAS0449	Putative binding-protein-dependent transport	1.62
BCAS0461	Putative lipoprotein	3.69
BCAS0463	Putative membrane protein	1.64
BCAS0477	Putative lipoprotein	2.07
BCAS0482	Conserved hypothetical protein	4.89[c]
BCAS0513	Putative phage tail protein	1.54
BCAS0519	Hypothetical phage protein	1.64
BCAS0543	Putative phage transcriptional regulator	1.84
BCAS0545	Hypothetical phage protein	1.55
BCAS0547	Putative phage DNA-binding protein	1.54
BCAS0552	Hypothetical phage protein	1.72
BCAS0569	Conserved hypothetical protein	2.31[d]
BCAS0574	Amino acid ABC transporter ATP-binding protein	3.67
BCAS0575	Putative binding-protein-dependent transport	2.02
BCAS0577	Periplasmic solute-binding protein	1.54
BCAS0587_J_0	Aminopyrrolnitrin oxidase PrnD (fragment)	2.33
BCAS0588	Putative membrane protein (fragment)	1.52
BCAS0672	Hypothetical protein	1.91
BCAS0713	Putative short-chain oxidoreductase	1.66
BCAS0730	Putative Na+ dependent nucleoside transporter	2.13
BCAS0750	Putative exported protein	1.82
pBCA001	Putative partition protein	1.93
pBCA002	Putative partitioning protein	1.52
pBCA008	Conserved hypothetical protein	2.48[d]
pBCA009	Conserved hypothetical protein	1.74[d]
pBCA010	Putative membrane protein	3.19
pBCA012	Hypothetical protein	3.34
pBCA013	Putative exported protein	6.32
pBCA014	Putative membrane protein	3.28
pBCA015	Hypothetical protein	2.71
pBCA016	Conserved hypothetical protein	6.54[d]
pBCA017	Conserved hypothetical protein	3.24[d]
pBCA018	Hypothetical protein	8.91
pBCA019	Putative membrane protein	2.40

(Continued)

Table A1 | Continued

Gene	Annotation or predicted function[a]	Fold change[b]
pBCA020	Putative TraG conjugative transfer protein	5.51
pBCA021	Putative TraH conjugative transfer protein	13.21
pBCA022	Conserved hypothetical protein	8.09[c]
pBCA023	Conserved hypothetical protein	5.09[d]
pBCA024	Conserved hypothetical protein	10.16[c]
pBCA025	Putative TraF conjugative transfer protein	7.10
pBCA026	Putative membrane protein	10.57
pBCA027	Putative conjugative transfer protein TraN	14.73
pBCA028	Conserved hypothetical protein	5.03[d]
pBCA029	Putative membrane protein	8.60
pBCA030	Putative conjugative transfer protein TrbC	6.06
pBCA031	Putative TraU conjugative transfer protein	6.92
pBCA032	Putative TraW conjugative transfer protein	8.96
pBCA033	Putative peptidase protein	4.97
pBCA034	Putative membrane protein	6.01
pBCA035	GntR family regulatory protein	18.91
pBCA036	Putative membrane protein	13.82
pBCA037	Putative membrane protein	7.33
pBCA037a	Hypothetical protein	11.90
pBCA038	Hypothetical protein	9.54
pBCA039	Hypothetical protein	1.98
pBCA040	Hypothetical protein	2.04
pBCA041	Putative TraC conjugative transfer protein	9.20
pBCA042	Type IV secretion system TraV	19.71
pBCA043	Thiol:disulfide interchange protein DsbC	7.91
pBCA044	Putative TraB conjugative transfer protein	3.00
pBCA045	Putative exported protein TraK	12.43
pBCA046	Putative TraE conjugative transfer protein	16.87
pBCA047	Type IV conjugative transfer system protein TraL	46.07
pBCA048	Putative membrane protein	55.79
pBCA049	Putative transglycosylase protein	4.97
pBCA050	Hypothetical protein	8.74
pBCA051	LamB/YcsF family protein	159.40
pBCA052	Putative exported protein	789.20
pBCA053	Putative extracellular solute-binding protein	480.70
pBCA054	LuxR family regulatory protein	3.90
pBCA056	Hypothetical protein	4.34
pBCA057	Putative conjugative transfer protein	4.80
pBCA058	Thiol:disulfide interchange protein DsbD	7.43
pBCA059	Putative TraD conjugative transfer protein	4.13
pBCA060	Hypothetical protein	6.97
pBCA062	Conserved hypothetical protein	2.52[d]
pBCA065	Conserved hypothetical protein	1.53[c]
pBCA076	Conserved hypothetical protein	1.55[c]
pBCA077	Conserved hypothetical protein	1.66[d]
pBCA087	NUDIX hydrolase family protein	1.53
pBCA088	Amidohydrolase family protein	1.64
pBCA090	Putative integrase	1.68
pBCA095	Putative ligase	1.59

[a]*Derived from B. cenocepacia J2315 (Holden et al., 2009) at http://www.burkholderia.com (Winsor et al., 2008) or http://www.microbesonline.org (Dehal et al., 2009).*

[b]*Fold change of RNA recovered from rat lungs (in vivo) relative to RNA isolated from in vitro grown cultures as determined by microarray analysis.*

[c]*Conserved hypothetical protein in one or more members of the Bcc and in B. pseudomallei.*

[d]*Conserved hypothetical protein in one or more members of the Bcc.*

Table A2 | *Burkholderia cenocepacia* genes induced during culture *in vitro*.

Gene	Annotation or predicted function[a]	Fold change[b]
BCAL0046	Putative fatty-acid CoA ligase	1.56
BCAL0057	Putative membrane protein	2.17
BCAL0112	Conserved hypothetical protein	1.82
BCAL0113	B-type flagellar hook-associated protein 2	2.71
BCAL0114	Flagellin (type II)	8.29
BCAL0121	Aquaporin Z	3.29
BCAL0126	Chemotaxis protein MotA	2.19
BCAL0127	Chemotaxis protein MotB	2.03
BCAL0128	Chemotaxis two-component response regulator	2.96
BCAL0129	Chemotaxis two-component sensor kinase CheA	2.38
BCAL0130	Chemotaxis protein CheW	1.63
BCAL0132	Chemotaxis protein methyltransferase	2.52
BCAL0133	Putative chemoreceptor glutamine deamidase cheD	2.47
BCAL0134	Chemotaxis response regulator protein-glutamate	2.04
BCAL0135	Chemotaxis protein CheY	1.52
BCAL0136	Chemotaxis protein CheZ	2.09
BCAL0140	Flagellar biosynthetic protein FlhB	2.46
BCAL0143	Putative flagellar biosynthesis protein	1.71
BCAL0147	5,10-Methylenetetrahydrofolate reductase	2.17
BCAL0154	Histone-like nucleoid-structuring (H-NS)	1.97
BCAL0168	Hypothetical protein	2.50
BCAL0169	Conserved hypothetical protein	2.42
BCAL0179	Hypothetical protein	1.87
BCAL0203	Phosphatidylethanolamine-binding protein	1.56
BCAL0212	Putative phenylacetic acid degradation NADH	1.63
BCAL0233	30s Ribosomal protein S10	1.59
BCAL0339	Putative lipoprotein	1.60
BCAL0341	Conserved hypothetical protein	1.75
BCAL0342	Conserved hypothetical protein	1.68
BCAL0343	Conserved hypothetical protein	1.86
BCAL0344	Conserved hypothetical protein	1.58
BCAL0345	Conserved hypothetical protein	1.78
BCAL0356	Putative quinone oxidoreductase	1.51
BCAL0404	Phenylacetate-coenzyme A ligase	1.59
BCAL0406	Probable enoyl-CoA hydratase PaaG	1.56
BCAL0412	Conserved hypothetical protein (pseudogene)	2.11
BCAL0413	Conserved hypothetical protein	1.67
BCAL0431	Conserved hypothetical protein	1.86
BCAL0432	Putative membrane protein	1.61
BCAL0434	Putative exported protein	2.13
BCAL0505	Integrase/recombinase	1.71
BCAL0511	Putative deoxygenases	1.60
BCAL0514	Putative membrane protein	2.52
BCAL0522	Flagellum-specific ATP synthase FliI	1.84
BCAL0523	Flagellar assembly protein FliH	1.63
BCAL0527	Flagellar protein FliS	3.38
BCAL0528	Conserved hypothetical protein	2.80
BCAL0543	Major facilitator superfamily protein	1.64
BCAL0561	Flagella synthesis protein FlgN	1.94
BCAL0562	Negative regulator of flagellin synthesis	2.56
BCAL0567	Flagellar hook protein 1 FlgE1	1.57
BCAL0568	Flagellar basal-body rod protein FlgF (putative	1.66
BCAL0576	Flagellar hook-associated protein 1 (HAP1)	3.18
BCAL0577	Flagellar hook-associated protein 3 (HAP3)	3.20
BCAL0621	Putative cyclic-di-GMP signaling protein	1.56
BCAL0705	Putative d-amino acid aminotransferase	1.55
BCAL0706	Conserved hypothetical protein	1.75
BCAL0744	Appr-1-p processing enzyme family protein	1.74
BCAL0771	Non-heme chloroperoxidase	1.82
BCAL0808	P-loop ATPase protein family protein	1.88
BCAL0812	Sigma-54 modulation protein	1.91
BCAL0813	Putative RNA polymerase sigma-54 factor	2.13
BCAL0831	Putative storage protein	4.32
BCAL0833	Putative Acetoacetyl-CoA reductase	1.78
BCAL0834	Putative membrane protein	2.15
BCAL0842	Putative membrane protein	2.26
BCAL0928	Conserved hypothetical protein	3.58
BCAL0947	Putative membrane protein	1.55
BCAL1055	Histidine transport system permease protein	1.74
BCAL1056	Histidine transport system permease protein	1.81
BCAL1057	Histidine ABC transporter ATP-binding protein	1.98
BCAL1058	AraC family regulatory protein	2.22
BCAL1059	Succinylornithine transaminase	1.81
BCAL1060	Putative arginine *N*-succinyltransferase, alpha	1.63
BCAL1061	Putative arginine *N*-succinyltransferase, beta	1.86
BCAL1062	Succinylglutamic semialdehyde dehydrogenase	1.90
BCAL1063	Succinylarginine dihydrolase	2.58
BCAL1064	Putative succinylglutamate desuccinylase	2.00
BCAL1065	Periplasmic solute-binding protein	1.91
BCAL1146	AraC family regulatory protein	1.73

(Continued)

Table A2 | Continued

Gene	Annotation or predicted function[a]	Fold change[b]
BCAL1155	Putative maleate cis–trans isomerase	3.29
BCAL1159	Putative 2,3-dihydroxybenzoate-AMP ligase	1.52
BCAL1167	Putative exported protein	1.74
BCAL1168	Conserved hypothetical protein	1.71
BCAL1221	Putative porin	1.54
BCAL1233	Putative heat shock Hsp20-related protein	1.65
BCAL1273	Phosphate ABC transporter ATP-binding protein	1.55
BCAL1282	Putative membrane protein	2.39
BCAL1291	Putative membrane protein	1.54
BCAL1292	Putative membrane protein	1.75
BCAL1299	Conserved hypothetical protein	1.51
BCAL1300	Conserved hypothetical protein	1.98
BCAL1316	Conserved hypothetical protein	1.56
BCAL1326	Conserved hypothetical protein	8.68
BCAL1357	Putative exported protein	1.56
BCAL1359	Conserved hypothetical protein	1.54
BCAL1360	Hypothetical protein	1.85
BCAL1373	LysR family regulatory protein	1.94
BCAL1390	Endoglucanase precursor	2.00
BCAL1394	Putative exported protein	1.51
BCAL1396	Putative membrane protein	1.72
BCAL1418	Major facilitator superfamily protein	2.31
BCAL1435	Inositol 2-dehydrogenase	2.41
BCAL1452	Putative methyl-accepting chemotaxis protein	1.75
BCAL1525	Flp type pilus subunit	12.95
BCAL1525a	Putative flp type pilus leader peptidase	4.62
BCAL1526	Putative flp type pilus assembly protein	2.62
BCAL1527	Flp type pilus assembly protein	2.05
BCAL1528	Flp type pilus assembly protein	2.87
BCAL1529	Flp pilus type assembly-related protein	1.93
BCAL1530	Flp pilus type assembly protein	3.56
BCAL1531	Flp type pilus assembly protein	2.02
BCAL1532	Flp type pilus assembly protein	2.40
BCAL1533	Putative lipoprotein	2.15
BCAL1534	Putative exported protein	2.81
BCAL1535	Putative membrane protein	1.71
BCAL1573	Hypothetical phage protein	1.52
BCAL1574	Hypothetical phage protein	1.56
BCAL1577	Hypothetical phage protein	2.26
BCAL1596	Hypothetical phage protein	1.66
BCAL1597	Hypothetical phage protein	1.78
BCAL1610	Periplasmic cystine-binding protein	1.59
BCAL1640	Major facilitator superfamily protein	3.38
BCAL1668	Periplasmic solute-binding protein	2.02
BCAL1677	Putative type-1 fimbrial protein	1.74
BCAL1730	Precorrin-4 C11-methyltransferase	1.71
BCAL1775	Putative demethylase oxidoreductase	1.85
BCAL1791	Conserved hypothetical protein	2.23
BCAL1818	Metallo-beta-lactamase superfamily protein	1.52
BCAL1900	Thioredoxin	1.96
BCAL1913	Putative acetoin catabolism protein	1.64
BCAL1949	Glyoxylate carboligase	1.62
BCAL2027	Conserved hypothetical protein	2.11
BCAL2054	Putative HEAT-like repeat protein	2.58
BCAL2059	Putative 2′–5′ RNA ligase	1.81
BCAL2122	Malate synthase A	1.65
BCAL2143	Ubiquinol oxidase polypeptide I	1.59
BCAL2192	Conserved hypothetical protein	1.74
BCAL2193	Ferredoxin, 2Fe–2S	1.79
BCAL2197	Putative iron–sulfur cluster scaffold protein	2.08
BCAL2198	Cysteine desulfurase	1.69
BCAL2208	Dihydrolipoamide acetyltransferase component of	1.62
BCAL2210	Two-component regulatory system, sensor kinase	1.56
BCAL2253	Conserved hypothetical protein	1.73
BCAL2254	Conserved hypothetical protein	1.56
BCAL2297	Conserved hypothetical protein	1.57
BCAL2305	Putative potassium channel subunit	1.75
BCAL2309	Putative copper-related MerR family regulatory	1.52
BCAL2375	Putative membrane protein	1.79
BCAL2385	Methylglyoxal synthase	1.67
BCAL2479	Putative IstB-like ATP-binding protein	2.81
BCAL2494	Putative exported protein	53.57
BCAL2531	Hypothetical protein	1.56
BCAL2614	LysR family regulatory protein	6.70
BCAL2645	Putative OmpA family membrane protein	1.66
BCAL2671	LysR family regulatory protein	1.74
BCAL2746	Putative citrate synthase	1.79
BCAL2751	Putative ketopantoate reductase	1.68
BCAL2775	Putative 4Fe–4S cluster-binding ferredoxin	1.67
BCAL2792	Putative tryptophan 2,3-dioxygenase	1.70
BCAL2793	Major facilitator superfamily protein	1.68
BCAL2847	Putative methionine aminopeptidase	1.55
BCAL2904	Conserved hypothetical protein	3.85
BCAL2969	Hypothetical phage protein	1.56
BCAL2969a	Hypothetical protein	1.68
BCAL2971	Hypothetical phage protein	1.55
BCAL2973	Putative exported protein	1.64
BCAL2998	Transglycosylase associated protein	2.82
BCAL3006	Cold shock-like protein	3.81
BCAL3018	Conserved hypothetical protein	2.19
BCAL3109	Urease accessory protein	1.69
BCAL3178	LysR family regulatory protein	1.72
BCAL3179	Probable d-lactate dehydrogenase	1.91

(Continued)

Table A2 | Continued

Gene	Annotation or predicted function[a]	Fold change[b]
BCAL3211	Conserved hypothetical protein	1.66
BCAL3227	Conserved hypothetical protein	2.10
BCAL3231	Hypothetical protein	1.63
BCAL3234	Glycosyltransferase	1.69
BCAL3239	Glucosyltransferase	1.84
BCAL3368	Putative regulatory protein	1.85
BCAL3427	Histone H1-like protein	2.68
BCAL3428	Ribonucleoside-diphosphate reductase beta chain	1.58
BCAL3457	Cell division protein FtsZ	1.71
BCAM0010	2-Amino-3-ketobutyrate coenzyme A ligase	2.03
BCAM0011	Threonine 3-dehydrogenase	1.71
BCAM0028	Putative FHA-domain protein	1.58
BCAM0030	Conserved hypothetical protein	8.45
BCAM0031	Conserved hypothetical protein	5.26
BCAM0032	Conserved hypothetical protein	1.71
BCAM0064	Conserved hypothetical protein	1.89
BCAM0067	Putative short-chain dehydrogenase	2.24
BCAM0069	Conserved hypothetical protein	1.57
BCAM0070	Putative hydrolase	1.66
BCAM0096	ABC transporter ATP-binding protein	2.32
BCAM0103	Major facilitator superfamily protein	1.65
BCAM0186	Lectin	2.64
BCAM0188	*N*-acyl-homoserine lactone dependent regulatory	1.57
BCAM0190	Putative aminotransferase – class III	2.44
BCAM0191	Putative non-ribosomal peptide synthetase	2.05
BCAM0192	Conserved hypothetical protein	1.65
BCAM0194	Conserved hypothetical protein	1.94
BCAM0210	Putative transferase	1.71
BCAM0288	Two-component regulatory system, response	1.52
BCAM0446	Outer membrane efflux protein	187.90
BCAM0485	LacI family regulatory protein	4.99
BCAM0487	Conserved hypothetical	1.53
BCAM0504	CsbD-like protein	2.24
BCAM0505	Putative membrane-attached protein	1.67
BCAM0507	CsbD-like protein	2.40
BCAM0521	Putative IstB-like ATP-binding protein	2.85
BCAM0522	Putative integrase	1.76
BCAM0589	Conserved hypothetical protein	1.68
BCAM0622	Two-component regulatory system, sensor kinase	1.58
BCAM0623	Two-component regulatory system, response	1.62
BCAM0633	Conserved hypothetical protein	2.67
BCAM0634	Hypothetical protein	10.80
BCAM0717	Putative Gram-negative porin	2.44
BCAM0753	Putative membrane protein	2.18
BCAM0780	Putative helicase	1.59
BCAM0851	Conserved hypothetical protein	1.83
BCAM0917	Putative DNA primase	1.64
BCAM0918	RNA polymerase sigma factor RpoD	1.52
BCAM0942	Putative exported protein	1.59
BCAM0953	Extracellular solute-binding protein	1.80
BCAM0957	Putative pepstatin-insensitive carboxyl	1.64
BCAM1041	Putative phage coiled coil domain protein	2.06
BCAM1123	ABC transporter ATP-binding protein	1.52
BCAM1138	Major facilitator superfamily protein	1.77
BCAM1140	Putative aldehyde oxidase/xanthine	1.52
BCAM1141	Putative isochorismatase	1.81
BCAM1142	Conserved hypothetical protein	1.76
BCAM1143	Putative hydrolase	1.86
BCAM1144	Putative Asp/Glu/Hydantoin racemase	2.22
BCAM1146	Putative flavoprotein monooxygenase	2.33
BCAM1147	Isoquinoline 1-oxidoreductase alpha subunit	1.98
BCAM1164	Conserved hypothetical protein	1.87
BCAM1175	Putative iron–sulfur cluster protein	1.60
BCAM1213	Putative membrane protein	2.19
BCAM1255	Putative exported protein	1.88
BCAM1265	Putative amino acid permease	1.80
BCAM1316a	Conserved hypothetical protein	2.00
BCAM1316b	Conserved hypothetical protein	1.54
BCAM1335	Glycosyltransferase	1.52
BCAM1358	Gluconate 2-dehydrogenase cytochrome *c* subunit	1.52
BCAM1411	Putative short-chain dehydrogenase	1.53
BCAM1412	Conserved hypothetical protein	10.28
BCAM1413A	Conserved hypothetical protein	24.61
BCAM1414	Conserved hypothetical protein	3.86
BCAM1424	Methyl-accepting chemotaxis protein	1.68
BCAM1443	Putative exported protein	2.64
BCAM1473	Putative di-haem cytochrome *c* peroxidase	1.67
BCAM1491	Putative exported protein	1.56
BCAM1572	Methyl-accepting chemotaxis protein	1.93
BCAM1573	Alpha, alpha-trehalose-phosphate synthase	1.64
BCAM1588	Putative lyase	1.74
BCAM1602	Conserved hypothetical protein	1.59
BCAM1623	Thiolase	2.75
BCAM1643	AMP-binding protein	1.76
BCAM1704	2,3-Butanediol dehydrogenase	1.79
BCAM1710	Putative enoyl-CoA hydratase/isomerase	1.58
BCAM1711	Phenylacetate-coenzyme A ligase	1.57
BCAM1733	Putative membrane protein	2.36
BCAM1734	Putative cytochrome *c*	1.73
BCAM1735	Putative oxidoreductase	1.89
BCAM1736	Conserved hypothetical protein	1.84

(Continued)

Table A2 | Continued

Gene	Annotation or predicted function[a]	Fold change[b]
BCAM1744	Serine peptidase, family S9	1.67
BCAM1777A	Putative exported protein	4.61
BCAM1804	Methyl-accepting chemotaxis protein	2.10
BCAM1869	Conserved hypothetical protein	1.85
BCAM1871	Conserved hypothetical protein	2.64
BCAM1881	Hypothetical phage protein	1.86
BCAM1882	Hypothetical phage protein	1.80
BCAM1919	Hypothetical phage protein	2.12
BCAM1920	Hypothetical phage protein	1.90
BCAM1927	Putative exported protein	1.94
BCAM2021	Methyl-accepting chemotaxis protein	1.94
BCAM2024	Putative membrane protein	2.65
BCAM2048	Type III secretion ssytem protein	1.69
BCAM2052	Putative type III secretion system protein	1.85
BCAM2053	Putative type III secretion system protein	1.98
BCAM2067	Putative undecaprenyl pyrophosphate synthetase	1.54
BCAM2087	Putative lipoprotein	2.24
BCAM2105	MerR family regulatory protein	1.64
BCAM2106	Non-heme chloroperoxidase	1.64
BCAM2167	Conserved hypothetical protein	1.51
BCAM2169	Putative outer membrane autotransporter	1.73
BCAM2198	Serine peptidase, family S49	2.78
BCAM2199	Putative membrane protein	2.03
BCAM2207	Conserved hypothetical protein	1.90
BCAM2210	Putative membrane protein	2.59
BCAM2215	Putative copper resistance protein C precursor	1.55
BCAM2307	Zinc metalloprotease ZmpB	2.28
BCAM2312	Putative ABC-type glycine betaine transport	2.59
BCAM2321	Putative electron transfer flavoprotein alpha	1.74
BCAM2325	Putative dipeptidase	1.75
BCAM2333	Putative glutathione-independent formaldehyde	1.73
BCAM2366	Putative proline iminopeptidase	1.57
BCAM2374	Putative methyl-accepting chemotaxis protein	2.01
BCAM2377	Putative exported protein	3.99
BCAM2378	Putative Xaa-Pro dipeptidyl-peptidase	1.63
BCAM2403	Conserved hypothetical protein	1.97
BCAM2419	Putative outer membrane protein A precursor	1.79
BCAM2444	Putative exported protein	2.52
BCAM2523	Conserved hypothetical protein	2.31
BCAM2545	Major facilitator superfamily protein	1.72
BCAM2563	Methyl-accepting chemotaxis protein	1.62
BCAM2564	Putative aerotaxis receptor	3.44
BCAM2625	Conserved hypothetical protein	2.00
BCAM2640	Putative methyltransferase	1.75
BCAM2657	Putative exported protein	1.55
BCAM2670	Conserved hypothetical protein	2.03
BCAM2674	Putative cytochrome oxidase subunit I	1.88
BCAM2677	Putative membrane protein	1.76
BCAM2690	Putative thioesterase	1.71
BCAM2711	H-NS histone family protein	1.77
BCAM2712	Conserved hypothetical protein	1.57
BCAM2748	Putative sigma factor	1.53
BCAM2754	Putative ketoreductase	1.70
BCAM2771	Putative dihydrodipicolinate synthetase	1.61
BCAM2806	Putative sugar ABC transporter ATP-binding	2.87
BCAM2837_J_0	Two-component regulatory system, response	1.87
BCAM2837_J_1	Two-component regulatory system, response	2.42
BCAS0018	MarR family regulatory protein	1.55
BCAS0040	Major facilitator superfamily protein	1.55
BCAS0074	Conserved hypothetical protein	1.52
BCAS0085	Organic hydroperoxide resistance protein	1.53
BCAS0172	Putative dehydrogenase	1.51
BCAS0173	Putative tautomerase	1.60
BCAS0189	Conserved hypothetical protein	1.82
BCAS0190	Putative H-NS family DNA-binding protein	2.38
BCAS0225	LysR family regulatory protein	2.71
BCAS0226	Putative hydrolase	1.99
BCAS0256	Putative porin protein	1.51
BCAS0263	Two-component regulatory system, response	3.60
BCAS0264	Two-component regulatory system, sensor kinase	2.41
BCAS0290	Conserved hypothetical protein	1.73
BCAS0291	Periplasmic solute-binding protein	2.74
BCAS0292	Conserved hypothetical protein	10.91
BCAS0293	Nematocidal protein AidA	51.98
BCAS0294	Putative GtrA-like family protein	3.06
BCAS0295	Glycosyltransferase	1.52
BCAS0299	Flp type pilus subunit	1.68
BCAS0399	Citrate-proton symporter	5.66
BCAS0400	Putative periplasmic solute-binding protein	2.00
BCAS0403	Hypothetical protein	2.12
BCAS0406	Putative exported protein	1.64
BCAS0409	Zinc metalloprotease ZmpA	6.72
BCAS0452	Putative membrane protein	1.56
BCAS0462	Putative alpha-galactosidase	2.14
BCAS0467	Putative transcriptional regulator – DeoR	1.67

(Continued)

Table A2 | Continued

Gene	Annotation or predicted function[a]	Fold change[b]
BCAS0481	Putative lipoprotein	1.86
BCAS0510	Hypothetical phage protein	2.29
BCAS0540	Hypothetical phage protein	1.72
BCAS0548	Hypothetical phage protein	1.69
BCAS0572	Putative exported protein	1.70
BCAS0573	Putative exported protein	1.72
BCAS0576	Putative binding-protein-dependent transport	1.52
BCAS0579	Putative exported protein	2.01
BCAS0595	Putative sugar efflux transporter	1.53
BCAS0596	Conserved hypothetical protein	1.58
BCAS0661C	Hypothetical protein	1.83
BCAS0662	Conserved hypothetical protein	1.91
BCAS0669	Hypothetical protein	1.90
BCAS0700	Putative oxygen-insensitive NAD(P)H	1.52
BCAS0717	Hypothetical protein	2.26
BCAS0773	Putative exported protein	1.64
pBCA055	Putative membrane protein	18.16

[a] *Derived from B. cenocepacia J2315 (Holden et al., 2009) at http://www.burkholderia.com (Winsor et al., 2008) or http://www.microbesonline.org (Dehal et al., 2009).*

[b] *Fold change of RNA isolated from in vitro grown cultures relative to RNA recovered from rat lungs (in vivo) as determined by microarray analysis.*