Mammalian Solute Carrier (SLC)-like transporters of Legionella pneumophila

Ashley Best1, Snake Jones1 & Yousef Abu Kwaik1,2

Acquisition of nutrients during intra-vacuolar growth of L. pneumophila within macrophages or amoebae is poorly understood. Since many genes of L. pneumophila are acquired by inter-kingdom horizontal gene transfer from eukaryotic hosts, we examined the presence of human solute carrier (SLC)-like transporters in the L. pneumophila genome using I-TASSER to assess structural alignments. We identified 11 SLC-like putative transporters in L. pneumophila that are structurally similar to SLCs, eight of which are amino acid transporters, and one is a tricarboxylate transporter. The two other transporters, LstA and LstB, are structurally similar to the human glucose transporter, SLC2A1/Glut1. Single mutants of lstA or lstB have decreased ability to import, while the lstA lstB double mutant is severely defective for uptake of glucose. While lstA or lstB single mutants are not defective in intracellular proliferation within Acanthamoeba polyphaga and human monocyte-derived macrophages, the lstA lstB double mutant is severely defective in both host cells. The two phenotypic defects of the lstA lstB double mutant in uptake of glucose and intracellular replication are both restored upon complementation of either lstA or lstB. Our data show that the two glucose transporters, LstA and LstB, are redundant and are required for intracellular replication within human macrophages and amoebae.

Legionnaire’s disease, an atypical pneumonia, is a result of inhalation of the bacteria Legionella pneumophila1–3. Within the human host, L. pneumophila primarily reside and replicate within alveolar macrophages4–6. Infection of humans is considered to be “accidental”, as the natural hosts for L. pneumophila are protozoa in the aquatic environment7,8. Growth within either host occurs through manipulation of evolutionarily conserved pathways, to avoid fusion to the lysosomes and to remodel the vacuole to become ER-derived, which is designated as the Legionella-containing vacuole (LCV)9–14. The Dot/Icm type IVb translocation system, which translocates >320 effector proteins into the host cytosol, is required for biogenesis of the LCV15 and for successful intracellular replication in macrophages and amoebae16–19, and subsequent killing of the host cell20. A plethora of host cell processes are modulated by the translocation of redundant effector proteins21 that allow L. pneumophila to evade innate immunity and acquire nutrients22–25.

L. pneumophila relies on host amino acids (such as, serine, cysteine, and alanine) to feed into tricarboxylic acid (TCA) cycle as the main source of carbon and energy26–28. The bacteria are in such high demand for amino acids that endogenous amounts within the host are below the threshold needed to support robust intracellular replication22,29. To raise host cellular levels of amino acids, L. pneumophila translocates the AnkB effector, which is post-translationally modified by the host cell30–32, and hijacks the host ubiquitin-proteasome machinery to degrade proteins33–35, but the host cell also undergo metabolic reprogramming in response to infection36–38. Early studies pointed to a preference for amino acids as an energy source and in vitro studies identified auxotrophies for seven amino acids in L. pneumophila: threonine, arginine, isoleucine, methionine, leucine, cysteine, and valine26–28. Many of these auxotrophies are shared with the amoeba host which may allow the bacterium to synchronize growth with that of the host, avoiding deleterious growth during times of environmental stress39–40. Legionella can enter into a viable but non-culturable (VNBC) state when encountering nutritional stress, which has only been shown to be recovered by co-culturing with amoebae41.

Nutritional virulence studies on L. pneumophila42,43 have focused on the generation and utilization of amino acids27,42,44,45. Only recently has glucose metabolism been studied for its role during intracellular replication46,47. Glycolysis plays a

1Department of Microbiology and Immunology, School of Medicine, University of Louisville, Louisville, KY, United States. 2Center for Predictive Medicine, University of Louisville, Louisville, KY, United States. Correspondence and requests for materials should be addressed to Y.A.K. (email: abukwaik@louisville.edu)
minimal role in glucose catabolism, but is predominately metabolized through the ED pathway while the pentose phosphate pathway (PPP) functions only to generate mannose and histidine46–48. A gene cluster encoding enzymes for glucose catabolism, through the Entner-Doudoroff (ED) pathway of *L. pneumophila* has been shown to be required for growth in the A549 epithelial cell line, A/J mouse macrophages, and *Acanthamoeba culbertsoni*, indicating the importance of the ED pathway in intracellular replication of *L. pneumophila*. Initial studies focused on poly-3-hydroxybutyrate (PHB), a 4-carbon storage molecule that is generated by metabolizing glucose, through the ED pathway into pyruvate, which gets converted into acetyl-CoA, then PHB. PHB is synthesized in late stages of growth and catabolized during stationary growth into acetyl-CoA to feed into the TCA cycle46,50. The primary usage of glucose by *L. pneumophila* is considered to be conversion into PHB46,50.

How nutrients are imported by *L. pneumophila* is not well understood51,52. To date, only one amino acid transporter, PhtA, of *L. pneumophila* has been shown to import threonine and is required for intracellular replication in macrophages53. Given that numerous genes in *L. pneumophila* have been acquired by inter-kingdom horizontal gene transfer from eukaryotic hosts, we sought to identify nutrient transporters in *L. pneumophila* based on their similarity to the human solute carrier (SLCs) transporters due to the lack of well annotated amoebal genomes54,55. This superfamily of transporters consists of over 65 families, grouped based on substrate specificity and tissue tropism56. They are considered to be part of a larger, evolutionarily conserved group of transporters known as the Major Facilitator Superfamily (MFS)57,58.

Eleven putative amino acid SLC-like transporters were identified, including a citrate transporter, 7 amino acids transporters and 2 glucose transporters. We focused our studies on the two putative SLC-like glucose transporters, LstA and LstB, to further understand the import of glucose and its role in intracellular replication of *L. pneumophila*.

### Results

**Identification of human SLC-like transporters in *L. pneumophila***. We utilized BLAST search of the *L. pneumophila* Philadelphia strain genome against all human SLC transporters of amino acids and glucose. In addition, since *L. pneumophila* utilizes pyruvate and citrate to feed its TCA cycle, we search for similarity of the *L. pneumophila* genome to the mono and tri-carboxylates SLC13 and transporters 22,26. Using the primary amino acid sequences from the human amino acid transporter families, SLC1, 3, 7, 17, 36, 38, and 43 against the genome of *L. pneumophila* strain AA100/130b, eight putative SLC-like amino acid transporters in *L. pneumophila* were identified by BLAST with similarity of 56–42% and identity of 25–37% (Table 1). In addition, one putative SLC-like transporter of tricarboxylates similar to the SLC13 family, *lpg*2876 (24%/51%), was identified. In addition, using the primary amino acid sequence from the human SLC2 and SLC5 family, we identified two putative SLC-like glucose transporters, *lpg*0970 (33%/50%) and *lpg*1653 (30%/48%) (Table 1). Structural modeling of these proteins was done using the Iterative Threading Assembly Refinement (I-TASSER) server, which is a bioinformatics algorithm for predicting three-dimensional structure based on fold recognition59–61. Structural alignment was performed using TM-align, an algorithm that uses known or predicted protein structure to align proteins and determine structural similarity59–61. A TM-score is given for each alignment where, 1.0 indicates an exact copy, 0.0 – <0.3 indicates random structural similarity, and 0.5 – <1.0 indicating shared structural topology. *L. pneumophila* proteins were compared to the human SLCs to determine structural homology, as measured by TM-scores (Table 1). Structural comparisons of these SLC-like transporters of *L. pneumophila* with human SLCs showed high structural similarity (TM-scores 0.78–0.977) (Table 1 and see Supplementary Fig. S1). Few homologs of mammalian SLCs have been identified within amoebae, which also can be used to identify *L. pneumophila* transporters; these are designated as CtrABC in *Dictyostelium discoideum* (see Supplementary Fig. S2). We have designated these putative transporters as *Legionella* SLC-like transporters, LstA-K (Table 1).

Since the uptake and the role of glucose in intracellular growth and metabolism of *L. pneumophila* is not well understood, we focused our studies on the two putative SLC-like transporters of *L. pneumophila* that shared

| Amino acid | Amino acid identity (BLAST) | Amino acid similarity (BLAST) | Putative substrates | Representative SLC, TM-score |
|------------|-----------------------------|-----------------------------|---------------------|-----------------------------|
| LstA (*lpg*0241) | 33% | 50% | Glucose and other monosaccharides | SLC2a1 (0.903) |
| LstB (*lpg*1653) | 30% | 48% | Glucose and other monosaccharides | SLC2a1 (0.922) |
| LstC (*lpg*0026) | 37% | 56% | Cationic amino acids (arginine, lysine, ornithine) | SLC7a1 (0.953) |
| LstD (*lpg*0049) | 25% | 44% | Cationic amino acids (arginine, lysine, ornithine) | SLC7a5 (0.954) |
| LstE (*lpg*0228) | 25% | 42% | Cationic amino acids (arginine, lysine, ornithine) | SLC7a1 (0.848) |
| LstF (*lpg*2821) | 25% | 53% | Cationic amino acids (arginine, lysine, ornithine) | SLC7a1 (0.985) |
| LstG (*lpg*0970) | 25% | 44% | Cationic amino acids (arginine, lysine, ornithine) | SLC7a4 (0.855) |
| LstH (*lpg*1691) | 27% | 45% | Cationic amino acids (arginine, lysine, ornithine) | SLC7a2 (0.756) |
| LstI (*lpg*2245) | 25% | 53% | Alanine, serine, cysteine, and threonine | SLC4a4 (0.961) |
| LstJ (*lpg*0886) | 29% | 54% | Neutral amino acids (glutamine, asparagine) | SLC1a5 (0.912) |
| LstK (*lpg*2876) | 24% | 51% | Succinate, citrate, isocitrate, α-ketoglutarate | SLC1a3 (0.931) |

Table 1. SLC-like putative proteins in *L. pneumophila* are homologous human SLCs. Eleven transporters in *L. pneumophila* were identified by BLAST amino acid sequence homology with human SLC transporters. Predicated structures, generated by I-TASSER, were used to determine structural similarity with human SLCs by TM-score.
strong structural similarity to SLC2a1/Glut1, LstA and LstB (Fig. 1a,b). LstA of *L. pneumophila* and Glut1 of humans have a TM-score of 0.903, and LstB and Glut1 a TM-score of 0.922, indicating very strong structural similarity (Fig. 1a,b,d). Members within the Glut family do not share this degree of similarity (Glut1 and Glut3, TM-score of 0.88). When comparing alignment of LstA to LstB, the TM-score is 0.959, indicating potential redundancy (Fig. 1c,d). These two *L. pneumophila* proteins are smaller in size than their human counterpart proteins but the secondary structural alignment is conserved. LstA and LstB are likely members of the major facilitator superfamily (MFS), which are important transporters that have been maintained, in all domains of life, with little deviation through evolutionary history \(^5\). LstA (Lpg0421) has been previously designated as YwtG, but based on its eukaryotic SLC-like structure and function (see below) and as one of a large family of eukaryotic SLC-like proteins in *L. pneumophila*, we have designated it as LstA \(^4\) .

**Glucose import by LstA and LstB.** Predicted substrate binding, by I-TASSER, for LstA and LstB indicate glucose as a putative substrate \(^5\) . Given their high degree of structural similarity to Glut1, we hypothesized that both of these putative transporters were involved in the transport of glucose of *L. pneumophila*. To test if either LstA or LstB were required for the uptake of glucose, null mutants were generated and uptake of glucose

---

**Figure 1.** The predicted structure of two putative glucose transporters, LstA and LstB, of *L. pneumophila*. Structural alignment between (a) Glut1 (blue) and LstA (red) (b) Glut1 (blue) and LstB (red), and (c) LstA (red) and LstB (blue), are shown using TM-align. (d) TM-scores, indicating structural similarity between Glut1, LstA, and LstB, as calculated by TM-align.
Our data show that uptake of glucose by transporters is most likely to be redundant in their function to import glucose. Our findings indicate that transporters are required for growth within hMDMs and human monocyte-derived macrophages. We determined intracellular replication of glucose transporters by deletion of glucose transporters will best highlight the intracellular need for glucose without altering the host cell response to infection.

The LstA and LstB glucose transporters are required for growth in Acanthamoeba polyphaga and human monocyte-derived macrophages. We determined intracellular replication of glucose transporters by deletion of glucose transporters will best highlight the intracellular need for glucose without altering the host cell response to infection.

Discussion

L. pneumophila generates copious amounts of host amino acids for carbon and energy but it is also reliant on host glucose. Hauslein et al. described L. pneumophila metabolism as being "bipartite", where amino acids serve as the major energy supply in the exponential phase and carbohydrates at the post-exponential phase are used in anabolic processes. The role of glucose during intracellular infection can be difficult to study; methods for altering the levels of glucose affect the host cells, which may have detrimental effects on intracellular growth of L. pneumophila.

The LstA and LstB glucose transporters are required for growth in Acanthamoeba polyphaga and human monocyte-derived macrophages. We determined intracellular replication of glucose transporters by deletion of glucose transporters will best highlight the intracellular need for glucose without altering the host cell response to infection.

Figure 2. Glucose uptake in L. pneumophila by lstA and lstB. Uptake of 14C-glucose measured in counts per minute (CPM), of WT L. pneumophila (black), single mutants (white), and double mutants (grey), was determined. Addition of unlabeled glucose (10 mM) was used as a negative control (checkered). Data points represent mean CPM ± SD, n = 4 and are representative of three independent experiments.

was analyzed by liquid scintillation using 14C-glucose. L. pneumophila strains were grown to post-exponential phase in the presence of 0.1% uniformly labelled 14C-glucose. Broth grown WT L. pneumophila was able to effectively take up 14C-glucose in vitro (Fig. 2). As a control, excess, unlabeled glucose (10 mM) was added, which abolished uptake of 14C-glucose (Student t-test, p < 0.001) (Fig. 2). The lstA and lstB mutants had significantly reduced uptake of 14C-glucose compared to the WT strain (Student t-test, p < 0.001), but glucose uptake was more reduced in the lstA mutant (Fig. 2). Complementation of the single mutants with the respective gene on a plasmid (lstA.C and lstB.C) restored uptake of glucose to that of the WT strain levels (Fig. 2).

To determine whether LstA and LstB were redundant, a lstA/lstB double mutant, was generated. Loss of both transporters abolished uptake of 14C-glucose compared to the WT strain (Student t-test, p < 0.001) (Fig. 2). Upon supplementation of excess, unlabeled glucose (10 mM) uptake of labeled glucose was inhibited in the complemented double mutants (Student t-test, p < 0.001) (Fig. 2). Interestingly, complementation with a single transporter, (lstA.C or lstB.C) restored uptake of 14C-glucose to the double mutant similar to the WT strain levels (Fig. 2). These data show that LstA and LstB are glucose transporters.

The LstA and LstB glucose transporters are required for growth in Acanthamoeba polyphaga and human monocyte-derived macrophages. We determined intracellular replication of glucose transporter single mutants, lstA and lstB, and the double mutant lstA/lstB in human monocyte-derived macrophages (hMDMs) and A. polyphaga. Single transporter null mutants, lstA and lstB, replicated similarly to WT L. pneumophila in A. polyphaga or hMDMs (Figs 3a and 4a), which is consistent with the idea that they are redundant transporters. Given that the lstA/lstB double mutant resulted in a severely diminished uptake of glucose, we determined the ability of the double mutant to replicate intracellularly. In vitro cultures of lstA/lstB grew similarly to the WT strain (see Supplementary Fig. S3).

Interestingly the lstA/lstB double mutant was severely defective for growth in A. polyphaga and hMDMs (Two-way ANOVA, p < 0.001) (Figs 3b and 4b). Complementation of the double mutant, with individual single transporters, lstA.C or lstB.C, restored intracellular growth of the double mutant in A. polyphaga and hMDMs to almost that of WT L. pneumophila (Figs 3b and 4b). These data show that the two glucose transporters, LstA and LstB, are required for intracellular growth of L. pneumophila within hMDMs and A. polyphaga and these two transporters are most likely to be redundant in their function to import glucose. Our data show that uptake of glucose is required for intracellular replication of L. pneumophila within evolutionarily distant host cells.

Discussion

L. pneumophila generates copious amounts of host amino acids for carbon and energy but it is also reliant on host glucose. Hauslein et al. described L. pneumophila metabolism as being "bipartite", where amino acids serve as the major energy supply in the exponential phase and carbohydrates at the post-exponential phase are used in anabolic processes. The role of glucose during intracellular infection can be difficult to study; methods for altering the levels of glucose affect the host cells, which may have detrimental effects on intracellular growth of L. pneumophila independent of the glucose level. The glucose analog, 2-deoxy-D-glucose (2-DG) causes autophagy in macrophages. Increasing the levels of glucose in macrophages increases the inflammatory response, while starving cells of mimics glucose treatment with 2-DG. Therefore, removing the L. pneumophila's ability to access glucose by deletion of glucose transporters will best highlight the intracellular need for glucose without altering the host cell response to infection.

Our findings indicate that L. pneumophila utilizes two redundant glucose transporters, LstA and LstB, both of which transport glucose, and are required for growth within hMDMs and A. polyphaga. Surprisingly, the need for intracellular glucose is immediate, despite the fact that glucose is thought to be imported by L. pneumophila at the post-exponential phase and is thought to be primarily used in the late stages of growth for PHB synthesis. Consistent with this idea, lstA is highly upregulated in the post-exponential growth phase in vitro, when glucose...
Figure 3. LstA and LstB are required for growth of *L. pneumophila* in amoebae. Intra-vacuolar replication of the (a) the WT strain; the two single transporter mutants, *lstA* and *lstB*; and the complemented single mutant, *lstA*.C and *lstB*.C, was determined in *A. polyphaga*. (b) The WT strain; the *dotA* mutant; the double mutant, *lstA/lstB*; and the complemented double mutant, *lstA/lstB lstA*.C and *lstA/lstB lstB*.C was also determined in *A. polyphaga*. The number of CFUs was determined at 2, 8, and 24 hrs post-infection. Data points represent mean CFUs ± SD, *n* = 3 and are representative of three independent experiments.

Figure 4. LstA and LstB are required for growth of *L. pneumophila* in hMDMs. Intra-vacuolar replication of the (a) the WT strain; the two single transporter mutants, *lstA* and *lstB*; and the complemented single mutant, *lstA*.C and *lstB*.C, was determined in hMDMs. (b) The WT strain; the *dotA* mutant; the double mutant, *lstA/lstB*; and the complemented double mutant, *lstA/lstB lstA*.C and *lstA/lstB lstB*.C was also determined hMDMs. The number of CFUs was determined at 2, 8, and 24 hrs post-infection. Data points represent mean CFUs ± SD, *n* = 3 and are representative of three independent experiments.
is being utilized\textsuperscript{47,66}. However, \textit{lstB} expression remains unchanged throughout the growth phases\textsuperscript{46}. This could represent dual usages for glucose; \textit{LstB} could transport low basal levels of glucose throughout intracellular growth while \textit{LstA} transports large amounts of glucose when the demand has increased during late stages of growth\textsuperscript{46,47}.

Interestingly, \textit{LstA} has been shown to be induced intracellularly during post-exponential growth of \textit{L. pneumophila} in THP and \textit{Acanthamoeba castellanii}, relative to growth in vitro\textsuperscript{49,50}. This supports the idea that glucose is required for intracellular replication but not for \textit{in vitro} growth of \textit{L. pneumophila}\textsuperscript{46}. However, loss of either of the two transporters, \textit{lstA} or \textit{lstB}, is not sufficient to affect intracellular growth, which is most likely due to functional redundancy.

\textit{LstA} is situated adjacent to a glucose utilization gene cluster, which is important for the catabolism of glucose via the ED pathway\textsuperscript{46,47}. Conflicting reports have shown that the gene cluster is required for growth in \textit{A. j.\textsubscript{Clulibertoni}} and \textit{A. polyphaga}, which is molecularly similar and acts as a competitive inhibitor of myo-inositol transport\textsuperscript{66–70}. Therefore, it is possible that \textit{LstB} has dual, or multi-, substrate specificity. Within \textit{L. pneumophila}, \textit{myo-inositol} is also metabolized into acetyl-CoA, which could also support the generation of PHB\textsuperscript{65–67}. Supplementation of glucose \textit{in vitro} does not enhance the growth of \textit{L. pneumophila}\textsuperscript{47}. This may suggest the intracellular requirement for glucose does not support replication as a source of carbon and energy. Given that PHB is generated from glucose and is essential for survival outside of the host, lack of glucose uptake could be triggering stress response genes that prevent \textit{L. pneumophila} from replicating\textsuperscript{59}. If this were the case, starving host cells of glucose, would prevent the replication of intracellular \textit{L. pneumophila}; however, starving host cells of glucose triggers cell death by autophagy\textsuperscript{71}. Glucose is an important requirement for generating a reactive oxygen species (ROS) by amoebae and human macrophages in response to invading pathogens\textsuperscript{64,65}. Uptake of glucose by \textit{L. pneumophila} could serve dual purposes of sequestering glucose from the host, to dampen the immune response and to provide the precursor for PHB.

In summary, we have identified two redundant glucose transporters, \textit{LstA} and \textit{LstB}, which are required for intracellular replication of \textit{L. pneumophila} in macrophages and amoebae. The requirement for glucose uptake by \textit{L. pneumophila} is essential for intracellular growth in hMDMs and \textit{A. polyphaga}, but not during growth \textit{in vitro}. This presents an interesting question in \textit{L. pneumophila} biology; why is glucose import required only during intracellular replication? This should be the focus of future studies.

Materials and Methods

Strains and cell lines. \textit{L. pneumophila} strain AA100/130b (ATCC BAA-74) and the T4SS-deficient mutant, \textit{dotA}, were grown on Buffered Charcoal Yeast Extract (BCYE) agar, as we previously described\textsuperscript{72}. To generate isogenic mutants in \textit{lstA} and \textit{lstB}, ~2 kb of flanking DNA on either side was amplified using primers listed in Table S2 and cloned into the shuttle vector, pBCSK- \textit{LstAKO} and pBCSK- \textit{LstBKO} (Table S1). The entire gene of either \textit{lstA} or \textit{lstB} was deleted via inverse PCR using the primers listed in Table S2, resulting in pBCSK- \textit{LstAKO} and pBCSK- \textit{LstBKO} (Table S1). The kanamycin cassette from the Ex-Tn5 transposon was amplified using primers listed in Table S2 and the resulting PCR product was subcloned into pBCSK- \textit{LstAKO} and pBCSK- \textit{LstBKO} between the flanking regions of either \textit{lstA} or \textit{lstB}, using standard molecular procedures, resulting in pBCSK- \textit{LstAKAN} and pBCSK- \textit{LstBKAN} (Table S1). Each resulting plasmid was independently introduced into \textit{L. pneumophila} AA100/130b via natural transformation, as we previously described\textsuperscript{73}. After three days, natural transformants were recovered by plated on BCYE supplemented with 50 \textmu{g}/ml kanamycin, to generate \textit{L. pneumophila \textit{lstA}} and \textit{L. pneumophila \textit{lstB}} (Table S2). To confirm deletion of either \textit{lstB} or \textit{lstB}, the forward primer for sequencing and the reverse primer for generation of the knockout, listed in Table S2 were used. To generate double mutants, a gentamycin cassette was amplified using primers listed in Table S2 and the resulting PCR product was subcloned into pBCSK- \textit{LstAKO} between the flanking regions of either \textit{lstA}, using standard molecular procedures, resulting in pBCSK- \textit{LstAGENT} (Table S1). The resulting plasmid was independently introduced into \textit{L. pneumophila} AA100/130b via natural transformation, as we previously described\textsuperscript{73}. After three days, natural transformants were recovered by plated on BCYE supplemented with 20 \textmu{g}/ml kanamycin and 5 \textmu{g}/ml gentamycin, to generate \textit{L. pneumophila \textit{lstB/lstA}} (Table S1). Deletions were confirmed using the same primers as described above.

To generate complement mutants of single deletions and double deletions, \textit{lstA} or \textit{lstB} with flanking upstream and downstream sequences were amplified by PCR using the primers listed in Table S2, and subcloned into pBCSK+, generating pBCSK- \textit{LstAC} and pBCSK- \textit{LstBC} (Table S1). The pBCSK- \textit{LstAC} plasmid was introduced into the \textit{lstA} and \textit{lstA/lstB} mutants and the pBCSK- \textit{LstBC} plasmid was introduced into the \textit{lstB} and \textit{lstA/lstB} mutants via electroporation as previously described (Table S1)\textsuperscript{51}. All complement mutants were selected on BCYE plates supplemented with 5 \textmu{g}/ml chloramphenicol, resulting in the following complement strains: \textit{LstAC}, \textit{LstBC}, \textit{LstA/lstB \textit{LstAC}}, and \textit{LstA/lstB \textit{LstBC}} (Table S1).

Human monocyte-derived macrophages (hMDMs) were isolated from healthy adult donors and cultured in RPMI 1640 (Corning) supplemented with 10% fetal bovine serum, as previously described\textsuperscript{72}. All methods were carried out and approved in accordance to the University of Louisville InstitutionalReview Board guidelines and blood donors gave informed consent as required by the University of Louisville Institutional Review Board (IRB).
Structural comparison of glucose transporters. Predicted structures were generated via I-TASSER server from the Zhang Lab (https://zhanglab.ccmb.med.umich.edu/I-TASSER/). Structures generated from I-TASSER were aligned using TM-align to determine structural alignment and TM-scores for similarity (https://zhanglab.ccmb.med.umich.edu/TM-align/).

Glucose uptake assay. Uptake of glucose was assayed by growing WT L. pneumophila, IstA, IstB, and complemented mutant strains individually in the presence of 14C-label glucose (specific activity, 3.3 MBq/μmol) (PerkinElmer) and the presence of glucose followed into the acid-insoluble fraction, as previously described. One milliliter cultures were grown in Buffered Yeast Extract (BYE) broth supplemented with 0.1% D-[U-14C] glucose (specific activity, 3.3 MBq/μmol) at 37°C, shaking to post-exponential phase (>OD550 2.0). For control, 10 mM sterile glucose was added to broth cultures with 0.1% D-[U-14C] glucose. Samples normalized to 10^6 bacteria in 5 ml 1% Triton X-100 for 30 mins, and then incubated for 30 mins with 5 ml of chilled 10% (w/v) trichloroacetic acid, on ice. To capture radioactivity, samples were filtered through nitrocellulose filters (0.45-μm pore size; Millipore) and rinsed three times with chilled 5% trichloroacetic acid. Radioactivity of the whole sample was determined by liquid scintillation (Tri-Carb 2910 TR, PerkinElmer) with BetaBlend scintillation cocktail (MP Biomedical).

Intracellular replication. L. pneumophila; the isogenic single mutants, dotA, IstA and IstB; the double isogenic mutant IstB/IstA; and the complement mutants IstA.C, IstB.C, IstB/IstA IstB.C, and IstB/IstA IstC.C were grown to post-exponential phase on BCYE plates at 37°C prior to infection and used to infect hMDMs or A. polyphaga, as previously described. A total of 1 × 10^5 host cells per well were plated into infected 96 well plates and infected with L. pneumophila at an MOI of 10 for 1 h then treated with gentamicin to kill remaining extracellular bacteria, as previously described. Host cells were lysed with sterile water (hMDMs) or 0.02% Triton X-100 (A. polyphaga) at various timepoints over a 24 h timecourse and L. pneumophila CFUs were determined by plating serial dilutions onto BCYE agar.

Data availability. All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

References
1. McDade, J. E. et al. Legionnaires’ disease: isolation of a bacterium and demonstration of its role in other respiratory disease. N Engl J Med 297, 1197–1203 (1977).
2. Fraser, D. W. et al. Legionnaires’ disease: description of an epidemic of pneumonia. N Engl J Med 297, 1189–1197 (1977).
3. Tsai, T. F. & Fraser, D. W. The diagnosis of Legionnaires’ disease. Ann Intern Med 89, 413–414 (1978).
4. Molmeret, M., Horn, M., Wagner, M., Santic, M. & Abu Kwaik, Y. Anceboae as training grounds for intracellular bacterial pathogens. Appl Environ Microbiol 71, 20–28 (2005).
5. Richards, A. M., Von Dwingelo, J. E., Price, C. T. & Abu Kwaik, Y. Cellular microbiology and molecular ecology of Legionella-amoeba interaction. Virulence 4, 307–314, https://doi.org/10.4161/viru.24290 (2013).
6. Nash, T. W., Libby, D. M. & Horwitz, M. A. Interaction between the legionnaires’ disease bacterium (Legionella pneumophila) and human alveolar macrophages. Influence of antibody, lymphokines, and hyaluronidase. J Clin Invest 74, 771–782 (1984).
7. Shuman, H. A., Purcell, M., Segal, G., Hales, L. & Wiater, L. A. Intracellular multiplication of Legionella pneumophila: Human pathogen or accidental tourist? Curr Top Microbiol Immunol 225, 99–112 (1998).
8. Boamah, D. K., Zhou, G., Ensminger, A. W. & O’Connor, T. J. From Many Hosts, One Accidental Pathogen: The Diverse Protozoan Pathogens. PLoS Pathog 5, e1000467, https://doi.org/10.1371/journal.ppat.1000467 (2009).
9. Molmeret, M., Horn, M., Wagner, M., Santic, M. & Abu Kwaik, Y. Anceboae as training grounds for intracellular bacterial pathogens. Appl Environ Microbiol 71, 20–28 (2005).
10. Richards, A. M., Von Dwingelo, J. E., Price, C. T. & Abu Kwaik, Y. Cellular microbiology and molecular ecology of Legionella-amoeba interaction. Virulence 4, 307–314, https://doi.org/10.4161/viru.24290 (2013).
11. Nash, T. W., Libby, D. M. & Horwitz, M. A. Interaction between the legionnaires’ disease bacterium (Legionella pneumophila) and human alveolar macrophages. Influence of antibody, lymphokines, and hyaluronidase. J Clin Invest 74, 771–782 (1984).
12. Shuman, H. A., Purcell, M., Segal, G., Hales, L. & Wiater, L. A. Intracellular multiplication of Legionella pneumophila: Human pathogen or accidental tourist? Curr Top Microbiol Immunol 225, 99–112 (1998).
13. Boamah, D. K., Zhou, G., Ensminger, A. W. & O’Connor, T. J. From Many Hosts, One Accidental Pathogen: The Diverse Protozoan Pathogens. PLoS Pathog 5, e1000467, https://doi.org/10.1371/journal.ppat.1000467 (2009).
14. Molmeret, M., Horn, M., Wagner, M., Santic, M. & Abu Kwaik, Y. Anceboae as training grounds for intracellular bacterial pathogens. Appl Environ Microbiol 71, 20–28 (2005).
15. Richards, A. M., Von Dwingelo, J. E., Price, C. T. & Abu Kwaik, Y. Cellular microbiology and molecular ecology of Legionella-amoeba interaction. Virulence 4, 307–314, https://doi.org/10.4161/viru.24290 (2013).
16. Nash, T. W., Libby, D. M. & Horwitz, M. A. Interaction between the legionnaires’ disease bacterium (Legionella pneumophila) and human alveolar macrophages. Influence of antibody, lymphokines, and hyaluronidase. J Clin Invest 74, 771–782 (1984).
17. Shuman, H. A., Purcell, M., Segal, G., Hales, L. & Wiater, L. A. Intracellular multiplication of Legionella pneumophila: Human pathogen or accidental tourist? Curr Top Microbiol Immunol 225, 99–112 (1998).
18. Boamah, D. K., Zhou, G., Ensminger, A. W. & O’Connor, T. J. From Many Hosts, One Accidental Pathogen: The Diverse Protozoan Pathogens. PLoS Pathog 5, e1000467, https://doi.org/10.1371/journal.ppat.1000467 (2009).
19. Molmeret, M., Horn, M., Wagner, M., Santic, M. & Abu Kwaik, Y. Anceboae as training grounds for intracellular bacterial pathogens. Appl Environ Microbiol 71, 20–28 (2005).
20. Richards, A. M., Von Dwingelo, J. E., Price, C. T. & Abu Kwaik, Y. Cellular microbiology and molecular ecology of Legionella-amoeba interaction. Virulence 4, 307–314, https://doi.org/10.4161/viru.24290 (2013).
21. Nash, T. W., Libby, D. M. & Horwitz, M. A. Interaction between the legionnaires’ disease bacterium (Legionella pneumophila) and human alveolar macrophages. Influence of antibody, lymphokines, and hyaluronidase. J Clin Invest 74, 771–782 (1984).
22. Shuman, H. A., Purcell, M., Segal, G., Hales, L. & Wiater, L. A. Intracellular multiplication of Legionella pneumophila: Human pathogen or accidental tourist? Curr Top Microbiol Immunol 225, 99–112 (1998).
23. Rolando, M. et al. Legionella pneumophila effector RomA uniquely modifies host chromatin to repress gene expression and promote intracellular bacterial replication. Cell Host Microbe 13, 395–405, https://doi.org/10.1016/j.chom.2013.03.004 (2013).

24. Fontana, M. F. et al. Secreted bacterial effectors that inhibit host protein synthesis are critical for induction of the innate immune response to virulent Legionella pneumophila. PLoS pathogens 7, e1001289, https://doi.org/10.1371/journal.ppat.1001289 (2011).

25. Luo, Z. Q. Legionella secreted effectors and innate immune responses. Cell Microbiol, https://doi.org/10.1111/1462-5882.2011.01713.x (2011).

26. Tesh, M. J., Morse, S. A. & Miller, R. D. Intermediary metabolism in Legionella pneumophila: utilization of amino acids and other compounds as energy sources. J.Bacteriol. 154, 1104–1109 (1983).

27. Schuender, E. et al. Amino Acid Uptake and Metabolism of Legionella pneumophila Hosted by Acanthamoeba castellanii. The Journal of biological chemistry 289, 21040–21054, https://doi.org/10.1074/jbc.M114.570085 (2014).

28. Fonseca, M. V. & Swanson, M. S. Nutrient salvaging and metabolism by the intracellular pathogen Legionella pneumophila. Front Cell Infect Microbiol 4, 12, https://doi.org/10.3389/fcimb.2013.00012 (2014).

29. Manske, C. & Hilbi, H. Metabolism of the vacuolar pathogen Legionella and implications for virulence. Frontiers in Cellular and Infection Microbiology 4, https://doi.org/10.3389/fcimb.2014.00125 (2014).

30. Price, C. et al. Host FIH-Mediated Asparaginyl Hydroxylation of Translocated Legionella pneumophila Effectors. Front Cell Infect Microbiol 7, 54, https://doi.org/10.3389/fcimb.2017.00054 (2017).

31. Qiu, J. & Luo, Z.-Q. Hijacking of the Host Ubiquitin Network by Legionella pneumophila. Frontiers in Cellular and Infection Microbiology 7, https://doi.org/10.3389/fcimb.2017.00487 (2017).

32. Kubori, T., Bui, X. T., Hubber, A. & Nagai, H. Legionella RavZ Plays a Role in Preventing Ubiquitin Recruitment to Bacteria-Containing Vacuoles. Frontiers in Cellular and Infection Microbiology 7, https://doi.org/10.3389/fcimb.2017.00384 (2017).

33. Al-Quadan, T. & Kwiska, J. A. Molecular Characterization of Exploitation of the Polyubiquitination and Farnesylation Machineries of Dictyostelium Discoideum by the AnkB F-Box Effector of Legionella Pneumophila. Frontiers in microbiology 2, 23, https://doi.org/10.3389/fmicb.2011.00023 (2011).

34. Lomma, M. et al. The Legionella pneumophila F-box protein Lpp2082 (AnkB) modules ubiquitination of the host protein parvin B and promotes intracellular replication. Cell Microbiol 12, 1272–1291, https://doi.org/10.1111/j.1462-5882.2010.01467.x (2010).

35. Price, C. T., Al-Khodor, S., Al-Quadan, T. & Abu Kwaik, Y. Indispensable role for the eukaryotic-like ankyrin domains of the ankyrin B effector of Legionella pneumophila within macropaghes and amoebae. Infection and immunity 78, 2079–2088, https://doi.org/10.1128/IAI.01450-09 (2010).

36. Eisenreich, W., Heesemann, J., Rudel, T. & Goebel, W. Metabolic host responses to infection by intracellular bacterial pathogens. Front Cell Infect Microbiol 3, 24, https://doi.org/10.3389/fcimb.2013.00024 (2013).

37. Tesh, M. J. & Miller, R. D. Amino acid requirements for Legionella pneumophila growth. J.Clin.Microbiol. 13, 865–869 (1981).

38. Price, C. & Abu Kwaik, Y. Amoebae and Mammals Deliver Different Pathogenic Products to Acanthamoeba. PLoS Pathogens 6, e1000923 (2010).

39. Price, C. T., Richards, M. A., Von Dwingle, J. E., Samara, H. A. & Abu Kwaik, Y. Amoeba-host Legionella synchronization of amino acid auxotrophy and its role in bacterial adaptation and pathogenic evolution. Environmental microbiology 16, 350–358, https://doi.org/10.1111/1462-2920.2012290 (2014).

40. Abu Khweek, A. et al. Biofilm-derived Legionella pneumophila evades the innate immune response in macrophages. Frontiers in Cellular and Infection Microbiology 3, https://doi.org/10.3389/fcimb.2013.00018 (2013).

41. Garcia, M. T., Jones, S., Pelaz, C., Millar, R. D. & Abu Kwaik, Y. Acanthamoeba polyphaga resuscitates viable non-culturable Legionella pneumophila and promotes intracellular replication. Front Cell Microbiol 7, 2099, https://doi.org/10.3389/fcimb.2016.02099 (2016).

42. Bruckert, W. M., Price, C. T. & Abu Kwaik, Y. Rapid nutritional remodeling of the host cell upon attachment of Legionella pneumophila. Infection and immunity 82, 72–82, https://doi.org/10.1128/IAI.01079-13 (2014).

43. Eylert, E. et al. Isotopolog profiling of Legionella pneumophila: role of serine and glucose as carbon substrates. The journal of biological chemistry 285, 22232–22243, https://doi.org/10.1074/jbc.M1126878 (2010).

44. Harada, E., Iida, K., Shiota, S., Nakayama, H. & Yoshida, S. Glucose metabolism in Legionella pneumophila: dependence on the Entner-Doudoroff pathway and connection with intracellular bacterial growth. Journal of bacteriology 192, 2889–2899, https://doi.org/10.1128/JB.01655-09 (2010).

45. Hauslein, I., Manske, C., Goebel, W., Eisenreich, W. & Hilbi, H. Pathway analysis using (13)C-glycerol and other carbon tracers reveals a bipartite metabolism of Legionella pneumophila. Molecular microbiology 100, 229–246, https://doi.org/10.1111/mmi.13313 (2016).

46. James, B. W., Mauchline, W. S., Dennis, P. J., Keevil, C. W. & Wait, R. Poly-3-hydroxybutyrate in Legionella pneumophila. Front Cell Infect Microbiol 5, https://doi.org/10.3389/fcimb.2015.00111 (2015).

47. Gilmainer, N. et al. Growth-Related Metabolism of the Carbon Storage Poly-3-Hydroxybutyrate in Legionella pneumophila. The journal of biological chemistry, https://doi.org/10.1074/jbc.M115.693481 (2016).

48. Yamn, A., Brennan, S. L., Johnson, R. C., Rubenstein, G. L. & Cambone, E. D. Identification of Conserved ABC Importers Necessary for Intracellular Survival of Legionella pneumophila in Multiple Hosts. Frontiers in Cellular and Infection Microbiology 7, https://doi.org/10.3389/fcimb.2017.00485 (2017).

49. Price, C. T., Richards, M. A. & Abu Kwaik, Y. Nutrient generation and retrieval from the host cell cytosol by intra-vacuolar Legionella pneumophila. Front Cell Infect Microbiol 4, 111, https://doi.org/10.3389/fcimb.2014.00111 (2014).

50. Sauer, J. D., Bachman, M. A. & Swanson, M. S. The phagosomal transporter A couples threonine acquisition to differentiation and replication of Legionella pneumophila in macrophages. Proceedings of the National Academy of Sciences of the United States of America 102, 9924–9929, https://doi.org/10.1073/pnas.0502767102 (2005).

51. de Felipe, K. S. et al. Evidence for acquisition of Legionella type IV secretion substrates via interdomain horizontal gene transfer. Journal of bacteriology 187, 7716–7726, https://doi.org/10.1128/JB.01872-10 (2015).

52. Gomez-Yelmono, L. et al. Excessive vacuolation events and horizontal gene transfer shaped the Legionella pneumophila genomes. BMC Genomics 12, 536, https://doi.org/10.1186/1471-2164-12-536 (2011).

53. Schlessinger, A., Yee, S. W., Sali, A. & Giacominini, K. M. SLC classification: an update. Clin Pharmacol Ther 94, 19–23, https://doi.org/10.1038/clpt.2013.73 (2013).

54. Yang, J. et al. The I-TASSER Suite: protein structure and function prediction. Nat Methods 12, 7–8, https://doi.org/10.1038/nmeth.3213 (2015).
Acknowledgements
The Y.A.K. lab is supported by Public Health Service Awards R01AI120244 from the NIAID and by the Commonwealth of Kentucky Research Challenge Trust Fund. A.B. was supported by a National Science Foundation Graduate Research Fellowship (NSF GRFP) under Grant No. DGE-1144204.

Author Contributions
Y.A.K. and A.B. conceived the ideas and designed the experiments. A.B. and S.J. performed the experiments. A.B. analysed all the data. A.B. and Y.A.K. wrote the main manuscript text and A.B. prepared all figures. All authors have read and approved of the final manuscript.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-018-26782-x.

Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2018