Burkholderia thailandensis strain E555 is a surrogate for the investigation of Burkholderia pseudomallei replication and survival in macrophages

A. Kovacs-Simon 1*, C. M. Hemsley 1, A. E. Scott 2, J. L. Prior 1,2 and R. W. Titball 1

Abstract

Background: Burkholderia pseudomallei is a human pathogen causing severe infections in tropical and subtropical regions and is classified as a bio-threat agent. B. thailandensis strain E264 has been proposed as less pathogenic surrogate for understanding the interactions of B. pseudomallei with host cells.

Results: We show that, unlike B. thailandensis strain E264, the pattern of growth of B. thailandensis strain E555 in macrophages is similar to that of B. pseudomallei. We have genome sequenced B. thailandensis strain E555 and using the annotated sequence identified genes and proteins up-regulated during infection. Changes in gene expression identified more of the known B. pseudomallei virulence factors than changes in protein levels and used together we identified 16% of the currently known B. pseudomallei virulence factors. These findings demonstrate the utility of B. thailandensis strain E555 to study virulence of B. pseudomallei.

Conclusions: A weakness of studies using B. thailandensis as a surrogate for B. pseudomallei is that the strains used replicate at a slower rate in infected cells. We show that the pattern of growth of B. thailandensis strain E555 in macrophages closely mirrors that of B. pseudomallei. Using this infection model we have shown that virulence factors of B. pseudomallei can be identified as genes or proteins whose expression is elevated on the infection of macrophages. This finding confirms the utility of B. thailandensis strain E555 as a surrogate for B. pseudomallei and this strain should be used for future studies on virulence mechanisms.

Keywords: Burkholderia pseudomallei, Burkholderia thailandensis strain E555, Transcriptome, Proteome, Virulence
B. thailandensis is a close relative of B. pseudomallei and is also found in the soil in tropical regions of the world [17]. The genomes of B. pseudomallei and B. thailandensis are very similar with two highly syntenic chromosomes, which have similar numbers of coding regions, similar assignments of encoded proteins to families and similar numbers of horizontally acquired genomic islands [18]. However, infections of humans with B. thailandensis are rare and the bacterium can be handled outside of a high containment laboratory. Several previous studies have used B. thailandensis as a surrogate for B. pseudomallei, to study virulence mechanisms [19–37]. These studies have used B. thailandensis isolates, such as strain E264, that replicate more slowly than B. pseudomallei in cell cultures [38, 39]. These strains lack the capsular polysaccharide of B. pseudomallei, which is a key virulence determinant [14, 40]. However, a minority of B. thailandensis strains possess a B. pseudomallei-like capsule [21, 40], though they are not virulent in murine models of disease [40]. In this study we set out to assess one of these strains as a surrogate for B. pseudomallei, in the hope that this would more faithfully mimic the behavior of B. pseudomallei in cell culture, and could be used to study virulence.

Results
Genome sequencing
We selected B. thailandensis strain E555 (a natural isolate previously described by Sim et al. [40]) for our study because, unlike most B. thailandensis strains, it possesses a capsular polysaccharide which is similar to B. pseudomallei [40, 41]. We sequenced the B. thailandensis strain E555 available in our laboratory. Sequence data are available at DDBJ/ENA/GenBank under the accession SJET00000000. In B. pseudomallei, the wcb operon, which includes capsular polysaccharide genes, consists of 20 genes (wcbA-T [42, 43]) of which 19 are also encoded in the B. thailandensis strain E555 genome (wcbN is not present) (Additional file 3: Table S1 C). In addition to the capsule, B. thailandensis strain E555 has been shown to exhibit several B. pseudomallei-like phenotypes such as colony wrinkling, resistance to human complement binding, and survival in macrophages [40]. However, despite these similarities, B. thailandensis strain E555 shows the same level of virulence in mice as other B. thailandensis strains [40]. Using RAST [44] we predicted 6508 open reading frames in our B. thailandensis strain E555 genome sequence data (ORFs; Additional file 3: Table S1 A and B). We compared the amino acid sequences of B. pseudomallei strain K96243 proteins to B. thailandensis strain E555 and B. thailandensis strain E264 and found 4535 proteins in both B. thailandensis strain E555 and strain E264, 94 only in B. thailandensis strain E555 and 380 only in B. thailandensis strain E264. The remaining 914 proteins were not found in either B. thailandensis strain and of these 233 were encoded by genes located in the genome islands in B. pseudomallei.

Infection model
We first infected J774A.1 mouse macrophage cells with B. thailandensis strain E555 and the number of intracellular bacteria was measured at intervals. We compared this data with data previously obtained in our laboratory using an identical method with B. pseudomallei strain K96243 or B. thailandensis strain E264 which is commonly used as a surrogate for B. pseudomallei [39]. B. thailandensis strain E555 and B. pseudomallei strain K96243 had similar growth rates in macrophages (doubling times are 2.02 h for B. pseudomallei strain K96243 and 2.06 h for B. thailandensis strain E555 over the 4 h period between 4 h and 8 h post-infection), while the growth of B. thailandensis strain E264 was slower and showed a different growth pattern (p values between B. pseudomallei strain K96243 and B. thailandensis strain E264 were p < 0.001 at 4 h, 6 h and 8 h post-infection, see Fig. 1).

Analysis of the global bacterial transcriptome
To investigate how B. thailandensis strain E555 adapts to the intracellular environment in J774A.1 mouse macrophage cells, the transcriptional and translational landscapes of the bacteria were profiled, using RNA-seq or mass spectrometry, after overnight growth in broth culture and during macrophage infection. Nine genes (Additional file 4: Table S2) were selected to validate the data generated from the RNA-seq study using RT-qPCR (the 23S rRNA was used as an internal control) [45, 46] (Fig. 2).

We identified broadly similar numbers of genes expressed in broth-cultured bacteria (n = 5071) or in bacteria from macrophages (n = 4378 and n = 5112 at 5 and 6 h post-infection) and most (n = 3893) were expressed in all 3 samples (Fig. 3 a and Additional file 5: Table S3). The abundance of transcripts at 5 h or 6 h post-infection (Fig. 3 a) showed a high correlation (R = 0.904; Additional file 1: Figure S1). Unique transcripts were more abundant at 6 h (n = 329) than at 5 h (n = 91) post-infection indicating that the adaptation of the bacteria to the intracellular environment was still in
progress between 5 h and 6 h post-infection. Expression of all of the \textit{wcb} genes ($n = 19$) encoded in the \textit{B. thailandensis} strain E555 genome was detected in the transcriptomes of bacteria grown in broth and bacteria grown in macrophages (at 5 h and 6 h post-infection) (Additional file 7: Table S5.F). Overall, we concluded that the RNA-seq analysis provided a robust picture of the \textit{B. thailandensis} transcriptome.

Analysis of the global bacterial proteome

For proteomics, we used an immunomagnetic method to first purify bacterial cells from infected macrophages. The protocol was assessed by visualising the protein extracts separated by SDS-PAGE. We tested three different antibodies against lipopolysaccharide, capsular polysaccharide or against \textit{B. pseudomallei} cells, each conjugated separately onto magnetic beads. Using

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**Fig. 1** Intracellular survival of \textit{Burkholderia} strains in mouse macrophages. J774A.1 mouse macrophages were exposed to \textit{B. thailandensis} strain E555 at an MOI of 10 for 2 h. Following removal of the extracellular bacteria, infected macrophage cells were incubated with kanamycin (1 mg/ml in the first 2 h and 0.25 mg/ml afterwards) for 1, 2, 4 and 6 h and the mean numbers (with standard errors of the mean) of intracellular bacteria were determined. \textit{B. thailandensis} strain E264 and \textit{B. pseudomallei} strain K96243 values were adapted from Wand et al. [39]. There was no statistically significant difference between the growth rate of \textit{B. thailandensis} strain E555 and \textit{B. thailandensis} strain E264, or between the growth rate of \textit{B. thailandensis} strain E555 and \textit{B. pseudomallei} strain K96243 at any time point ($p > 0.05$). In contrast, the difference between the growth rate of \textit{B. pseudomallei} strain K96243 and \textit{B. thailandensis} strain E264 was significant: $p < 0.001$ at 4 h, 6 h and 8 h post-infection, (indicated by ***)

**Fig. 2** Validation of RNASeq data by RT-qPCR. RNASeq (green) and RT-qPCR (blue) data at 6 h post-infection in macrophages. Tested genes: [i] 3 genes (Fig869/728.6.peg.5909*, 6274, 1473) with significantly increased, [ii] 4 genes (Fig869/728.6.peg.4093*, 5186, 1728, 355) with significantly decreased and [iii] 2 genes (Fig869/728.6.peg.5948*, 731) without significantly increased level of expression during macrophage infection (a known virulence factor* was included in each group). \textit{B. pseudomallei} homologs of these genes are shown in brackets. Internal control: 23S rRNA. Expression data is detailed in Additional file 4: Table S2. Horizontal lines show 2-fold change in expression cut-off. Asterisks on the graph indicate statistical significance.
antibodies against capsular polysaccharide, with an incubation time of 25 min at 4 °C, was the most efficient way of recovering bacteria from macrophage lysates (data not shown). Bacterial proteins were then identified by mass spectrometry (Additional file 6: Table S4). We detected lower numbers of proteins than corresponding gene transcripts. For over 90% of the proteins we identified the corresponding mRNA transcripts but conversely we could not detect the proteins corresponding to the majority of the transcripts (Fig. 4). These transcripts were not close to the limits of detection, suggesting that the inability to detect the corresponding protein was not linked to low levels of gene expression (Additional file 1: Figures S2 and S3). In contrast to the transcriptome data, not all of the proteins encoded by the wcb operon were found in the proteome; we detected 12 in broth grown bacteria and 7 in bacteria isolated from macrophages (Additional file 7: Table S5). Compared with transcriptome mapping, our proteomic analysis revealed the over-representation of cytoplasmic proteins. In contrast, proteins of unknown location were under-represented compared with transcriptome data sets (Fig. 5), possibly reflecting the limitations of pSORTb in predicting exported proteins [47], which would be difficult to detect in our proteome samples.

**Intracellular metabolism**

We investigated which cellular processes are required and which are dispensable in bacteria during infection of macrophages. We found up-regulation, of several fatty acid biosynthetic enzymes both in the 6 h transcriptome and in the 6 h proteome. Genes encoding proteins with roles in the valine, leucine and isoleucine biosynthetic and degradation pathways were also up-regulated at 6 h post-infection. In addition, four (argT, hisM, hisQ, hisP) out of five components of a lysine, arginine, ornithine and histidine ABC-type transporter and a branched chain amino acid transporter (livK, livH, livM, livG, livF) were up-regulated 6 h after infection. We found down-regulation of a number of genes involved in nitrogen metabolism (metabolic enzymes and two-component systems). Linked to this pathway, glutamate metabolism was down-regulated. Finally our findings that cysteine and methionine metabolism were downregulated in bacteria isolated from macrophages suggests that these pathways are dispensable during infection.
Comparison of the significantly regulated transcriptome and proteome

We next identified genes and proteins that were significantly ($p < 0.05$) up- or down-regulated at least two-fold in bacteria isolated from macrophages, compared with broth-cultured bacteria. We found that 11% of genes (265 up-regulated and 438 down-regulated) were differentially regulated at 5 h post-infection (Additional file 7: Table S5), and 15% of genes (396 up-regulated and 583 down-regulated) were differentially regulated at 6 h post-infection (Additional file 7: Table S5 and Additional file 1: Figure S4.A). A total of 242 genes were up-regulated at both 5 h and 6 h post-infection. 23 genes were up-regulated only at 5 h post-infection and 154 genes were up-regulated only at 6 h post-infection. Among the 23 genes, 6 were known virulence factors of which 5 are components of the T3SS-3. Despite the larger number of up-regulated genes, only an additional 10 genes are known virulence factors of the 154 genes specific for 6 h post-infection (Additional file 7: Table S5.D). For our subsequent studies we used transcript data at 6 h post-infection.

In parallel, the proteomic landscape was investigated. We applied the same statistical criteria as with the transcriptomic data and identified 109 up-regulated and 364 down-regulated proteins 6 h post-infection (Additional file 7: Table S5 and Additional file 1: Figure S4.B). The number of up- or down-regulated proteins was smaller than the number of up- or down-regulated genes (Fig. 6). At 6 h post-infection we found 45% overlap between significantly up-regulated proteins and genes and 34% overlap between significantly down-regulated proteins and genes (Fig. 6). We found one up-regulated protein but with decreased corresponding mRNA, and 16 genes with decreased protein levels but with increased corresponding mRNA levels (Additional file 7: Table S5).

The groups which showed the greatest degree of enrichment in bacteria isolated from macrophages were genes encoding proteins involved in translation, and proteins involved in post-translational modification functions (Fig. 6 a). Proteins associated with amino acid transport and metabolism, and energy production were over-represented in the significantly down-regulated proteome but not in the significantly down-regulated transcriptome (Fig. 7 b).
Virulence factors
We compiled a list of 326 genes encoding virulence factors of *B. pseudomallei* which had been identified from previous experimental studies (Additional file 2: Supplementary References, Additional file 8: Table S6.A). Of these we found 267 in both *B. thailandensis* strain E555 and strain E264, 17 only in *B. thailandensis* strain E555 and 19 only in *B. thailandensis* strain E264. The remaining 23 virulence-associated factors were not found in either *B. thailandensis* strain (Additional file 8: Table S6.A).
Table S6). Of the 17 candidate virulence-associated factors found in both *B. thailandensis* strain E555 and *B. pseudomallei* but not in *B. thailandensis* strain E264, 9 are implicated in capsule biosynthesis, 6 genes encoded hypothetical proteins, 1 encoded a cell surface protein and 1 a non-ribosomal peptide synthetase. Of the 23 virulence-associated factors found only in *B. pseudomallei* strain K96243, 16 are hypothetical proteins or have either unknown or predicted function, 6 are predicted to be involved in transport, metabolism or catabolism of amino acids, lipids or secondary metabolites, and 1 is predicted to have a role in intracellular trafficking and secretion. These proteins might merit further attention as potential virulence determinants of *B. pseudomallei*. All or most of the genes in T6SS-1, T6SS-2, T6SS-3, T6SS-5 and T6SS-6 are present in the genome of the *B. thailandensis* strain E555 but the T6SS-4 locus is missing (only two genes, the homologs of BPSS0166 and BPSS0185, are present).

In bacteria isolated from macrophages at 6 h post-infection expression of 284 of the virulence associated genes and 102 of the virulence associated proteins were detected (Fig. 4 b). Most (97%) of the virulence-associated factors detected in the proteome were also detected in the transcriptome (Fig. 4 b). Of these, 47 genes and 16 proteins were up-regulated compared to broth grown bacteria (Fig. 6 a) and some virulence-associated factors were down-regulated (Fig. 6 b).

We found that 14 (56%) of the 25 most highly up-regulated genes in *B. thailandensis* isolated from macrophages are known virulence factors of *B. pseudomallei* (Table 1). 11 of these genes encode components of the type VI secretion system-1 (T6SS-1) (Fig. 8 b) and the remaining genes encode BsaN, BimA and BprB which are associated with virulence [13, 48–50]. We also found that 10 of the 30 type III secretion system-3 (T3SS-3) genes were upregulated at 6 h post-infection (Fig. 8 a, Additional file 5: Table S3.D and Additional file 7: Table S5). One of the T3SS-3 genes (*bsaN*) was in the 25 most up-regulated genes (Table 1). RT-PCR data validated the up-regulation of *bsaR*, a component of the T3SS-3, and *tssD-5 (hcpI)*, a component of the T6SS-1 (Fig. 2).

Four genes involved in iron acquisition were up-regulated in *B. thailandensis* strain E555 isolated from macrophages (Table 1; Bt E555 fig|869,728.6.peg.2756, 3100, 6277 and 3095; in *B. pseudomallei* BPSS0244, BPSL1779, BPSS0362 and BPSL1783, respectively) highlighting the importance of iron acquisition during infection.

The known importance of lipopolysaccharide [51] during infection by *B. pseudomallei* was mirrored by our finding of increased expression of the *rfbA-rfbB* lipopolysaccharide ABC-transporter genes and increased expression of lipopolysaccharide biosynthetic genes and proteins in *B. thailandensis* strain E555 within macrophages. We also found up-regulation of *B. thailandensis* strain E555 fig|869,728.6.peg.5896 (BPSS1490 in *B. pseudomallei*) which encodes a peptidoglycan biosynthetic enzyme (Table 1). Of the capsule genes only *webA* and *webK* were significantly up-regulated (in the 6 h transcriptome and in the 6 h proteome, respectively).

The role of the SyrP-like protein Bt E555 fig|869,728.6.-peg.3093 (BPSL1785 in *B. pseudomallei*) in virulence has not been tested experimentally in *B. pseudomallei* but there is evidence that it plays a role in the virulence of *Pseudomonas syringae* through the control of syringomycin production [52] and this gene was highly up-regulated in *B. thailandensis* strain E555 isolated from macrophages (Table 1).

Using our data we investigated whether up-regulated genes or up-regulated proteins were more likely to be virulence-associated factors. Significantly up-regulated genes identified 16% of the known virulence-associated factors, whilst significantly up-regulated proteins revealed only 5% (Table 2 and Additional file 9: Table S7). However, the proteome analysis identified virulence factors with higher degree of confidence than the transcriptome analysis (14.7% versus 11.9%). Combining the significantly up-regulated gene and protein datasets did not significantly improve the predictive power. But looking at the genes common in both the significantly up-regulated transcriptome and the significantly up-regulated proteome increased the likelihood of detection of virulence factors (22%; Table 2). In comparison, the likelihood of randomly identifying a virulence-associated factor from the genome is approximately 5%.

Some proteins, such as those which are membrane located or secreted, are difficult to recover while the corresponding transcripts are not subject to these constraints. Since the most likely cellular locations of virulence factors are at the cell surface and in the extracellular milieu, this might limit the utility of proteomics for the identification of virulence factors. However, extracellular, outer membrane and periplasmic proteins were found in similar proportions in the proteomes and in the corresponding transcriptomes. Nevertheless, all proteins are initially translated in the cytoplasm allowing their detection in this cellular compartment. Therefore, proteome analysis might also provide confident data for identification of classic virulence candidates, especially because the majority of cellular processes occur at protein level and not at transcript level.

**Discussion**

Central to this study is the proposition that *B. thailandensis* is a surrogate for understanding the interactions of *B. pseudomallei* with host cells. Most previous studies have used *B. thailandensis* strain E264 for these studies,
Table 1 List of 25 most up-regulated genes in the transcriptome of *B. thailandensis* strain E555 during infection of macrophages after 6 h post-infection

| Bt E555 log2 fold change in T6 transcriptome | Bt E555 gene function assigned by RAST | Bp Sanger ID | Bp gene name | Bp gene product | Significantly upregulated in T5 transcriptome? | Significantly upregulated in T6 proteome? |
|---------------------------------------------|----------------------------------------|--------------|--------------|----------------|---------------------------------------------|-----------------------------------------|
| 5909                                        | Hcp                                    | BPSS1498     | tssD-5 (hcp1) | type VI secretion system protein | Yes                                         | Yes                                     |
| 5906                                        | N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28) | BPSS1490 | –           | N-acetylmuramoyl-L-alanine amidase | Yes                                         | Yes                                     |
| 5907                                        | Uncharacterized protein ImpB            | BPSS1496     | tssB-5       | type VI secretion system protein | Yes                                         | Yes                                     |
| 5908                                        | Uncharacterized protein ImpC            | BPSS1497     | tssC-5       | type VI secretion system protein | Yes                                         | Yes                                     |
| 5910                                        | Uncharacterized protein similar to VCA0109 | BPSS1499 | tssE-5       | type VI secretion system protein | Yes                                         | Yes                                     |
| 5897                                        | Plasmid pIB6 ORFA DNA                  | BPSS1491     | –           | hypothetical protein | Yes                                         | Yes                                     |
| 2756                                        | TonB-dependent hemin, ferrichrome receptor | BPSS0244 | –           | BhuR (Outer membrane heme receptor) | Yes                                         | No                                      |
| 5913                                        | ClpB protein                           | BPSS1502     | tssH-5       | type VI secretion system protein | Yes                                         | No                                      |
| 5911                                        | Protein ImpG/VasA                      | BPSS1500     | tssF-5       | type VI secretion system protein | Yes                                         | No                                      |
| 3093                                        | SyrP-like protein                      | BPSSL1785    | –           | hypothetical protein | Yes                                         | Yes                                     |
| 3100                                        | ABC-type siderophore export system, fused ATPase and permease components | BPSSL1779 | –           | siderophore biosynthesis related ABC transport protein | Yes                                         | No                                      |
| 3113                                        | Non-ribosomal peptide synthetase modules, pyoverdine | BPSSL1778 | –           | siderophore related no-ribosomal peptide synthase | No                                          | No                                      |
| 5914                                        | VgrG protein                           | BPSS1503     | tssI-5       | type VI secretion protein | Yes                                         | No                                      |
| 5915                                        | hypothetical protein                   | BPSS1504     | tagAB-5      | type VI secretion system-associated protein | Yes                                         | Yes                                     |
| 5839                                        | Fig. 00464790: hypothetical protein    | BPSS13352    | –           | hypothetical protein | Yes                                         | Yes                                     |
| 5920                                        | Uncharacterized protein ImpJ/VasE      | BPSS1509     | tssK-5       | type VI secretion system protein | Yes                                         | Yes                                     |
| 5922                                        | IcmF-related protein                   | BPSS1511     | tssM-5       | type VI secretion system protein | Yes                                         | Yes                                     |
| 6277                                        | Hemin uptake protein                   | BPSS0362     | –           | hypothetical protein | Yes                                         | No                                      |
| 3095                                        | Ferrichrome transport system permease protein PhuB (TC 3.3.1.14.3) | BPSS1783 | –           | iron-hydroxamate transporter permease subunit | No                                          | No                                      |
| 5952                                        | Type III secretion                     | BPSS1546     | bsaN         | regulator of type III secretion | Yes                                         | No                                      |
| Bt E555 | log₂ fold change in T6 transcriptome | Bt E555 gene function assigned by RAST | Bp Sanger ID | Bp gene name | Bp gene product | Significantly upregulated in T5 transcriptome? | Significantly upregulated in T6 proteome? |
|---------|-------------------------------------|----------------------------------------|--------------|--------------|----------------|-----------------------------------------------|------------------------------------------|
| 5900    | 6.4369                              | thermoregulatory protein (LcrF, VirF, transcription regulation of virulence plasmid) | no homolog   |              | system effector proteins BsaN (T3SS-3)         | Yes                                           | No                                      |
| 5899    | 6.4344                              | Fig. 00456189: hypothetical protein    |              | bimA         | Burkholderia intracellular motility A, BimA    | Yes                                           | Yes                                     |
| 5919    | 6.1933                              | Autotransporter adhesin                | BPSS1492     | bimA         | type VI secretion system protein TssJ-5       | Yes                                           | Yes                                     |
| 3092    | 6.1826                              | putative lipoprotein                  | BPSS1508     | tssJ-5       | hypothetical protein                           | Yes                                           | No                                      |
| 5927    | 6.1495                              | putative lipoprotein                  | BPSS1522     | bprB         | two-component response regulator              | Yes                                           | No                                      |

Known virulence factors of *B. pseudomallei* are shown in bold in the first column.
but our work reveals that unlike *B. thailandensis* strain E264, *B. thailandensis* strain E555 grows at similar rate to *B. pseudomallei* in macrophages. A previous report showed that a *B. pseudomallei* mutant lacking capsular polysaccharide showed no difference in intracellular growth rate compared to the wild type [53]. Therefore the similar behaviour of *B. thailandensis* strain E555 to *B. pseudomallei* in host cells is unlikely to be due simply to the presence of the similar capsule in these different bacteria. Consistent with this we find that the overall genetic makeup of *B. thailandensis* strain E555 is more similar to that of *B. pseudomallei* strain K96243 than to the overall genetic makeup of *B. thailandensis* strain E264.

In this study we also compared the relative utilities of transcriptomic or proteomic approaches to identify...
genes which are differentially regulated after infection. Of the 6508 total genes encoded by the *B. thailandensis* strain E555 genome, RNA-seq analysis detected expression of approximately 88% of the genes during growth in broth or in macrophages. In parallel mass spectrometry, identified 42% of the predicted proteins. Similar findings have been reported in other bacteria [54–57]. Our finding that the transcriptome and proteome did not precisely mirror each other is not unexpected. In an *Escherichia coli* single-cell study [57] the correlation coefficient between mRNA and protein levels of the same gene averaged zero for the genes tested. Although according to the central dogma of molecular biology that transcription and translation are tightly linked in prokaryotes [58], the lack of correlation between mRNA and the encoded protein has been attributed to differences in the stability of these molecules [57] and in translation efficiency. Translation efficiency has a large impact on transcript-protein correlation. For example transcripts that have weak Shine–Dalgarno sequences are translated less efficiently [59, 60]. Secondary structure of the mRNA [61] and codon bias (large codon bias correlates with highly expressed genes [62, 63] and proteins [64]) also have an influence on translation efficiency. Untranslated RNA molecules are also considered to be responsible for the differences between the transcriptomic and the proteomic results. However, the most important factor responsible for low correlations between mRNA and protein expressions is the long half-life of proteins relative to mRNAs. Transcripts are instantaneous messengers that are degraded in 3 to 8 min [65], while proteins are accumulated products with a typical half-life of ~20 h in *E. coli* [66, 67]. Finally, the reduced ability to recover some membrane-located or secreted proteins would limit the ability to detect them. These differences found between the transcriptomic and the proteomic data highlight the value of profiling both the transcriptome and the proteome.

Our comparison of the transcriptomes and proteomes of *B. thailandensis* strain E555 showed that most of the detected proteins were also present in the corresponding transcriptomes. We looked for transcripts and proteins that are significantly up-regulated during infection and found that an analysis of the transcriptome revealed more virulence-associated factors than an analysis of the proteome. However, although the proteome data revealed fewer virulence factors, they were identified with a higher degree of confidence. We found some examples of opposite patterns of gene and protein expression after infection (Additional file 7: Table S5). This raises the question of which method to use identifying candidate virulence factors. Among these genes we detected 1 virulence-associated factor (Fig.869,728.6.-peg.5292) which was significantly up-regulated in the transcriptome but down-regulated in the proteome.

Both analyses generated false negative results. For example, deletion of the treA gene (BPSS0671) results in reduced intracellular survival of *B. pseudomallei* [68] but, compared to broth grown bacteria expression of the trehalase A gene in *B. thailandensis* strain E555 (Fig.869,728.6.peg.4715) was reduced during macrophage infection and the protein was not detected in culture or in macrophages. We also found numbers of other known virulence factors in the significantly down-regulated groups (Additional file 7: Table S5). This may reflect the different roles of virulence factors in different cell types or different hosts or at different stages of infection.

In summary, we combined a transcriptomic and proteomic approach to elucidate the changes involved in the adaptation of *B. thailandensis* strain E555 to macrophage cells. The differences found between the transcriptomic and the proteomic data suggested various post-transcriptional mechanisms and the complexity of the bacterial adaptation.

**Conclusions**

In contrast with *B. thailandensis* strain E264, which has been used widely as a surrogate model for *B. pseudomallei*, we show that the pattern of growth of *B. thailandensis* strain E555 in macrophages more closely mirrors that of *B. pseudomallei*. Using this infection model we have shown that many of the known virulence factors of *B. pseudomallei* can be identified as genes or proteins.

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**Table 2** Summary of bacterial virulence factors detected in the transcriptome and in the proteome during infection of macrophages

| Groups | Description | Total number of genes | Number of virulence factors | Virulence factors in proportion of the total known virulence factors in *B. thailandensis* E555 (302) | proportion of the genes detected in the group (column 3) |
|--------|-------------|-----------------------|-----------------------------|-------------------------------------------------------------------------------------------------|--------------------------------------------------|
| A      | Significantly up-regulated in the transcriptome | 396                   | 47                          | 15.6%                                                                                           | 11.9%                                            |
| B      | Significantly up-regulated in the proteome   | 109                   | 16                          | 5.3%                                                                                           | 14.7%                                            |
| A + B  | All genes in Groups A and B                  | 456                   | 52                          | 17.2%                                                                                           | 11.4%                                            |
| A = B  | Common genes in Groups A and B               | 49                    | 11                          | 3.6%                                                                                           | 22.4%                                            |

See the lists of genes in each group in the Additional file 9: Table S7.
whose expression is elevated on the infection of macrophages. This finding further confirms the utility of *B. thailandensis* strain E555 as a surrogate for *B. pseudomallei*. The identification of up-regulated genes provided a more comprehensive identification of virulence factors than the identification of up-regulated proteins.

**Methods**

**Bacterial strain and cell culture growth conditions**

*B. thailandensis* strain E555 (gift from Patrick Tan, Genome Institute of Singapore) was grown on Luria-Bertani (LB) agar or in LB broth (200 rpm) at 37 °C. J774A.1 mouse macrophage cells (European Collection of Cell Cultures, ECACC; Catalog No. 91051511) were maintained at 37 °C under 5% CO₂ atmosphere in DMEM (with 4.5 g/l Glucose, 4.0 mM L-Glutamine and Sodium Pyruvate; Gibco 11,995,073) supplemented with 10% heat inactivated fetal bovine serum (FBS Gold; PAA A15–751).

**Macrophage infection model**

For infection assays, 1.5 × 10⁶ cells/well in 24-well cell culture plates or 6 × 10⁵ cells/T75 flask (approximately 7.9 × 10⁴ cells/cm²) were seeded and an overnight culture of bacteria was diluted to 1.5 × 10⁶ cells/ml or to 6 × 10⁷ cells/10 ml in Leibovitz L-15 Media (with 2.05 mM L-Glutamine; Gibco 21,083,027), respectively. J774A.1 monolayers were washed once with L-15 medium before addition of bacteria at a multiplicity of infection of 10 (1 ml/well or 10 ml/T75 flask). Murine macrophages with the bacterial cells were incubated at 37 °C for 2 h to allow bacterial internalisation to occur. Extracellular bacteria were removed by washing the macrophage cells three times with warm PBS (Gibco 10,010,023). Fresh media containing kanamycin (1 mg/ml, which was replaced with 0.25 mg/ml kanamycin after 2 h of incubation) was then added to each well or flask to suppress the growth of extracellular bacteria. Infected cells were incubated in the presence of kanamycin at 37 °C until washed three times with PBS at appropriate time points. To determine the number of intracellular bacteria, macrophages were lysed in 0.1% Triton X-100 (Sigma-Aldrich T8787) in PBS for 5 mins, the lysis mixture was serially diluted and plated out on LB agar plates, which were then incubated overnight at 37 °C to allow bacteria to grow. Unless otherwise stated, all infections were performed as three independent experiments, each with three technical replicates. Macrophages infected in T75 flasks were processed for transcriptome and proteome analysis as detailed below.

**Genome sequencing and assembly**

DNA were prepared for sequencing using a Promega Wizzard Genomic DNA Purification kit according to the manufacturer’s instructions. Sequencing was performed at the University of Exeter Sequencing Facility using an ‘Illumina HiSeq 2500 System’ benchtop sequencing instrument (read length: 100bp, read type: paired end). Illumina adapters were removed and sequences quality trimmed using ea-utils [69]. SPAdes [70] was used to perform a de-novo assembly of the samples. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession SJE70000000.

**Bacterial RNA extraction, sequencing and reverse transcription quantitative real-time PCR (RT-qPCR) for transcriptional studies**

Total RNA was extracted from infected macrophages (internalised CFU per replicate approximately 1.5 × 10⁷ and 2 × 10⁷ at 5 and 6 h, respectively) and from bacterial cultures, and the RNA integrity number (RIN) was determined. RIN was more than 9 with all the extracts indicating no evidence of RNA degradation. After the final washing step at 5 or 6-h post-infection (2-h infection + 3 or 4-h kanamycin treatment), 2 ml TRIzol® Reagent (Invitrogen 15,596,026) was added to the flasks to lyse both the macrophage and bacterial cells. Cell lysates were stored at −80 °C until further processing. When thawed, total RNA (total eukaryotic and total prokaryotic RNA) was extracted using the Direct-zol™ RNA MiniPrep Kit (Zymo Research R2052). Contaminating DNA was digested by DNase I treatment (Ambion AM2222) which was confirmed by reverse transcriptase PCR (Qiagen OneStep RT-PCR Kit 210,212) using primers *glt1*-F (5’ CGCACCATGACATCTATTCG 3’) vs *glt1*-R (5’ ACCGGATTGACGTTCTTCAG 3’). DNA-free RNA was treated with the TruSeq RNA Library Preparation Kit v2 (Illumina RS-122-2001) and the Ribo-Zero Gold rRNA Removal Kit (Epidemiology) (Illumina MRZE724). Eukaryotic mRNA was first removed using poly-A selection, the remaining RNA was then enriched for bacterial mRNA by depleting both the bacterial and macrophage rRNA and the RNA-seq libraries were prepared to the guidelines of the manufacturer. Control bacterial mRNA was obtained from the overnight broth cultures of bacteria which were used to infect macrophage cells. RNA from broth culture was isolated as described above (including the poly-A depletion step). RNA was isolated from 3 separate assays (biological replicates) from both infected macrophages and control bacteria. RNA-seq libraries were created using the Illumina TruSeq Stranded mRNA Library Prep Kit according to the manufacturer’s protocol. The concentration, quality and integrity of all RNA and DNA samples were analysed using the Agilent 2100 Bioanalyser. Sequencing was performed at the University of Exeter Sequencing Facility using an ‘Illumina MiSeq System’ benchtop sequencing instrument (read length:
75 bp, read type: paired end). Reads from RNASeq were mapped to the *B. thailandensis* strain E555 genome assembly using TopHat [71]. Cufflinks [72] was used for transcript assembly of individual samples. All assemblies were merged to create a reference transcript, which was used to calculate gene counts with HTSeq [73]. DESeq [74] was then used to find differentially expressed genes. Transcripts with a *p*-value<0.05 and more than 2-fold differential expression were considered significantly expressed.

The preparation of cDNA from total RNA extracted from culture and infected macrophages was carried out using the Superscript III Reverse Transcriptase Synthesis System (Invitrogen 18,080,051) with random hexamers according to the manufacturer's recommendations. RT-qPCR was performed with primers annealing to internal regions of the target genes (Additional file 10: Table S8) using Platinum® SYBR® Green qPCR SuperMix-UDG according to the manufacturer’s manual (Invitrogen 11,733,038). For the adjustment of cDNA amounts, the housekeeping gene 23S rRNA [75] was used as internal standard.

**Bacterial protein extraction and mass spectrometric analysis for proteomic studies**

In contrast to the RNA, prokaryotic and eukaryotic proteins are indistinguishable. Therefore, intact bacterial cells were first extracted from infected macrophages and bacterial proteins were then isolated from the bacterial cells. Macrophages were infected as described above using the same conditions as used for the 6 h transcriptome samples (i.e., internalised CFU per replicate approximately 2 × 10^7 at 6 h post-infection). Following the final washing step at 6-h post-infection (2-h infection + 4-h kanamycin treatment) infected macrophages in T75 flasks were lysed in 0.1% Triton X-100 in PBS for 5 mins to release the intracellular bacteria and whole bacterial cells were isolated using a modified immunomagnetic separation technique described previously by Twine et al. [76]. Briefly, Dynabeads® M-270 Epoxy (Life Technologies 14311D) were covalently attached to goat α-mouse IgG (H + L) secondary antibodies (Invitrogen A16068) as per the manufacturer’s instructions (20 μg of antibodies per 1 mg of magnetic beads were used) and magnetic beads coated with secondary antibodies were labelled with mouse α-capsule primary antibodies (CPS 4V1H12 [12]) (17.5 μg of α-CPS antibodies per 1 mg of bead – secondary antibody complexes), which recognise surface antigens of *B. thailandensis* strain E555 cells. Magnetic bead – α-mouse – α-CPS complexes were then used to purify the bacteria. 1 mg of beads coated with both antibodies was mixed with macrophage cell lysate per T75 flask and incubated for 25 min with constant agitation (35 rpm) at 4 °C to capture the bacterial cells. The beads-bacteria complexes were separated from the cell debris on a magnetic stand, washed, and immediately re-suspended in BugBuster® Protein Extraction Reagent (Novagen 70,584) to lyse the bacterial cells and release the proteins. Bacterial purification was completed in about 45 min. Control extracts included bacterial proteins isolated by BugBuster® Protein Extraction Reagent from the overnight broth cultures of bacteria which were used to infect macrophage cells.

Mass spectrometric analysis of protein samples isolated from 3 biological replicates of both infected macrophages and bacterial cultures was done by the University of Bristol Proteomics Facility. Proteomics was performed as described previously using an UltiMate™ 3000 nano HPLC system in line with an LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific) [77]. The raw data files were processed and quantified using Proteome Discoverer software v1.2 (Thermo Scientific) and searched against *B. thailandensis* strain E555 RAST ORFs using the SEQUEST algorithm. The reverse database search option was enabled and all peptide data was filtered to satisfy false discovery rate (FDR) of 5%. Abundance of each protein in each sample was calculated using the average area measurements of the three most abundant peptides matching to each protein (Top3 method) [78]. This value was then expressed in the fraction of the signal derived from the 100 most abundant proteins detected in each sample which was then compared for each protein in LB broth and macrophages. Statistical significance of the fold change difference was calculated using R with q-values reported after 5% FDR correction. All proteins with a q-value<0.05 and more than 2-fold change difference were considered significantly regulated.

**Online and bioinformatic tools**

Genome sequence of *B. thailandensis* strain E555 was annotated using the web-based RAST server (Rapid Annotation using Subsystem Technology) [44]. Cellular localisation of the predicted proteins encoded in the *B. thailandensis* strain E555 genome was predicted using PSORTb 3.0 [79] (Additional file 3: Table S1.D). Protein homologs in *B. pseudomallei* strain K96243, *B. thailandensis* strain E555 and *B. thailandensis* strain E264 were identified by blast search. Proteins were considered homologs if they met the following criteria: (i) sequence identity > 30% if alignment length/query length > 90% [80], (ii) sequence identity > 40% if alignment length/query length between 70 and 90%, (iii) sequence identity > 55% if alignment length/query length between 50 and 70% (Additional file 3: Table S1.C). Correlation coefficients between the samples were calculated using the formula built-in the Microsoft Excel Software. *B. thailandensis* strain E555
genes were classified into functional groups based on clusters of orthologous gene (COG) designations; functional classes of *B. thailandensis* genes and proteins were determined based on the categorization of their homologs in *B. pseudomallei* (Sheet C of Additional file 4: Table S2 in reference [81], Additional file 3: Table S1 D). Statistical analysis of growth curves was performed using Graph Pad Prism 5.03 software One-way ANOVA Kruskal-Wallis test with Dunns post test. DESeq [74] was used to determine fold change and the *p* value of differential expression in the RNASeq data. Statistical significance (*q* value) of the fold change difference in the proteome was calculated using R. To identify pathways that are up- or down-regulated during infection we used Keg Array 1.2.4a software which maps the genes/proteins to KEGG pathways. Up-regulation or down-regulation of genes/proteins with at least 1.5-fold increase or decrease in expression difference was considered to be necessary or dispensable respectively during macrophage infection.

**Additional file**

**Additional file 1:** Figure S1. Abundance of transcripts detected in the bacterial transcriptome both at 5 h and 6 h post infection.

**Figure S2.** Abundance of bacterial transcripts detected in culture and in macrophages. **Figure S3.** Abundance of bacterial proteins detected in culture and in macrophages. **Figure S4.** Volcano plots of gene expression for *B. thailandensis* strain E555 during macrophage infection (versus in vitro growth) at transcript and protein level. (DOCX 1997 kb)

**Additional file 2:** Supplementary References. (DOCX 17 kb)

**Additional file 3:** Table S1. Details of *B. thailandensis* strain E555 genes and proteins. (XLSX 6051 kb)

**Additional file 4:** Table S2. Gene expression results and details of the genes subjected to RT-qPCR analysis. (XLSX 15 kb)

**Additional file 5:** Table S3. Transcripts detected in vitro and in vivo. (XLSX 2144 kb)

**Additional file 6:** Table S4. Proteins detected in vitro and in vivo. (XLSX 1005 kb)

**Additional file 7:** Table S5. Significantly regulated genes, capsule expression and all expression data. (XLSX 2279 kb)

**Additional file 8:** Table S6. Known virulence genes. (XLSX 208 kb)

**Additional file 9:** Table S7. Lists of genes in each group shown in Table 2. (XLSX 321 kb)

**Additional file 10:** Table S8. Primers used in RT-qPCR. (DOCX 15 kb)

**Abbreviations**

CFU: Colony forming unit; CFU: Colony-forming unit; COG: Clusters of orthologous gene; CPS: Capsule polysaccharide; DMEM: Dulbecco’s modified Eagle’s medium; DNA: Deoxyribonucleic acid; LB: Luria-Bertani; mRNA: Messenger ribonucleic acid; RAST: Rapid Annotation using Subsystem Technology; RNA-Seq: RNA sequencing; RT-qPCR: Reverse transcription quantitative real-time polymerase chain reaction; SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis; T3SS: Type III secretion system; T6SS: Type VI secretion system

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**Availability of data and materials**

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Authors’ contributions**

AK did all laboratory works, analysed and interpreted the data, and drafted the manuscript. AK, CM and RT designed the study. RT coordinated the study and revised the manuscript. CM, AS and JP also revised the manuscript critically for important intellectual content. All authors read and approved the final version of the manuscript.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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**Author details**

1 College of Life and Environmental Sciences, University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter EX4 4QD, UK. 2 CBR Division, Defence Science and Technology Laboratory, Porton Down, Salisbury SP4 0JQ, UK.

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**References**

1. Limmathurotsakul D, Golding N, Dance DA, Messina JP, Pigott DM, Moyes CL, Rolim DB, Bertherat E, Day NP, Peacock SJ, et al. Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. Nat Microbiol. 2016;1:15008.

2. Wieringa WJ, Vrk HS, Torres AG, Currie BJ, Peacock SJ, Dance DAB, Limmathurotsakul D. Melioidosis. Rev Dis Primers. 2018;18(1):107.

3. Perumal Samy R, Stiles BG, Sethi G, Lim LHK. Melioidosis: clinical impact and burden of melioidosis. Nat Rev Dis Primers. 2017;11(5):s004738.

4. Peacock SJ. Melioidosis. Curr Opin Infect Dis. 2006;19(5):421–8.

5. Dance DAB. Melioidosis. Curr Opin Infect Dis. 2002;15:127–32.

6. Gan YM, Chua KL, Chua HH, Liu BP, Hii CS, Chong HL, Tan P. Characterization of *Burkholderia pseudomallei* infection and identification of novel virulence factors using a *Caenorhabditis elegans* elegans host system. Mol Microbiol. 2002;44:1185–97.

7. Holden MTG, Titball RW, Peacock SJ, Cerdeno-Tarraga AM, Atkins T, Crossman LC, Pitt T, Churcher C, Mungall K, Bentley SD, et al. Genomic plasticity of the causative agent of melioidosis, *Burkholderia pseudomallei*. Proc Natl Acad Sci U S A. 2004;101(39):14240–5.

8. Howe C, Sampath A, Spotnitz M. The pseudomallei group: a review. J Infect Dis. 1971;124(6):598–606.

9. Zilinskas RA. A brief history of biological weapons programmes and the use of animal pathogens as biological warfare agents. Rev Sci Tech. 2017;36(2):415–22.

10. Titball RW, Burnett MN, Bancroft GJ, Brett P. *Burkholderia pseudomallei* and *Burkholderia mallei* vaccines: are we close to clinical trials? Vaccine. 2017;35(44):5981–9.

11. Dance D. Treatment and prophylaxis of melioidosis. Int J Antimicrob Agents. 2014;43(4):310–8.
32. Peano C, Chiaramonte F, Motta S, Pietrelli A, Jaillon S, Rossi E, Consolandi C, Stone JK, DeShazer D, Brett PJ, Burtnick MN. Melioidosis: molecular aspects of pathogenesis. Expert Rev Anti-Infect Ther. 2014;12(12):1487–98.
31. Shan J, Korbsrisate S, Withatanung P, Adler NL, Clokie MR, Galyov EE. Generation of Burkholderia pseudomallei contributes to survival in serum by reducing complement factor C3b deposition. Infect Immun. 2005;73(2):1106–15.
30. Moule MG, Hemsley CM, Seet Q, Guerra-Assuncao JA, Lin J, Sarkar-Tyson M, Clark TG, Tan PB, Tibbett RW, Cucci J, et al. Genome-wide saturation mutagenesis of Burkholderia pseudomallei K96243 predicts essential genes and novel targets for antimicrobial development. MBio. 2014;5(6):e00926–13.
29. Cucci J, Easton A, Bancroft G, Oyston PC, Tibbett RW, Wren BW. Development of signature-tagged mutagenesis in Burkholderia pseudomallei to identify mutants important in survival, attenuation and pathogenesis. Infect Immun. 2007;75:1186–95.
28. Brett P, Deshazer D, Woods D. Burkholderia thailandensis sp. nov., a Burkholderia pseudomallei-like species. Int J Syst Bacteriol. 1998;48:3177–20.
27. Yu Y, Kim HS, Chua HH, Lin CH, Sim SH, Lin D, Derr A, Engels R, DeShazer D, Birren B, et al. Genomic patterns of pathogen evolution revealed by comparison of Burkholderia thailandensis, the causative agent of melioidosis, to avirulent Burkholderia thailandensis. BMC Microbiol. 2006;6(6):46.
26. Le Guillouzer S, Groele MC, Deziel E. The complex quorum sensing circuitry of Burkholderia thailandensis is both hierarchically and Homeostatically organized. MBio. 2017;8(6):e01686–17.
25. Gupta A, Bedre R, Thapa SS, Sabrin A, Wang G, Dassanayake M, Grove A. Global awakening of cryptic biosynthetic gene clusters in Burkholderia thailandensis. ACS Chem Biol. 2017;12(2):302–21.
24. Yu Y, Kim HS, Chua HH, Lin CH, Sim SH, Lin D, Derr A, Engels R, DeShazer D, Birren B, et al. Genomic patterns of pathogen evolution revealed by comparison of Burkholderia thailandensis, the causative agent of melioidosis, to avirulent Burkholderia thailandensis. BMC Microbiol. 2006;6(6):46.
23. Whiteley L, Haug M, Klein K, Willmann M, Bohm E, Chiantia S, Schwarz S. Cholesterol and host cell surface proteins contribute to cell-cell fusion induced by the Burkholderia type VI secretion system. PLoS one. 2017;12(10):e0185715.
22. Whiteley L, Haug M, Klein K, Willmann M, Bohm E, Chiantia S, Schwarz S. Cholesterol and host cell surface proteins contribute to cell-cell fusion induced by the Burkholderia type VI secretion system. PLoS one. 2017;12(10):e0185715.
21. Whiteley L, Haug M, Klein K, Willmann M, Bohm E, Chiantia S, Schwarz S. Cholesterol and host cell surface proteins contribute to cell-cell fusion induced by the Burkholderia type VI secretion system. PLoS one. 2017;12(10):e0185715.
20. Whiteley L, Haug M, Klein K, Willmann M, Bohm E, Chiantia S, Schwarz S. Cholesterol and host cell surface proteins contribute to cell-cell fusion induced by the Burkholderia type VI secretion system. PLoS one. 2017;12(10):e0185715.
19. Whiteley L, Haug M, Klein K, Willmann M, Bohm E, Chiantia S, Schwarz S. Cholesterol and host cell surface proteins contribute to cell-cell fusion induced by the Burkholderia type VI secretion system. PLoS one. 2017;12(10):e0185715.
52. Zhang JH, Quigley NB, Gross DC. Analysis of the syrP gene, which regulates syringomycin synthesis by Pseudomonas syringae pv. Syringae. Appl Environ Microbiol. 1997;63(7):2277–8.

53. Phewiakian A, Wongratanaachewin S, Chareonsudjai S. Role of Burkholderia pseudomallei in the invasion, replication and induction of apoptosis in human epithelial cell lines. Southeast Asian J Trop Med Public Health. 2010; 41(5):1164–76.

54. Munir RI, Spicer V, Kotkkin OV, Shamsurin D, Zhang X, Taillefer M, Blunt W, Cieck N, Sparling R, Levin DB. Transcriptomic and proteomic analyses of core metabolism in Clodstridium termidris CT112 during growth on alpha- cellulose, xylan, cellulose and xyllose. BMC Microbiol. 2016;16:93.

55. Gunji SP, Roshnani Y, Frentz BR, Aebolrad R. Correlation between protein and mRNA abundance in yeast. Mol Cell. Biol. 1999;19(3):120–30.

56. Yeung ES. Genome-wide correlation between mRNA and protein in a single cell. Angew Chem Int Ed Engl. 2011;50(3):583–5.

57. Taniguchi Y, Choi P1, Li GW, Chen H, Babu M, Hearn J, Emili A, Xie XS. Quantifying E. coli proteome and transcriptome with single-molecule sensitivity in single cells. Science. 2010;329(5991):533–8.

58. Gowrishankar J, Harinarayanan R. Why is transcription coupled to translation in bacteria? Mol Microbiol. 2004;54(3):598–603.

59. Shine J, Dalargano L. The 3′-terminal sequence of Escherichia coli 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. Proc Natl Acad Sci U S A. 1974;71(14):1342–6.

60. Shine J, Dalargano L. Determinant of citron specificity in bacterial ribosomes. Nature. 1975;254(5495):34–8.

61. Grossman AD, Zhou YN, Gross DC, Heilig J, Christie GE, Calendar R. Mutations in the rpoH (htpR) gene of Escherichia coli K-12 phenotypically suppress a temperature-sensitive mutant defective in the sigma 70 subunit of RNA polymerase. J Bacteriol. 1985;161(3):939–43.

62. Jansen R, Bussemaker HH, Gerstein M. Revisiting the codon adaptation index from a whole-genome perspective: analyzing the relationship between gene expression and codon occurrence in yeast using a variety of models. Nucleic Acids Res. 2003;31(8):2242–51.

63. Kliman RM, Irving N, Santiago M. Selection conflicts, gene expression, and codon usage trends in yeast. J Mol Evol. 2003;57(1):98–109.

64. Wu G, Nie L, Freeland SJ. The effects of differential gene expression on coding sequence features: analysis by one-way ANOVA. Biochem Biophys Res Commun. 2007;358(4):1105–10.

65. Bernstein JA, Khodursky AB, Lin PH, Lin-Chao S, Cohen SN. Global analysis of mRNA decay and abundance in Escherichia coli at single-gene resolution using two-color fluorescent DNA microarrays. Proc Natl Acad Sci U S A. 2002;99(15):9697–702.

66. Jaffke AL, Levy HR. Protein turnover in growing cultures of Escherichia coli. J Biol Chem. 1953;217(2):947–57.

67. Mandelstam J. Turnover of protein in growing and non-growing populations of Escherichia coli. Biochem J. 1958;69(1):110.

68. Vanaporn M, Sarkis MR, Kasai K, Hata S, Ishida K. Analysis of the syrP gene, which regulates syringomycin synthesis by Pseudomonas syringae. J Biol Chem. 2010;285(15):11018–26.

69. Piras GS, Pistelli R, Pelucchi R, Barbieri S, Silani E, Berisio R, et al. Lpin-1, a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19(5):455–77.

70. Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics. 2009;25(11):110–15.

71. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, Pimentel H, Salzberg SL, Rinn JL, Pachter L. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and cufflinks. Nat Protoc. 2012;7(3):562–78.

72. Anders S, Pyl PT, Huber W. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics. 2015;31(2):166–9.

73. Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biol. 2010;11(10):R106.

74. Jirtroasuwit S, Ong C, Juntawisong N, Ooi WF, Hemley CA, Vattanaviboon P, Tilbally RW, Pan P, Kotsirthane S. Transcriptional profiles of Burkholderia pseudomallei reveal the direct and indirect roles of sigma E under oxidative stress conditions. BMC Genomics. 2014;15:787.

75. Twine SM, Mykytczuk NC, Pett MD, Shen H, Sjostedt A, Wayne Conlan J, Kelly JF. In vivo proteomic analysis of the intracellular bacterial pathogen, Francisella tularensis, isolated from mouse spleen. Biochem Biophys Res Commun. 2006;343(4):1621–33.

76. Gaggs R, Harper MT, Pope RJ, Savage JS, Williams CM, Mundell SJ, Heesom KJ, Bass M, Mellor H, Poole AW. RhoG protein regulates platelet granule secretion and thrombus formation in mice. J Biol Chem. 2013;288(47):34217–29.

77. Ahne E, Molzahn L, Glatter T, Schmidt A. Critical assessment of proteome-wide label-free absolute abundance estimation strategies. Proteomics. 2013;13(17):2567–78.

78. Yu NY, Wagner JR, Laird MR, Weli G, Rey S, Lo R, Dao P, Sahinalp SC, Esters M, Foster L, et al. PSORTb 3.0: improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. Bioinformatics. 2010;26(13):1608–15.

79. Rost B. Twilight zone of protein sequence alignments. Protein Eng. 1999;12(1):65–94.

80. Ooi WF, Ong C, Nandi T, Kreisberg JF, Chua HH, Sun G, Chen Y, Mueller C, Conejero L, Eshaghi M, et al. The condition-dependent transcriptional landscape of Burkholderia pseudomallei. PLoS Genet. 2013;9(9):e1003795.