UNIVERSITY OF CALIFORNIA, SAN DIEGO

Comparative Analyses of Transport Proteins in the Genus *Leptospira*

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

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2014

The Thesis of Bora Buyuktimkin is approved and it is acceptable in quality and form for publication on microfilm and electronically:

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University of California, San Diego

2014

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ACKNOWLEDGEMENTS

I would first like to thank Professor Milton Saier, Jr., Ph.D., for his guidance as my advisor and mentor. His unwavering support and enthusiasm is unmatched and contagious. Additionally, I must thank members of Saier lab for their support and encouragement including: Andy, Justin, Daniel, Brian, Ake, Greg, Andrew K., Shounak, Andrew L., Scott, Ida, and Andre. Vamsee Reddy deserves specific acknowledgement as his computer work is quite literally the glue that holds this lab together.

The support that I had goes beyond the laboratory. Dr. Mary Boyle showed me the joy in teaching, while Dr. Angela Yu helped me handle the nerves of my first time teaching as a second year student. Dr. Keefe Reuther was a delight to work with as an incredibly relatable person. Dr. Tour gave me assurance and confidence in my research. The MQ was home away from home in its papers and people. Coach Johnson and the rowing team helped me build the courage and discipline I will use for the rest of my life.

I wish to thank my siblings: Arda, for generating the strangest collection of inside jokes; Barlas and Tuba for being the accomplished older brother and older sister I model myself after. Last, but most certainly not least, I wish to thank my parents. They are the kind, thoughtful, and insightful parents that every child should wish to have growing up and they have made all that I have accomplished possible. The restricted

research climate in the 1980s in Turkey and throughout Europe deserves mention as it prompted my parents to come to America with nothing more than a few pieces of oversized luggage to become postdocs, giving me access to the tools, resources, and support that have allowed me to grow as a person.

As this is my first rodeo, so to speak, I have had my fair share of ups and downs with this project. I stopped counting after 3 the number of times I started completely over from scratch, hours forever gone in lieu of studying, grading, or keeping up with my friends. However, had one of those earlier versions of that work come out its quality and my growth would have suffered for it. In these trials and tribulations I turned to television to keep myself sane, particularly the comedy show *30 Rock*. I found an offhand quote by the character Dr. Leo Spaceman that resonated with me: "Science is whatever we want it to be." My attitude toward science and biology prompted by this zany character's remark is summed up in the following quote:

The nitrogen in our DNA, the calcium in our teeth, the iron in our blood, the carbon in our apple pies were made in the interiors of collapsing stars. We are made of starstuff.

-Carl Sagan

Just as we humans are of cosmic origin, so are even the smallest and seemingly most insignificant bacteria. Then, all life exists with the same cosmic relevance, mysterious and spectacular. By understanding the organisms around us we better understand the universe, ourselves, and where we come from.

ABSTRACT OF THE THESIS

Comparative Analyses of Transport Proteins in the Genus *Leptospira*

by

Bora Buyuktimkin

Master of Science in Biology

University of California, San Diego, 2014

Professor Milton Saier Jr., Chair

Select species of the bacterial genus *Leptospira* are causative agents of leptospirosis, an emerging global zoonosis affecting nearly one million people worldwide annually. We examined two *Leptospira* pathogens, *L. interrogans* serovar Lai str. 56601 and *L. borgpetersenii* serovar Hardjo-bovis str. L550, as well as the free-living leptospiral saprophyte, *L. biflexa* serovar Patoc str. 'Patoc 1 (Ames)'. The transport proteins of these leptospires were identified and compared through bioinformatics to determine which proteins are related to pathogenesis, and

saprophytism. *L. biflexa* possesses a disproportionately high number of secondary carriers for metabolic uptake flexibility and environmental adaptability, as well as an increased number of inorganic cation transporters providing ionic homeostasis and effective osmoregulation in a rapidly changing environment. *L. interrogans* and *L. borgpetersenii* possess remarkably similar transporter proteomes (transportomes), with near-equivalent representation in most transporter families. The *Leptospira* pathogens possess complete sphingomyelinases, holins, and virulent outer membrane porins. These transport-related virulence factors, in conjunction with decreased transporter substrate versatility, indicates that pathogenicity arose in *Leptospira* as a result of progressively narrowing ecological niches and the emergence of a limited set of proteins responsible for host invasion. The variability of host tropism and mortality rates by infectious leptospires suggests that small differences in individual sets of proteins play important physiological and morphological roles.

INTRODUCTION

Leptospirosis is an emerging zoonotic disease that affects about 900,000 people annually worldwide. It is caused by members of the bacterial genus *Leptospira* within the Spirochaete phylum (Bharti et al. 2003). The disease poses a tremendous public health risk in tropical environments, especially as it is transmitted through contaminated water, infected tissue, and the urine of mammalian hosts (Bharti et al. 2003). Once infected, patients can potentially experience a variety of symptoms ranging from fever, myalgia, and fatigue to refractory shock, jaundice, renal failure, and pulmonary hemorrhage (Bharti et al. 2003). At-risk populations for this disease are primarily, but not exclusively associated with tropical climates in developed and underdeveloped countries. Cases throughout the United States have also been reported e.g. Hawaii, Baltimore, New Orleans, etc. (Duplessis et al. 2011, Vinetz et al. 1996, Toliver and Krane 2014). Factors that increase risk include conditions of slum living, recreational water activities, and flooding (Bharti et al. 2003). Nonetheless, leptospirosis is found globally, and various animals can serve as reservoirs for its transmissions to humans such as rats, bats, and marsupials as well as domesticated animals like dogs and cows (Paixao Mdos et al. 2014, Vashi et al. 2010, Ayral et al. 2014) Human to human transmission is rare, but it is believed that globalization and ecotourism contribute significantly to the emergence of this zoonosis (Bandara et al. 2014).

The causative agents of leptospirosis, *Leptospira* spp., are spiral-shaped, thin, aerobic, gram-negative bacteria of highly divergent spirochetes, whose primary

carbon sources are long-chain fatty acids (Adler and de la Pena Moctezuma 2010). Despite its threat to global public health, the genus of *Leptospira* is not entirely filled with pathogenic species (Cerqueira and Picardeau 2009). It is divided up into saprophytes (e.g. *L. biflexa*, *L. wolbachii*, *L. meyeri)*, pathogens (e.g. *L. interrogans*, *L. borgpetersenii*, *L. kmetyi*), and intermediate pathogens (e.g. *L. licerasiae*, *L. wolfii*, *L. broomii*) (Cerqueira and Picardeau 2009). Among these characteristically distinct leptospira, practice among scientists has been to distinguish leptospires from each other by means of serotyping and antigenic similarity instead of genetic similarity (Cerqueira and Picardeau 2009). Consequently, serotyping has resulted in the identification of over 230 different serovars among *Leptospira* with serovars crossing species lines (Cerqueira and Picardeau 2009). The limited ability of sequencing technology at the advent of the identification and characterization of members of *Leptospira* was a driving force for this behavior, but rapid sequencing technology such as qPCR has begun to enable scientists and clinicians to rapidly and effectively identify infecting leptospires (Gonzalez et al. 2013).

L. interrogans and *L. borgpetersenii* are two of many species that have been identified as pathogens of leptospirosis (Adler and de la Pena Moctezuma 2010). Their most common reservoir animals are rats and cows, respectively, although these two spirochetes are not exclusive to these two animals (Loffler et al. 2014, Fang et al. 2014). Working closely with these and other aforementioned animals, or being in proximity to them or their urine, gives increased likelihood to contracting the pathogen (Munoz-Zanzi et al. 2014). *L. biflexa*, on the other hand, is a free-living

saprophyte isolated from stream water, whose antigenic properties have been used as a basis for antigenic testing of pathogenic leptospires (Victoria et al. 2008).

In our study, the publicly available genomes of the following representative organisms were examined: *L. interrogans* serovar Lai str. 56601, *Leptospira borgpetersenii* serovar Hardjo-bovis str. L550, *Leptospira biflexa* serovar Patoc str. 'Patoc 1 (Ames)'. Table 1 shows the nomenclature for these organisms used in this study as well as additional information about the organisms. *L. interrogans* and *L. borgpetersenii* allow comparison and identification of hallmarks of pathogenic leptospires, and *L. biflexa* enables comparison for identification of transport proteins and mechanisms unlikely to be related to pathogenicity.

In general, the mechanisms of pathogenesis in leptospirosis are relatively poorly understood. However, there are several suggested mechanisms of *Leptospira* pathogenesis (Adler and de la Pena Moctezuma 2010). The coiled shape of *Leptospira* is relevant to its corkscrew-like motility through viscous media, which provides an efficient mechanism of dissemination after entry into various organs such as the lungs, liver, kidneys, eyes, and brain (Adler and de la Pena Moctezuma 2010) Genes associated with motility and methyl-accepting chemotaxis are known to play a role in virulence (Adler 2014). Among proposed factors that may facilitate virulence during migration through host tissues are hemolytic sphingomyelinases and phospholipases (Adler 2014). Additional components of *Leptospira* virulence promote adhesion and

invasion of host cells, although intracellular pathogenicity has not been demonstrated (Adler 2014).

Chronically infected animals (rats, bats, and marsupials) are usually asymptomatic but show high levels of leptospiral excretion through the urine, supporting the hypothesis that renal colonization is important for *Leptospira* in reservoir selection and pathogenesis (Adler and de la Pena Moctezuma 2010, Hsu et al. 2010). Kidney histological studies further support this hypothesis, as kidneys show interstitial nephritis during infection but no such damage in chronic carriers (Ferrer et al. 2014). Kidney nephritis, along with damage to connective tissues, evident from hemorrhagic manifestations in lungs, supports virulence mechanisms involving invasion and damage to connective tissues (Stevenson et al. 2007). Leptospiral lipopolysaccharide (LPS), known to be less toxic than the typical LPSs of other gram-negative bacteria, more strongly activates Toll-like receptor 2 (TLR2) than TLR4, conventionally achieved by gram-negative LPS in macrophages (Wang et al. 2012).

Ultimately, these hemolytic sphingomyelinase and phospholipase activities, together with the identified motility and chemotaxis factors of *Leptospira*, damage host tissue and activate the inflammatory response of the host immune system to potentially cause significant damage, eventually resulting in death of the host (Bharti et al. 2003). Identification of transporters relevant to pathogenesis might reveal the presence of pore-forming toxins, transporters facilitating basic nutrient uptake, and the protein secretion systems necessary to release proteinaceous virulence factors.

Furthermore, *Leptospira* pathogenic species are known to maintain poor viability in acidic urine relative to alkaline urine which suggests a preference of sodium cations (Na^+) in some transport systems that establish or utilize the proton motive force or the sodium motive force (Adler and de la Pena Moctezuma 2010).

Pathogenic species of *Leptospira* must encode the proteins that mediate virulence. Both *L. interrogans* and *L. borgpetersenii* might be expected to show similar pathogenesis-related transporters, but this postulate had not been examined. *L. biflexa* would be expected to have few, or incomplete sets of these proteins (Picardeau et al. 2008). Studies on *L. biflexa, L. interrogans, and L. borgpetersenii* have suggested that *L. biflexa* is most closely related to the common *Leptospira* ancestor, and that pathogenicity is an acquired feature (Picardeau et al. 2008) Consequently, the saprophytic and free-living nature of *L. biflexa* suggests that its genome enables it to live with high versatility in a range of environments (Picardeau et al. 2008, Bulach et al. 2006). The suggested flexibility of *L. interrogans* to live within a host and also in the external environment combined with the greater dependence of *L. borgpetersenii* for survival in the host in spite of its relatively small genome due to insertion sequence (IS)-mediated reduction (Bulach et al. 2006), suggests that increased host tropism and pathogenicity favor decreased versatility and reduced genetic diversity. The encoded transport proteins should reflect these characteristics.

METHODS

The spirochete genomes analyzed were the most complete and up to date versions for each organism at the time these studies were initiated. The FASTA formatted protein coding sequences of *Leptospira interrogans* serovar Lai str. 56601, *Leptospira biflexa* serovar Patoc str. 'Patoc 1 (Ames)', and *Leptospira borgpetersenii* serovar Hardjo-bovis str. L550 were used (Ren et al. 2003, Bulach et al. 2006, Picardeau et al. 2008). Each protein sequence from the respective proteomes was queried and blasted against the Transporter Classification Database (TCDB; www.tcdb.org) using the program GBlast (Reddy and Saier 2012). GBlast retrieves the TC top hit sequence, TC number, protein size in number of amino acyl residues, the number of predicted TMSs using the HMMTop 2.0 Program, the E-value for the query and hit proteins, regions of sequence similarity, and regions of TMS overlap. The low complexity filter was not used as it is normally of value only for larger datasets including proteins with multiple repeat elements. The Web-based Hydropathy, Amphipathicity and Topology (WHAT) program (Tusnady and Simon 2001, Zhai and Saier 2001) was used with a window size of 19 residues and an angle of 100 degrees (as is appropriate for alpha helices) to display the hydropathy plot for individual proteins in conjunction with TOPCONS for consensus prediction (TOPCONS, www.topcons.net) in order to resolve the differences in the number of TMSs between the proteins retrieved and their TCDB homologues. The plot generated by WHAT allows the user to judge if a program such as HMMTOP has missed a TMS or has predicted a TMS inappropriately. A cut-off E-value of 0.001 was used with the

GBlast program to eliminate false positives and proteins with unreliable degrees of sequence divergence.

Proteins with no predicted TMSs were eliminated so that only integral membrane proteins, primarily multispanning membrane proteins, were retrieved. Proteins with only an N-terminal signal sequence are numerous because these proteins include almost all secreted proteins that are exported via the general secretory (Sec) pathway or twin arginine translocase (TAT) (Pugsley 1993, Sargent et al. 2006). The topological prediction programs often miss these TMSs, recording the proteins to have zero TMSs. Consequently, the number of zero or one TMS proteins retrieved were not reliable and were therefore not always recorded. Furthermore, TMSs detected by GBlast are only of the α-helix-type transporters and not β-sheet-type porins. Transporters known to have β-sheet, porins found primarily in TC subclasses 1.B and partly in 1.C, were further analyzed for β strands using PRED-TMBB with all three decoding methods (Bagos et al. 2004).

Transport proteins thus obtained from query *Leptospira* sequences were tabulated, and unusual characteristics were identified based in part on topologies that differed from corresponding family members in TCDB as well as E-values obtained with GBlast. Unusual properties can result from events such as genetic deletion and fusion, sometimes resulting in the gain or loss of extra domains or the generation of multifunctional proteins. Such results can be reflective of the protein sequence, but they can be artifacts due to sequencing errors or incorrect initiation codon assignment.

In the latter cases, but not the former, the protein sequences were either corrected when possible or eliminated from our study.

Candidate proteins were examined in greater detail to estimate their probable substrate specificities on the basis of their predicted structures and numbers and degrees of sequence similarity with entries of known function in TCDB. Transport proteins were also classified into families and subfamilies of homologous transporters according to the classification system presented in TCDB (Saier et al. 2006, Saier et al. 2009, Saier et al. 2014). Regions of sequence similarity were examined to ensure that homology was in the transmembrane region(s) and not merely in hydrophilic domains (Youm and Saier 2012). The substrate specificities of particular homologues identified in the sequenced genomes were often predicted based on homology to functionally characterized proteins. Assignment to a family or subfamily within the TC system often allows prediction of substrate type with confidence (Busch and Saier 2002, Felce and Saier 2004, Saier 2000).

RESULTS

Three *Leptospira* genomes were analyzed for the occurrence of transport proteins using the Transporter Classification Database (TCDB; www.tcdb.org, (Yen et al. 2009)) and the GBlast program (Reddy and Saier 2012). The results are summarized according to TC subclass in Table 2. Examining the total number of transport proteins present in these three genomes, we see that *L. borgpetersenii* has the fewest at 260. The most found in any genome is 337 for *L. biflexa*, 77 more than in *L. borgpetersenii*. However, the two pathogenic species (*L. interrogans* and *L. borgpetersenii*) combined contained 110 unique transport proteins lacking in *L. biflexa*, the saprophyte.

Transport Protein Subclasses

TC subclass 1.A in TCDB includes all α-type channels except for holins which are found in subclass 1.E. 17, 21, and 26 of these α-type channel proteins were identified in *L. interrogans*, *L. borgpetersenii*, and *L. biflexa*, respectively. *L. biflexa* possesses the greatest number of unique families in this subclass, many of which are cationic channels including two mercuric ion channels, suggesting greater versatility in the saprophyte than in the pathogens. Furthermore, *L. interrogans* possesses eight proteins belonging to the 1.A.30 Outer Membrane Transporter Energizer (Mot-Exb) Superfamily compared to the fifteen found in both *L. biflexa* and *L. borgpetersenii*.

TC subclass 1.B includes outer membrane β-type porins. 38 were identified in *L. interrogans*, 32 in *L. borgpetersenii*, and 39 in *L. biflexa*. The distribution of these

porins does not suggest a major contribution to pathogenicity. Since these proteins localize to the outer membrane via β-strands instead of α-helixes, those containing zero or one predicted α-helical TMSs were included in our study.

TC subclass 1.C includes pore-forming toxins. *L. interrogans* encodes ten putative toxins showing sequence similarity to established toxins belonging to four families, whereas *L. borgpetersenii* contains eight and *L. biflexa* contains seven. The 1.C.67 family of SphH Hemolysins is notably absent in *L. biflexa* but present in *L. interrogans* and *L. borgpetersenii* with four and two members, respectively. Although hemolysins have not been unequivocally shown to be essential for leptospiral pathogenesis, their presence in pathogens is likely to be of significance (Adler 2014). Hemolysins have been shown to strongly induce proinflammatory cytokines (Wang et al. 2012). The three other families represented in subclass 1.C contain similar representation in the three leptospires examined. It should be noted that toxins with zero or one predicted transmembrane α-helices were included in this study as many secreted toxins can exist in both soluble and membrane integrated forms, and many are known to be pore-forming β-type toxins (see TCDB).

TC subclass 1.E consists of Holins. Both *L. interrogans* and *L. borgpetersenii* encode a protein that hits the Mycobacterial 4 TMS Phage Holin (MP4 Holin) (TC#1.E.40.3.6). Holins have a variety of proposed functions in prokaryotes, and may play a role in cell lysis and biofilm formation (Saier and Reddy 2014). The

presence of these holins in *L. interrogans* and *L. borgpetersenii* and their aforementioned functions may promote pathogenicity in these leptospires.

The largest number of transporters for all three species is found in TC subclass 2.A, secondary carriers. *L. interrogans* encodes 75, *L. borgpetersenii* 70, and *L. biflexa* 110. Given the substantial difference in the number of secondary carriers found in *L. biflexa*, the relative presence (or lack thereof) of a variety of transporters may help to distinguish between the two *Leptospira* pathogens and free living bacteria. *L. biflexa* appears to have much greater metabolic flexibility than its two pathogenic cousins.

TC subclass 2.C includes energizers for motility and outer-membrane transport. All three species encode a single TolB protein that is necessary to energize the stable construction of the outer membrane. The MotAB and ExbBD H^+ or Na^+ channel-forming proteins (TC#1.A.30) energizes outer membrane transport and periplasmic accumulation of solutes, dependent on TonB and outer membrane receptors (OMRs; TC#1.B.14). Further analysis of these energizers will be reported below.

TC subclass 3.A are pyrophosphate hydrolysis-driven primary active transporters, usually multi-component systems. With 56, 49, and 67 integral membrane transport proteins of this subclass found in *L. interrogans*, *L. borgpetersenii*, and *L. biflexa*, respectively, these proteins make up a significant

portion of the total transport proteins found in these organisms. The variety and wealth of transporters found in this subclass clearly play an important role in spirochetes. The 3.D TC subclass of ion-pumping electron carriers are represented in *L. interrogans*, *L. borgpetersenii*, and *L. biflexa* with 25, 30, and 32, respectively.

TC class 4 includes group translocators that are believed to modify their substrates in processes coupled to transport. TC 4.B subclass includes members of the nicotinamide ribonucleoside (NR) group translocating uptake permease (PnuC) family. Only *L. biflexa* encodes such a protein.

TC subclass 4.C includes fatty-acyl-coenzyme A ligases that activate fatty acids for lipid biosynthesis and may function in transport via group translocation. All three species contain one each. GBLAST revealed more fatty-acyl-coenzyme A ligases in all three leptospires, but the absence of transmembrane segments in addition to an unproven transport function in leptospires warranted their exclusion from our analyses.

All three *Leptospira* species have proteins belonging to TC subclass 4.D, putative group translocating glycosyl transferase. *L. interrogans* and *L. biflexa* encodes three, whereas *L. borgpetersenii* encodes four. Proteins in this family have demonstrated exopolysaccharide synthesis activities thought to be coupled to polysaccharide secretion. As exopolysaccharides can contribute to biofilm formation,

all three leptospires likely benefit from the presence of these proteins, for both free-living and/or host colonization purposes.

Subclass 5.A includes electron-carriers that transfer an electron pair from one side of the membrane to the other, thereby influencing cellular energetics. *L. interrogans* was found to have two, whereas *L. borgpetersenii* and *L. biflexa* have three. Among these are disulfide bond oxidoreductases and prokaryotic molybdopterin-containing oxidoreductases. These proteins might play a role in establishing the proton motive force, but they probably do not contribute to pathogenicity.

Subclass 5.B in TCDB consists of one electron transmembrane carriers. None of the leptospires were found to contain integral membrane carriers in this subclass. However, all three contain multiple copies of cytochrome c peroxidases (TC#5.B.3.1.1) for extracellular reduction of Fe_2O_3 (unpublished results).

Subclass 8.A represents auxiliary proteins with one in *L. interrogans* and *L. borgpetersenii*, and three in *L. biflexa*. All three encode a stomatin-like protein which may help with localization and insertion of proteins into the outer membrane. *L. biflexa* is the only one to encode a membrane fusion protein (TC#8.A.3.2.1), which probably functions with an ABC exporter.

Subclass 9.A in TCDB contains known transport proteins whose biochemical mechanism of transport is unknown. These proteins are generally poorly characterized from a mechanistic standpoint. All three leptospires have the same four subclass 9.A protein homologs (TC#9.A.8.1.4, 9.A.25.1.1, 9.A.40.2.2, and 9.A.58.2.4).

TC Subclass 9.B includes a variety of proteins that are putatively classified as transporters. Further study of a given 9.B protein might either confirm its involvement in transport, or warrant its removal from the TC classification system if a transport function is disproven. *L. interrogans* and *L. borgpetersenii* have 34 while *L. biflexa* has 38 of these proteins.

Transporter Superfamilies and Families

Figure 1 details protein families found in some, but not all three leptospiral species. 21 families are shown to be unique to *L. biflexa*, dwarfing the three families each unique to *L. interrogans* and *L. borgpetersenii*. This is reflective of the large differences in the numbers of total transporters (Table 1) and the disproportionately high number of 2.A carriers (Table 3). Analyses of these families may reveal key features of free-living saprophytes.

Unique families of transporters found in *L. biflexa* presented in Table 3 reveals ten families in the 2.A subclass. Families of transporters with substrates unique to *L. biflexa* are TC#1.A.72, 2.A.51, 2.A.56, 2.A.59, 2.A.102, and 4.B.1 that

transport mercury, chromate, dicarboxylates, arsenic, sulfites, and nicotinamide mononucleotide and related compounds, respectively. All of these substrates are transported only by *L. biflexa*, suggesting increased versatility over *L. borgpetersenii* and *L. interrogans*.

Families of transporters unique to *L. interrogans* and *L. borgpetersenii* (Table 3) are less numerous than those unique to *L. biflexa*. Members of TC#1.A.43 and TC#2.A.121 transport fluoride and sulfate, respectively. They are the only ones specific to *L. interrogans*. *L. borgpetersenii*, on the other hand, does not appear to have unique transporter families. Families belonging to both pathogens are TC#1.A.23, 1.C.67, 1.E.40, 2.A.114, 2.A.115 and 9.B.125 encoding small mechanosensitive ion channels, hemolysins, holins, peptide transporters, multidrug exporters, and unknown substrates, respectively. The families unique to *L. interrogans* may confer increased environmental versatility over *L. borgpetersenii*, but the shared transport families may play roles in pathogenesis.

Interesting Facets of Channel Proteins

A limited number of channel protein families were represented in the three *Leptospira* examined. Most channel proteins are involved in ionic and water homeostasis, but some also serve functions in stress responses. These will be described below.

Only a single member of the Voltage-gated Ion Channel Superfamily (TC#1.A.1) was identified, and this protein was found only in *L. biflexa*. It proved to be a 6 TMS cyclic nucleotide-dependent channel, almost certainly a potassium channel like those characterized in cyanobacteria and other spirochetes (Brams et al. 2014).

A single member of the MIP Family (TC#1.A.8) of aquaporins and glycerol facilitators was found in each of the three leptospires. These three proteins are probably aquaporins capable of transporting three-carbon compounds such as glycerol and dihydroxyacetone (Bienert et al. 2013). The high scores, all matching the same TCDB entry, suggest that these three proteins are orthologous.

Ammonium channels are prevalent in leptospires but in variable numbers; thus, *L. interrogans* has two dissimilar paralogs, and *L. biflexa* has three, but *L. borgpetersenii* has only one. Interestingly, it appears that one of these proteins in each organism hits the homolog from *Azospirillum brasilense* (TC#1.A.11.1.4) with excellent comparable scores. These three proteins are undoubtedly orthologs. It is worth noting that the *A. brasilense* protein is subject to multiple mechanisms of regulation which may be applicable to the spirochete proteins as well (Huergo et al. 2007).

The three *Leptospira* species examined possess either one or two homologs of Epithelial Chloride Channels (TC#1.A.13), characterized only in animals. Although

bacterial homologs have been identified, none has been characterized. However, our results reveal that these proteins from spirochetes exhibit the same topology as the mammalian proteins, suggesting a similar function. We suggest that these proteins will prove to exhibit chloride channel activities comparable to those found in eukaryotes.

The three leptospires display either zero or one Mechanosensitive Ion Channel (MscS; TC#1.A.23), and interestingly, *L. biflexa* is the one that lacks such a protein. All three organisms lack an MscL channel (TC#1.A.22). These proteins are known to function in osmotic adaptation (Pivetti et al. 2003).

All three spirochetes possess a member of the MgtE Family (TC#1.A.26) of magnesium uptake channels. These three proteins hit the same TC entry with the same high score, clearly indicating orthology.

The three *Leptospira* species examined all possess multiple paralogs of the H^+ or Na^+-translocating MotAB/ExbBD/TolQR channel-forming constituents (TC#1.A.30). While MotAB proteins function to energize motility (Lo et al. 2013, Nan et al. 2011), ExbBD channels energize transport across the outer membrane (Noinaj et al. 2010), and TolQR channels are believed to energize assembly of the outer membrane, promoting stability of this structure (Lazzaroni et al. 1999) All three spirochetes possess two MotAB energizers which presumably function in motility, possibly one utilizing the proton motive force and the other utilizing the

sodium motive force. On the other hand, the occurrence of ExbBD/TolQR energizers are variable in these three species with two in *L. interrogans*, four in *L. borgpetersenii*, and five in *L. biflexa*. These results suggest that *L. interrogans*, like *E. coli*, possesses the equivalent of one ExbBD complex and one TolQR complex (Tang and Saier 2014, Held and Postle 2002, Goemaere et al. 2007) However, the other two leptospires have an increased number of these H^+ or Na^+ channel proteins. The functions of these proteins will be interesting targets of future investigations.

Remaining families of channel proteins are present only in select *Leptospira* species. The CorA Metal Ion Transporter Family (TC#1.A.35) is only represented in *L. interrogans* and *L. biflexa*. Only *L. interrogans* possesses a member of the Camphor Resistance Family (TC#1.A.43). These proteins have recently been shown to be fluoride export channels which protect the bacterium against the toxic effects of fluoride (Stockbridge et al. 2013, Li et al. 2013). For both the Homotrimeric Cation Channel Family (TC#1.A.62) and the Mer Superfamily (TC#1.A.72) only *L. biflexa* has constituent channels. While the former proteins have not been characterized in bacteria, the latter function in the uptake of mercuric ions for the purpose of reduction to metallic mercury by a cytoplasmic mercuric reductase, a detoxification reaction (Pivetti et al. 2003).

Interesting Facets of β-type porins

β-type porins represent a significant portion of the channel proteins found in the *Leptospira* examined. The leptospiral outer membrane is of particular interest as

it contains cell surface antigens that can be used for vaccine production, and they can also serve as potential drug targets (Raja and Natarajaseenivasan 2013). Members of sixteen different families of outer membrane porins were identified in at least one of the three *Leptospira* species examined, and interestingly, fourteen of these families are represented in all three species. Just two of the families (POP; 1.B.5 and SAP; 1.B.16) were found only in *L. biflexa*, not in the two pathogenic species. While the POP Family is concerned with anion transport, the SAP Family mediates urea and short-chain amide transport.

Some of the families represented in all three organisms have only a single protein per organism, and these may be orthologs of each other as all three proteins hit the same TC entry (see for example 1.B.4, 1.B.6, 1.B.9, and 1.B.13). Striking differences occur in some of the other families, for example, the Outer Membrane Receptor (OMR) Family (TC#1.B.14), where each organism exhibits different sets of these pore-forming receptors. This fact can be explained by the different specificities of these receptors as illustrated in Table 2. Similar observations were made for the Outer Membrane Factor (OMF) Family (TC#1.B.17), and again, the different specificities of these porins provide an explanation. It seems likely that the complement of OMRs and OMFs reflect the specific environments in which these organisms are found.

The remaining families in this subclass consist of macromolecular transporters for protein secretion (TC#1.B.22; TC#1.B.48), outer membrane protein

insertion (TC#1.B.33), lipid export (TC#1.B.42), outer membrane lipid insertion (TC#1.B.46), and polysaccharide export for protection and biofilm formation (TC#1.B.55). All leptospires possess members of these families which represent core components of the *Leptospira* outer membrane proteome.

Interesting Facets of Secondary Carriers (TC Subclass 2.A)

In most organisms, the Major Facilitator Superfamily (MFS; TC#2.A.1) is the largest superfamily of secondary carriers. However, in the pathogenic leptospires, the MFS is poorly represented. Both species have only six MFS members and zero members of the related GPH Family (TC#2.A.2). By contrast, *L. biflexa* has fifteen MFS porters and two GPH porters. Thus, its MFS representation is almost three times that of the pathogens. Of particular note is the presence of multiple multidrug efflux pumps of MFS subfamily 21, nitrate/nitrite transporters, and several MFS families of unknown function. Also, while the pathogens possess only a single member of the APC amino acid transporters, *L. biflexa* has three such members, each derived from a separate subfamily.

Divalent cation transporters can mediate either uptake or efflux of these essential but potentially toxic substances. While the CDF Family (TC#2.A.4) catalyzes heavy metal export and has equal numbers of these proteins in all three leptospires, the ZIP Family (TC#2.A.5) and the NRAMP Family (TC#2.A.55) catalyze heavy metal uptake and are found only in *L. biflexa*. Interestingly, the

Chromate Resistance (CHR) Family (TC#2.A.51) and the Arsenical Resistance-3 (ACR3) Family (TC#2.A.59) are also restricted to *L. biflexa*.

The RND Superfamily is by far the largest superfamily of secondary carriers present in these spirochetes with sixteen in *L. interrogans*, fourteen in *L. borgpetersenii*, and twenty in *L. biflexa*. These proteins are divided about equally between heavy metal efflux pumps and multidrug resistance pumps. Only the SecD and SecF proteins, present in single copy in all three organisms, fall outside of these two groups. These two proteins function together as a single RND pump to facilitate proton-driven protein secretion via the General Secretory Pathway (Sec; TC#3.A.5) (Arkowitz and Wickner 1994). Finally, the two pathogens, but not *L. biflexa*, possess a single member of the poorly characterized putative Hydrophobe/Amphiphile Efflux-3 (HAE3) subfamily (TC#2.A.6.7) of the RND superfamily.

The Drug/Metabolite Transporter (DMT) Superfamily is the third largest superfamily in these spirochetes. *L. interrogans* has four such members, *L. borgpetersenii* has five, and *L. biflexa* has nine. Most of the top hits in TCDB have not been functionally characterized, so specific substrates cannot be assigned. However, all known members of this superfamily function in the transport of small metabolites and drugs.

Interestingly, all three leptospires have a single homolog of the Sweet family of putative sugar transporters (TC#2.A.123). The homologs identified have a 3 TMS

subunit structure. Several 7 TMS Sweet family members have been shown to transport sugars such as glucose and fructose (Chen et al. 2010), presently two 3D structures of a 3 TMS Sweet glucose transporter from *L* biflexa has been solved (Xu et al. 2014). Transport mediated by this protein appears to be that of a secondary carrier (Xu et al. 2014).

Table 2 reveals the presence of secondary carriers belonging to many other families, and almost all of these are well-represented in all three leptospires. These families will not be further discussed here.

Interesting Facets of Primary Active Transporters

TC subclass 3.A contains the largest superfamily of transporters found in all three *Leptospira* species, the ABC Superfamily (TC#3.A.1). While *L. interrogans* and *L. borgpetersenii* possess 28 and 27 of these proteins, respectively, *L. biflexa* possesses 41. The ABC Superfamily is represented in all domains of life and is known to transport a wide variety of substrates for both uptake and export. Of note, *L. biflexa* is the only leptospire to possess ABC transporters for putrescine/spermidine, phosphate, thiamine, zinc (Zn^{2+}), iron siderophores, and fatty acyl-CoA. However, all three organisms possess good representation of oligopeptide transporters, suggesting that these substances are important to the nutrition of these organisms. All three organisms have ABC uptake systems for sulfate and for lipids.

ABC efflux systems are present in numbers that are similar to those of the uptake systems in all three spirochetes. The primary substrates for these exporters are

1) lipids and lipoproteins, 2) proteins and peptides, 3) exopolysaccharides, and 4) multiple drugs. Most of these transporters, except for those specific for lipids, are found in similar numbers in the three spirochetes examined. Only a few ABC export systems are specific to *L. biflexa*. One of these is a putative organoanion (fatty acid?) exporter (TC#3.A.1.203.8), and the others undoubtedly exhibit specificity for specific proteins (TC#3.A.1.109, 3.A.1.110, 3.A.1.111). Interestingly, a single member of the Membrane Fusion Protein (MFP) Family (TC#8.A.1) was found exclusively in *L. biflexa*, correlating with the presence of these ABC protein exporters.

All three leptospires possess orthologous sets of the integral membrane components of the ATP synthases in the F-ATPase Superfamily (TC#3.A.2) for subunits a, b, and c. The reversibility of the enzyme for both the establishment of the proton motive force and ATP synthesis is a key characteristic of this system. Additionally, all three *Leptospira* have a H^+ or Na^+-translocating pyrophosphatase (TC#3.A.10). While the TC hit for *L. biflexa* (TC#3.A.10.1.1) is different from that for *L. interrogans* and *L. borgpetersenii* (TC#3.A.10.1.6), sequence comparison of these entries show that these proteins are probably orthologous.

Of note is the variance of P-type ATPases (TC#3.A.3) in *Leptospira* species. *L. interrogans* possesses six of these transporters with substrate specificities for magnesium (Mg^{2+}), copper (Cu^{2+}), and potassium (K^+). *L. borgpetersenii*, however, possesses only two, one specific for copper (Cu^{2+}) and the other for calcium (Ca^{2+}).

While the Mg^{2+} and K^{+} systems catalyze uptake, the Cu^{2+} and Ca^{2+} systems probably catalyze efflux. *L. biflexa* has six including a putative Na^{+}/K^{+} ATPase, two copper (Cu^{2+}) transporters, a calcium (Ca^{2+}) transporter, and a heavy metal (Co^{2+}, Zn^{2+}, Cd^{2+}) transporter. The diversity of these transporters presumably reflects the types of stress that these organisms encounter. Thus, most prokaryotic P-type ATPases function in stress relief (Chan et al. 2010, Thever and Saier 2009).

All three leptospires have proteins with sequence similarity to the three integral membrane components of the General Secretory Pathway (Sec) Family (TC#3.A.5), which transports most secreted proteins across the inner cytoplasmic membrane. The presence of SecDF (TC#2.A.6.4) as well as the associated YajC protein (TC#9.B.18) in all three species reveals the genus-wide presence of the integral membrane constituents of the general secretory pathway. In addition, we found the constituents of the outer membrane protein secreting Main Terminal Branch (MTB) Family (TC#3.A.15). These proteins proved to be distantly related to the MTB constituents previously tabulated in TCDB, and consequently we have entered all constituents of this system from *L. interrogans* into TCDB under TC# 3.A.15.4.1. The MTB Family is believed to export hundreds of proteins across the outer membranes of gram negative bacteria initially secreted across the cytoplasmic by the Sec system (Nivaskumar and Francetic 2014).

Not surprisingly, all motile leptospires possess the flagellar (Type III) secretion complex (TC#3.A.6). The constituents recorded in Table 2 include six

integral membrane constituents of this system whose near-identical E-values for all components suggest orthology of the entire system in this genus. Although *L. biflexa* is non-pathogenic, it is likely that motility, and hence flagelli, are essential for pathogenesis. However, the striking similarity between Type III secretion systems and flagellar export systems gives rise to the possibility that these systems export of virulence factors in addition to flagellar subunits as has been demonstrated for these systems in other bacteria (Lambert et al. 2012, Nguyen et al. 2000).

As expected, all leptospires have the Septal DNA Translocase (TC#3.A.12), involved in DNA transfer across the completed septa of newly divided cells. However, while these organisms lack a type IV protein secretion system involved in conjugation, they do possess components of Bacterial Competence-related DNA Transformation (DNA-T) systems (TC#3.A.11). Interestingly, while *L. interrogans* and *L. biflexa* appear to have all constituents of these systems, only two were found in *L. borgpetersenii*. Possibly this last organism has lost some of the constituents of these systems and therefore has lost competence. Surprisingly, nothing seems to have been published on competence in *Leptospira* species for DNA uptake.

None of the spirochetes examined appear to have a Na^+-transporting carboxylic acid decarboxylase of the NaT-DC Family (TC#3.B.1). These organisms do have decarboxylases, but they lack the integral membrane protein which is required for Na^+ extrusion. We therefore conclude that this mechanism for generating a sodium motive force is lacking in these organisms, in agreement with the

conclusion that these ion pumps are largely restricted to anaerobes (Granjon et al. 2010).

Constituents of most, but not all, of the primary proton pumping electron transfer complexes present in mitochondria and many aerobic bacteria were found in the leptospires. These include the proton-translocating NADH dehydrogenase (TC#3.D.1), proton-translocating transhydrogenase (TC#3.D.2), and proton-translocating cytochrome oxidase (TC#3.D.4), but not the proton-translocating quinol:cytochrome c reductase (TC#3.D.3). Additionally, leptospires possess prokaryotic succinate dehydrogenase (TC#3.D.10). These results are consistent with the conclusion that leptospires use electron transfer as a primary mechanism for generating a proton motive force, subsequently used for ATP synthesis. As expected, these aerobic bacteria possess members of the disulfide bond oxidoreductase D (DsbD) and Molybdopterin-containing Oxidoreductase (PMO) Family (TC#5.A.1 and 5.A.3, respectively), but surprisingly not the single electron transferring DsbB complex.

Possible Group Translocators (TC Class 4)

None of the leptospires possess a phosphoenolpyruvate-dependent sugar transporting phosphotransferase system (PTS) although such systems have been found in other spirochetes (Saier et al. 1977). However, *L. biflexa* appears to have a nicotinamide ribonucleoside uptake permease (TC#4.B), thought to function by a group translocation mechanism (Foster et al. 1990). Each spirochete also has a

membrane-associated acyl-CoA ligase (TC#4.C) that could function in transport (Black and DiRusso 2007). Finally, each leptospire possesses three or four polysaccharide synthase/exporters (TC#4.D), all of which give low scores to the proteins in TCDB. These putative enzyme/porters may catalyze vectorial glycosyl polymerization (Davis 2012, Hubbard et al. 2012).

Poorly Characterized Transporters (TC Class 9)

TC subclass 9.A represents known transport systems that function by an unknown mechanism of action. Three such systems are found in all three spirochetes, and no other members of this subclass were identified. The first of these families is the FeoB family of ferrous iron uptake transporters (TC#9.A.8). The second family is a multicomponent protein secretion system characterized only in Bacteroidetes (TC#9.A.25). Homologs of only one constituent of this family were identified, suggesting that the complete system is not present. Members of the third family, (HlyC/CorC; TC#9.A.40) may function as divalent cation channels. TC subclass 9.B includes putative transporters, where even transport function is not established. These proteins are listed in Table 2 but will not be discussed.

Transporter Substrates

The substrates of transporters found in these leptospires likely reflect the physiological characteristics of each organism. In Figure 3, the distribution of substrates by category and subcategory is shown. Each chart in the top row represents the percentage of substrate types in each category for a given leptospire, and the chart below corresponds to the subclasses.

All three spirochetes have very similar percentages of the various substrate categories and subcategories. The most obvious difference between the three species is the relatively larger size of certain categories and subcategories in *L. biflexa*. Whereas *L. interrogans* and *L. borgpetersenii* have 87 and 89 inorganic substrate transporters, respectively, *L. biflexa* has 123, suggesting that this organism must be capable of maintaining intracellular ionic homeostasis under a much greater range of environmental conditions than for the two pathogens. Similarly, *L. biflexa* has a greater number of transporters for each category of substrate except nonselective transporters. The same can be seen in most subcategories where *L. biflexa* has more transporters than the other two spirochetes.

Correlating with its greater capacity for maintaining ionic homeostasis, *L. biflexa* has 98 proteins involved in cation transport whereas *L. interrogans* and *L. borgpetersenii* have 68 and 71, respectively. The prevalence of these transporters correlates with the disproportionately high number of *L. biflexa* secondary carriers that can utilize protons (H^+) or sodium (Na^+) for symport or antiport. This fact also indicates a reliance on transport that is energized by the proton (or sodium) motive force over other energy-coupling mechanisms such as those driven by ATP hydrolysis. In addition to ionic homeostasis, cation symport and antiport facilitate osmotic regulation (Pivetti et al. 2003) and heavy metal resistance (Silver and Phung 1996). Other inorganic monovalent and divalent cationic substrates pumped by these three organisms include potassium (K^+), calcium (Ca^+), magnesium (Mg^{2+}), and

various cations of metals including copper, iron, zinc, cobalt, cadmium, mercury, and manganese.

Transporters specific for inorganic anions number 17 for *L. interrogans*, 15 for *L. borgpetersenii*, and 22 for *L. biflexa*. Anions, compared to cations, represent a much smaller proportion of the inorganic substrates transported by these leptospires. The latter play strong roles in redox processes, establishing, for example, the pmf or smf for energization. Anion transporters are found primarily in TC subclass 2.A, taking up or exporting bicarbonate, phosphate, arsenate, arsenite, telluride, chromate, chloride, and fluoride. Sulfate uptake, on the other hand, is mediated primarily by the CysPTWA ATP-dependent ABC system.

The three *Leptospira* species have only a small percentage of their transporters dedicated to organoanion transport. *L. biflexa* has four of these transporters, whereas *L. interrogans* has five and *L. borgpetersenii* has three. Fatty acids and other carboxylic acids, bile acids and their conjugates, taurine, and carnitine are the main substrates in of this subclass of carbon sources. These spirochetes also exhibit good representation of carboxylate transporters with six in *L. biflexa*, two in *L. borgpetersenii*, and three in *L. interrogans*. Pyruvate, malate, succinate, acetate, hydroxybenzoate, citrate, and fumarate are all probably transported. These leptospires have outer membrane porins dedicated to fatty acid and hydrophobic compound uptake (TC#1.B.9.3.3). Fatty acid group translocation

may be catalyzed by transporters in the FAT Family (TC#4.C.1) (Nevoigt and Stahl 1997, Patarakul et al. 2010, Brihuega et al. 2012).

Sugars and polyols taken up include glycerol, glycerol-3-phosphate, mono- and disaccharides. *L. biflexa* possesses eleven such proteins, whereas *L. interrogans* and *L. borgpetersenii* each possesses seven. As *Leptospira* utilize fatty acids as primary carbon sources, this subclass of carbon sources may play roles in osmoregulation, alternative metabolic pathways in differentially expressed genes, and membrane construction (Nevoigt and Stahl 1997, Patarakul et al. 2010, Brihuega et al. 2012).

L. biflexa has disproportionately high numbers of proteins involved in the transport of amines, amides, and polyamines (ten proteins compared to two each in *L. interrogans* and *L. borgpetersenii*). Among such transported substrates are putrescine, spermidine, ethanolamine, choline, and quaternary ammonium compounds. Transport of polyamines is associated with cellular growth and proliferation and may alleviate stress resulting from elevating external pH (Igarashi 2006, Grillo and Colombatto 1994, Tomitori et al. 2012).

All three leptospires have transporters for amino acids and their conjugates, primarily members of TC subclass 2.A. Constituents can be found in the APC (TC#2.A.3), DMT (TC#2.A.7), AGCS (TC#2.A.25), and ABC (TC#3.A.1) families.

There appears to be substantial diversity in the types of amino acids transported. Of additional note, the three *Leptospira* species possess at least seven homologs to a putative alanyl teichoic acid synthesis protein DltB (TC#2.A.50). These homologs possess domains that strongly match with a DltB domain (e-48), and another weaker match for an O-acyltransferase (e-24) (unpublished results). The strong match warrants inclusion of these proteins in this study as potential transporters of activated d-alanine, an amino acid conjugate.

The three spirochetes in this study possess several proteins that function in the transport of peptides and their conjugates. Peptides in this subcategory can be di-/tripeptides, oligopeptides, and peptidoglycan fragments. These transporters can additionally transport antibacterial agents, various nitrogen sources, and precursors of cell wall biosynthesis (Newstead 2014, Newstead 2011, Sobhanifar et al. 2013). These peptide transporters primarily belong to the POT/PTR and ABC families.

We found it of interest that the *Leptospira* have the Bacterial Competence-related DNA Transformation Transporter (DNA-T) Family (TC# 3.A.11), although *L. borgpetersenii* appears to have lost some constituents of the full system. Competence in these organisms seems not to have been reported. The low E-values obtained for some members of the DNA-T Family found in *L. interrogans* and *L. biflexa* suggest that uptake in these leptospires may exhibit unique features.

Proteins can be secreted by leptospires using multiple systems. The General Secretory Pathway (TC#3.A.5) and the outer membrane secreting Main Terminal Branch (MTB) (TC#3.A.15) probably provide the primary pathways for protein secretion across the two membranes of the cell envelope. However, flagellar proteins and possibly some virulence proteins are secreted by the Type III Secretory Pathway (TC#3.A.6). Finally, the Outer Membrane Insertion BAM complex (TC#1.B.33) probably inserts most outer membrane proteins into this structure.

A key feature of *Leptospira* is its outer membrane, composed of lipids, porins, lipoproteins, and lipopolysaccharide (LPS); this last constituent consists primarily of lipid A and O-antigen. The Outer Membrane Lipopolysaccharide Export Porin (LPS-EP) Family (TC#1.B.42), the Outer Membrane LolAB Lipoprotein Insertion Apparatus Family (TC#1.B.46), the Multidrug/Oligosaccharidyl-lipid/Polysaccharide Flippase Family (TC#2.A.66), and members of the ABC Superfamily (TC#3.A.1) are the primary systems dedicated to the export of lipids and LPS precursors to the outer membrane.

All three leptospires in this study possess proteins for the transport of drugs with sixteen in *L. borgpetersenii*, and twenty in both *L. interrogans* and *L. biflexa*. Multidrug resistance pumps are known to be prevalent in free living organisms which need to defend themselves against toxic substances produced by other microbes (Saier and Paulsen 2001). Further characterization of the members of the Drug/Metabolite Transporter Superfamily (TC#2.A.7) should provide a more

accurate representation of the substrates transported by members of this superfamily. Drug exporters function to protect the cell from endogenously produced antibiotics, to remove exogenous and harmful substances produced by other microbes, and to export drug-like secondary metabolites such as siderophores, lipids, signalling peptides and periplasmic redox cofactors (Saier and Paulsen 2001).

The rest of the "Drugs, vitamins, siderophores & cofactors" category of substrates in these three organisms is comprised of thirteen transporters in *L. interrogans* and *L. biflexa*, with only eight in *L. borgpetersenii*. All three leptospires transport Vitamin B_{12} (cobalamin), while only *L. biflexa* has a transporter designated as a Vitamin B_1 (thiamine) transporter, and it belongs to the ABC Superfamily (TC#3.A.1.17.8). Siderophore transporters, also of the ABC-type, are found in all three organisms, consistent with a need for iron in these aerobes.

Nonselective channels include α-type channels, β-barrel porins, and pore-forming toxins. The three leptospires examined have transporters in these subclasses with sixteen in *L. interrogans*, fifteen in *L. borgpetersenii*, and eleven in *L. biflexa*. These nonselective transporters can play receptor roles in the outer membrane, induce toxin-like effects in other bacteria, and regulate cellular osmolarity (see TCDB).

All three leptospires have a substantial proportion of transporters that are poorly defined, about 12%. This represents a diverse subset of all leptospiral proteins

with no clearly demarcated (Ren et al. 2003, Bulach et al. 2006, Picardeau et al. 2008). Nonetheless, these putative transport proteins with currently unknown substrates and/or mechanism of action are likely to serve significant roles in metabolism and pathogenicity of these important bacteria.

DISCUSSION

Members of the genus *Leptospira* cause leptospirosis, a zoonotic disease with global prevalence, affects nearly one million people annual with mortality ranging 5-25%. Current treatment of its wide variety of symptoms relies heavily on symptom management and antibiotic administration. Antibiotics currently in use to treat leptospirosis include penicillin, doxycycline, and cephalosporin (the efficacy of which remains mixed and questionable) (Brett-Major and Coldren 2012, Guerrier and D'Ortenzio 2013, Wei et al. 2012, Griffith et al. 2007, Griffith et al. 2006). Prophylaxis usually involves vaccines of typically heat-attenuated leptospires with limited and mixed results. Chemoprophylactic treatment involves continual administration of doxycycline, a procedure that has been shown to reduce the incidence of the disease (Guidugli et al. 2000, Brett-Major and Lipnick 2009, Ricaldi and Vinetz 2006). Variation in the susceptibility of different strains of *Leptospira* to these treatments has been reported, but instances of asymptomatic carriers in humans complicates the issue (Ressner et al. 2008, Ganoza et al. 2010). The causes of the variable clinical manifestations of leptospirosis are poorly understood, and the entire *Leptospira* genus is poorly characterized.

Comparative analyses of transport proteins should provide clues as to the metabolic, pathological, and drug resistance properties of these spirochetes. By comparing and contrasting two known pathogenic members, *Leptospira interrogans* and *Leptospira borgpetersenii*, with a free-living saprophyte, *Leptospira biflexa* (Ren et al. 2003, Bulach et al. 2006, Picardeau et al. 2008, Johnson 1996), we have

generated data that will help define the metabolic capabilities of these organisms, allow identification of transporters common to these leptospires, and distinguish transport systems required for pathogenic versus saprophytic life.

Bioinformatics is a powerful tool in analyzing and assessing biological data, and this field enables supplementation of findings in the wet lab. The results provided by GBlast enables rudimentary compilation of potential transport proteins within an organism's genome, but it currently has limits. Since sequence similarity is the primary method used to determine the match of an unknown query to a known entry in TCDB, functionally distinct proteins with variations in sequence may result in a false positive for a given protein being declared a transporter, depicting sequence convergence where divergence would otherwise be expected. A few examples of this occurred in our study, most notably with query entries matching with members of the Pore-Forming RTX Toxin Family (TC#1.C.11). For most of these proteins, the three leptospires examined were shown to have methyl-accepting chemotaxis protein domains, indicating that these toxin homologs may have other functions in these three organisms. All three organisms possess proteins that demonstrate strong sequence similarity to an activated D-alanine derivative exporter, belonging to the Glycerol Uptake Porter family (TC#2.A.50). Activated D-alanine transporters have been found in gram-positive bacteria, and it is likely these leptospiral proteins belong to the membrane-bound o-acyltransferase family.

Leptospires exhibit profound changes in their transcriptomes in response to a changing external environment (Xue et al. 2010, Caimano et al. 2014). The findings

in our study reflect the total potential transporters available to respective *Leptospira* species. Other investigators have studied the transcriptional profiles of these organisms under various environmental conditions, especially for specific pathovars. Future efforts will look to integrate transporter proteomic studies with transcriptomic studies and the bioinformatic analyses explored here.

Distinguishing Transporters of Three Leptospires

All identified transporters are compiled with their characteristics in Table 2, revealing many of the conclusions drawn in our study. We show that *L. borgpetersenii* possesses 260 transport proteins, *L. interrogans* possesses 270, and *L. biflexa* possesses 337. The two pathogenic leptospires have a substantially smaller complement of transporters than the free-living *L. biflexa*. This difference is believed to arise from marked decreases in the secondary carriers, primary active transporters, and channel proteins in both *L. interrogans* and *L. borgpetersenii* relative to *L. biflexa* as seen in figure 2. These transporters reflect the decreased transport capabilities and therefore metabolic diversity and potential for homeostatic control of the pathogens relative to the free-living saprophyte.

Figure 3 shows the very significant difference in the inorganic cation transporters of *L. biflexa* (98) compared to *L. interrogans* (68) and *L. borgpetersenii* (71). Transporters associated with these substrates include pmf and/or smf generators, osmotic and ionic homeostatic stress response regulators, and heavy metal resistance proteins. These cation transporters confer upon *L. biflexa* the ability

to survive in external environments through effective osmotic regulation, metabolic versatility, and by competing with other environmental microbes.

Reflecting the diversity of metabolites transported by *L. biflexa* are the increased numbers of transporters for carbon sources and amino acids relative to *L. interrogans* and *L. borgpetersenii*. Among these substrates are carboxylates, sugars, polyols, non-carboxylic organoanions, amines, amino acids & their conjugates, and peptides & their conjugates. With just two exceptions, *L. biflexa* has more transporters in each of these subcategories of substrates. This undoubtedly reflects its superior metabolic versatility, conferring the ability of this free-living organism to grow under a wide range of environmental conditions. Pathogenic species of *Leptospira* are known to lack proteins related to carbohydrate, nitrogen, and amino acid metabolism that correlate with their protracted growth in artificial media (Ricaldi et al. 2012). Many of the aforementioned transporters, as seen in Table 2, belong to the 2.A TC subclass, whose carriers are known to demonstrate lower affinities, but greater efficiencies at lower energy cost, than ABC transporters.

The relatively high affinity ABC transporters are well represented in all three leptospires, but *L. biflexa* has significantly more (67) of these transport proteins than *L. interrogans* (56) or *L. borgpetersenii* (49). Uptake systems for peptides and sulfate are also present, but *L. biflexa* possesses a system for putrescine/spermidine uptake, as well as ones for siderophore, zinc (Zn^{2+}), and vitamin acquisition. High affinity acquisition of putrescine, critical in cell survival, demonstrates a crucial component

of *L. biflexa* saprophytism (Tabor and Tabor 1984). Similarly, uptake systems for iron siderophores, thiamine, and zinc (Zn^{2+}) can serve to accumulate them in high concentrations within the cell for use as cofactors where pathogens might have better access within a host. Macromolecular ABC export systems are similarly well-represented in all three species, transporting proteins and polysaccharides. They also exhibit differential abilities to export drugs including antibiotics. *L. biflexa*, however, has homologs of exporters for fatty acyl CoA and putative adhesin proteins. Fatty acyl CoA export may function to acylate the outer membrane and adhesin proteins, likely to play a role in biofilm formation in *L. biflexa*.

Interspecies differences in transporter classes and substrate categories are limited in *Leptospira*. A basis for pathogenesis in the *Leptospira* species examined has no likely root in gross characteristics of their respective transporter proteomes. Various individual transport proteins serve as likely contributors to pathogenesis in *L. interrogans* and *L. borgpetersenii*. Both pathogens encode members of the SphH Hemolysin Family (TC#1.C.67) that are notably absent in *L. biflexa* (Lee et al. 2002, Narayanavari et al. 2012, Zhang et al. 2008). Sph2 (Uniprot # P59116; Table 2), is a sphingomyelinase with all active site residues essential for catalysis in vitro. Sphingomyelinase C (Uniprot # Q04XS2) of *L. borgpetersenii* is closest in sequence similarity to Sph2. Both pathogens, but not *L. biflexa*, possess proteins with strong sequence similarity to a member of the Mycobacterial 4 TMS Phage Holin Family (TC#1.E.40). The proposed roles of prokaryotic holins in cell lysis and biofilm formation indicate the potential role these proteins may play in pathogenesis (Saier

and Reddy 2014). Both *L. interrogans* and *L. borgpetersenii* possess a member of the Putative Peptide Transporter Carbon Starvation CstA Family (TC#2.A.114). Mutation of a homolog in *C. jejuni* revealed decreased host-pathogen interaction (Rasmussen et al. 2013). All three leptospires possess proteins exhibiting sequence similarity to a member (TC#1.B.6.1.20) of the OmpA-OmpF Porin family. The *L. interrogans* protein queried has been shown to be Loa22, a protein essential for leptospiral virulence (Ristow et al. 2007). The relative dissimilarity of the *L. biflexa* homolog could render it avirulent.

Transporter Hallmarks of Leptospira

Leptospira is a branch of a divergent phylum and represents a genetically isolated group of bacteria (Picardeau et al. 2008). Transporters identified in the leptospires in this study potentially serve novel roles for these gram-negative aerobes. All three leptospires possess a significant number of α-type channels, most of which transport inorganic ions and small metabolites. The largest representative of these channels are the MotAB/ExbBD/TolQR channel-forming constituents (TC#1.A.30) with roles of motility, energized outer membrane transport, and outer membrane stability, respectively. The presence of two MotAB energizers may permit one system to utilize the proton motive force utilization and the other the sodium motive force. *L. interrogans* is notably deficient in ExbBD/TolQR energizers relative to *L. borgpetersenii* and *L. biflexa*, which raises questions about the role of these energizers in *Leptospira* (Tang and Saier 2014, Held and Postle 2002, Goemaere et al. 2007).

Of strong clinical relevance is the leptospiral outer membrane proteome (surfaceome), constituted largely by a variety of β-type porins. It contains cell surface antigens for potential vaccine production and drug targets (Raja and Natarajaseenivasan 2013). As mentioned above, all three leptospires possess Loa22 (or a homolog), a surface-exposed porin, necessary for leptospiral virulence. Aside from this virulence factor, leptospires possess outer membrane transporters for the nonselective transport of small molecules, larger molecules including siderophores, proteins and membrane constituents. The leptospiral outer membrane plays a role in transport of substrates from the extracellular space to the periplasm.

The largest TC subclass identified in *L. interrogans*, *L. borgpetersenii*, and *L. biflexa* is 2.A, carrier proteins catalyzing uniport, antiport, and symport. The diversity of substrates transported is in part due to the Major Facilitator Superfamily (TC#2.A.1) as well as the 40 other families within this TC subclass. This distribution of transporters suggests an important role of secondary carriers in nutrient acquisition over primary active transporters such as ABC systems. The distribution of secondary carriers and primary active transporters reveals the prioritization of the acquisition and export of various molecules. Metabolic flexibility should dictate utilization of low-affinity secondary carriers, whereas a specific metabolic need might necessitate other higher-affinity systems.

P-type ATPase (TC#3.A.3) distribution varies between all three leptospires, but all three possess an exporter of copper (Cu^{2+}), indicating a critical need for strict intracellular copper regulation. Only *L. interrogans* possesses ATPases for the uptake of Mg^{2+} and K^{+}, suggesting these ions play critical roles for this organism to survive in the external environment and/or in the host. *L. biflexa* possess ATPases for Ca^{2+} export, heavy metal resistance (Co^{2+}, Zn^{2+}, Cd^{2+}), and Na^{+}/K^{+}. These proteins, unique to *L. biflexa*, likely highlight its effective osmoregulation and capacity for membrane potential maintenance.

All three leptospires possess multiple systems for protein secretion. The primary pathways for protein secretion across the two membranes of the cell envelope are probably provided by the General Secretory Pathway (TC#3.A.5) and the outer membrane secreting Main Terminal Branch (TC#3.A.15). Flagellar proteins and potential virulence proteins are secreted by the Type III Secretory Pathway (TC#3.A.6). This particular pathway is critical for virulence, as inhibition of flagellar motility in leptospires has been shown to render them avirulent (Lambert et al. 2012).

Common in all three leptospires are primary proton pumping electron transfer complexes inherently present in mitochondria and many aerobic bacteria including the proton-translocating NADH dehydrogenase (TC#3.D.1), proton-translocating transhydrogenase (TC#3.D.2), and proton-translocating cytochrome oxidase (TC#3.D.4). The presence of these proton-translocating systems is consistent with

the conclusion that electron transfer is used as a primary mechanism to generate a proton motive force, subsequently used for ATP synthesis.

As leptospires are genetically divergent compared to most well studied bacteria, they are expected to share a strong core of proteins and possess unique systems for pathogenesis and free-living (Ren et al. 2003, Bulach et al. 2006, Picardeau et al. 2008). A significant portion of the identified transporter proteome in these leptospires are incompletely characterized proteins from TC subclasses 9.A and 9.B. Further identification and characterization of these proteins, in addition to the remaining encoded non-transport proteins, should provide a more complete understanding of leptospiral pathogenesis and saprophytism.

Key attributes of *Leptospira* are aligned with their motile and chemotactic abilities. The embedded flagelli permitting cork-screw like motility favors these organisms in host dissemination and environmental survival (Islam et al. 2014). Transport proteins identified in this study, including a flagellar export system, chemotaxis proteins, and flagellar motor energizers, should play roles in survival and virulence. Chemotaxis toward specific molecules like glucose may facilitate tissue tropism of *Leptospira* pathogens (Islam et al. 2014). Revealed in *L. biflexa* is its efficacy to persist over long periods of time in distilled water by forming biofilms, and aggregation has also been suggested to be a mechanism of environmental survival and host colonization (Brihuega et al. 2012, Barragan et al. 2011). Transport

proteins that excrete exopolysaccharides, signaling molecules, and adhesion proteins should promote biofilm formation for persistence.

Transport proteins represent a subset (about 10%) of the entire proteome of an organism. However, intracellular processes are dependent on what materials are available in the cell. By providing an overview of the molecules transported and how they are imported and exported, conclusions about the metabolism and physiology of an organism can be drawn. In the case of *Leptospira*, the overall transportome (transporters of the proteome), reveals key characteristics of saprophytism and pathogenesis. *L. biflexa* demonstrates high flexibility and versatility in its transportome with a relatively large subset of secondary carriers and transporter families not found in the pathogens. In addition, *L. biflexa* possesses high-affinity transporters for critical cofactor import and increased numbers of uptake systems for carbon and nitrogen sources. Meanwhile, the pathogens possess remarkably similar transport protein profiles, suggesting the host tropism and environmental survival between the two relies on individual transporters and differences in the non-transporting proteome. Both possess factors that may be associated with pathogenesis, absent in *L. biflexa*, such as sphingomyelinases, holins, and virulence-related outer membrane porins. The increased versatility of *L. biflexa* as a free-living organism likely reflects the inverse as the decreased versatility in the pathogens forces them to realize progressively narrower ecological niches. As leptospirosis manifests itself in a variety of symptoms, small differences within individual proteins and the leptospiral proteome may play roles in determining virulence and mortality in

humans (Spichler et al. 2011). The findings reported here on these leptospiral transporters should improve our understanding of the pathology of leptospirosis and allow more specific experimentation with *L. biflexa* as a model system for the *Leptospira* genus.

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APPENDIX

Table 1. Overview of three *Leptospira* species and their basic traits.

Species Name	*Leptospira interrogans* serovar Lai str. 56601	*Leptospira borgpetersenii* serovar Hardjo-bovis str. L550	*Leptospira biflexa* serovar Patoc str. 'Patoc 1 (Ames)'
Chromosome I RefSeq	NC_004342.2	NC_008508.1	NC_010842.1
Chromosome I Size (Mb)	4.339	3.614	3.604
Chromosome II RefSeq	NC_004343.2	NC_008509.1	NC_010845.1
Chromosome II Size (Mb)	0.359	0.317	0.278
Plasmid p74 RefSeq			NC_010846.1
Plasmid p74 Size (Mb)			0.074
Total Genome Size (Mb)	4.698	3.931	3.956
Total Protein #	3,683	2,945	3,600
Transporters	270	260	337
Transporters as % of Proteinss	7.33	8.83	9.36
Pathogenic?	Yes	Yes	No

Table 2.
TC classification and functional prediction of transport-related proteins found in *L. interrogans*, *L. borgpetersenii*, and *L. biflexa*. Sequences were retrieved using GBlast with E-values of 0.001 or smaller. Transporters with E-values larger than e-12 are highlighted indicating low substrate-specificity confidence.

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)[1]	Comments	Uniprot#	Query TMS #	E-value[2]	Uniprot#	Query TMS #	E-value[2]	Uniprot #	Query TMS #	E-value[2]
1.A α-Type Channels															
1.A.1	Voltage-gated Ion Channel (VIC) Superfamily														
		1.A.1.5.16	Q10V66	6	Cation (K^+)								B0S9V7	7	6.9E-75
1.A.8	Major Intrinsic Protein (MIP) Family														
		1.A.8.2.6	D7V8E7	6	Sugars & Polyols (Glycerol)		Q8F0N7	6	4.7E-62	Q04YM4	6	2.9E-61	B0S9P8	6	1.0E-59
1.A.11	Ammonia Channel Transporter (Amt) Family														
		1.A.11.1.4	O67997	12	Amines, amides, polyamines (Ammonia)		Q8EZP6	12	1.6E-98	Q04XZ2	12	3.8E-99	B0SD95	12	8.5E-97
		1.A.11.2.3	Q93IP6	12	Amines, amides, polyamines (Ammonia)								B0S994	11	9.3E-97
		1.A.11.2.5	Q0IDE4	12	Amines, amides, polyamines (Ammonia)								B0SCI2	12	5.3E-97
		1.A.11.2.7	O28528	12	Amines, amides, polyamines (Ammonia)		Q8F074	12	2.5E-93						
1.A.13	Epithelial Chloride Channel (E-ClC) Family														
		1.A.13.4.1	F8CM01	3	Anion (Cl^-)		Q8EY02	3	9.0E-19	Q04X11	3	3.4E-20	B0SI02	3	3.1E-20
							Q8EY03	2	4.4E-18	Q04X10	3	1.4E-17			
1.A.23	Small Conductance Mechanosensitive Ion Channel (MscS) Family														
		1.A.23.4.3	P0AEB5	4	Nonselective					Q04YU2	6	1.3E-17			
		1.A.23.4.10	O25170	6	Nonselective		Q8F7F3	6	1.3E-13						
1.A.26	Mg^{2+} Transporter-E (MgtE) Family														
		1.A.26.1.2	Q5SMG8	5	Cation (Mg^{2+})		Q8F430	5	4.0E-56	Q051E5	5	1.1E-55	B0S9F9	5	6.6E-56
1.A.30	H^+ or Na^+-translocating Bacterial Flagellar Motor/ExbBD Outer Membrane Transporter Energizer (Mot-Exb) Superfamily														
		1.A.30.1.2	O06874	1	M^+ cation (H^+ or Na^+)					Q054U9	1	4.3E-16			
		1.A.30.1.3	P28612	1	M^+ cation (H^+ or Na^+)					Q051E6	1	4.5E-26	B0S9G1	1	9.4E-28
													B0S9P4	1	8.3E-11

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
		1.A.30.1.4	P39063	4	M^+ cation (H^+ or Na^+)		Q8F0B9	4	2.6E-47	Q04XW3	4	9.8E-48	B0SA55	4	4.0E-48
		1.A.30.1.4	P39064	1	M^+ cation (H^+ or Na^+)		Q8F0C0	3	4.6E-17	Q04XW4	1	6.2E-17	B0SA56	1	5.1E-16
							Q8EYN0	1	2.6E-06	Q055X0	1	1.0E-10			
		1.A.30.1.5	Q8E9J0	3	M^+ cation (H^+ or Na^+)		Q8F896	4	3.4E-40	Q054S6	3	7.8E-38	B0SEV2	4	1.2E-37
		1.A.30.2.1	P0ABV2	1	M^+ cation (H^+ or Na^+)		Q8F3T1	1	7.9E-10	Q051K3	1	7.3E-10			
		1.A.30.2.5	Q1CYB7	1	M^+ cation (H^+ or Na^+)								B0SFX9	1	3.9E-19
		1.A.30.2.5	Q1CYB8	3	M^+ cation (H^+ or Na^+)		Q8F3T0	3	1.6E-19	Q04WU1	3	6.9E-23	B0SFX8	3	2.0E-22
													B0SHM8	3	1.5E-19
													B0SI74	3	9.0E-23
		1.A.30.2.6	Q1DE41	1	M^+ cation (H^+ or Na^+)					Q04WU2	1	6.2E-20			
		1.A.30.2.6	Q1DE42	3	M^+ cation (H^+ or Na^+)					Q051K4	3	2.5E-19			
		1.A.30.2.7	Q1D0D2	1	M^+ cation (H^+ or Na^+)					Q04YU5	1	1.4E-07	B0S9Q3	1	1.6E-08
													B0SHM9	1	9.6E-08
		1.A.30.2.8	Q8EXJ4	3	M^+ cation (H^+ or Na^+)					Q04WS1	3	1.3E-75	B0SID9	3	2.0E-15
		1.A.30.2.8	Q8EXJ5	1	M^+ cation (H^+ or Na^+)					Q04WS0	1	1.0E-53	B0SID8	1	2.3E-09
		1.A.30.2.9	Q8F191	1	M^+ cation (H^+ or Na^+)		Q8F191	1	8.3E-76	Q04YU6	1	4.0E-70	B0S9Q4	1	1.1E-52
		1.A.30.2.9	Q8F192	4	M^+ cation (H^+ or Na^+)		Q8F192	4	9.6E-155	Q04YU7	6	5.1E-127	B0S9Q5	4	6.6E-79
1.A.35	CorA Metal Ion Transporter (MIT) Family														
		1.A.35.3.2	Q9WZ31	3	Cation (Mg^{2+}, Co^{2+})								B0SIH7	3	2.4E-45
		1.A.35.3.3	O31543	2	Cation (Mg^{2+}, Co^{2+})		Q8F2H8	3	9.4E-54						
1.A.43	Camphor Resistance (CrcB) Family														
		1.A.43.1.1	P37002	4	Anion (F^-)		Q8EZS4	3	3.0E-15						
1.A.62	Homotrimeric Cation Channel (TRIC) Family														
		1.A.62.2.1	A0M015	7	Cation (K^+, Na^+)								B0SDQ4	7	6.1E-41
1.A.72	Mer Superfamily														
		1.A.72.3.5	H6WCN3	3	Cation (Hg^{2+})								B0SBM2	3	4.1E-35
													B0SBR4	3	4.3E-35
1.B β-Barrel Porins															
1.B.4	Brucella-Rhizobium Porin (BRP) Family														
		1.B.4.3.1	Q72TA0	1	Nonselective		Q8F237	1	0	Q04X33	3	3.9E-12	B0SI14	1	6.2E-22

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
1.B.5	Pseudomonas OprP Porin (POP) Family														
		1.B.5.2.2	D3SLU1	0	Putative anion (Phosphate, pyrophosphate)								B0SFQ3	1	2.9E-09
1.B.6	OmpA-OmpF Porin (OOP) Family														
		1.B.6.1.14	K1LZ89	1	Nonselective		Q8EY80	1	2.0E-25	Q056P8	2	8.9E-27	B0S8Z3	1	5.8E-22
							Q8F9Y1	1	1.7E-18	Q04X85	1	2.0E-18	B0SE35	0	1.7E-40
							Q8F012	0	2.1E-44	Q056D3	0	1.4E-41	B0SH41	0	1.1E-17
		1.B.6.1.15	H6KWI2	1	Nonselective		Q8F081	2	4.9E-24	Q056A6	1	3.6E-24	B0SGL3	0	1.3E-26
		1.B.6.1.17	Q8F994	1	Nonselective		Q8F994	1	0	Q054Z0	1	0			
		1.B.6.1.20	B0SGK2	0	Nonselective		Q8F9H3	1	2.4E-54	Q04XF2	1	1.7E-54	B0SGK2	0	1.1E-108
1.B.9	FadL Outer Membrane Protein (FadL) Family														
		1.B.9.3.3	Q8F6Y8	1	Organoanion porin (Free fatty acids)		Q8F6Y8	1	0	Q04ZE4	1	0	B0SAZ7	1	5.1E-63
							Q8F8I8	1	7.0E-93						
1.B.13	Alginate Export Porin (AEP) Family														
		1.B.13.3.5	J4K5X9	1	Polysaccharides (Alginate)		Q8F2M3	1	0	Q04ZW7	1	0	B0SCZ8	1	4.8E-85
							Q8EYE4	1	3.5E-85	Q056K3	1	2.2E-85	B0SFZ4	1	4.6E-80
							Q8EZ15	1	5.4E-52	Q04XL2	1	8.0E-51	B0SIJ8	0	1.1E-57
1.B.14	Outer Membrane Receptor (OMR) Family														
		1.B.14.1.10	Q82VI7	0	Siderophores (Ferrioxamine)		Q8F2X2	2	1.1E-41						
		1.B.14.1.12	Q9HXB2	1	Siderophore (Ferric Citrate)					Q054C2	1	3.0E-25	B0S9W5	0	1.3E-26
		1.B.14.1.20	P13036	1	Siderophores (Ferric Citrate)		Q8F0M4	1	1.2E-28						
		1.B.14.2.6	Q93SS7	1	Siderophores (Heme)		Q8F1I6	1	7.7E-19						
		1.B.14.2.9	Q9JZN9	0	Siderophores (Heme)					Q04YG1	1	1.7E-30			
		1.B.14.3.2	Q1CY23	0	Vitamins (Cobalamin)		Q8F6F1	1	3.3E-44	Q053S9	1	7.8E-42	B0SBJ8	1	7.0E-27
		1.B.14.3.5	Q1CVX9	1	Vitamins (Cobalamin)		Q8F8I4	3	2.5E-36						
		1.B.14.6.6	Q8F853	2	Siderophores		Q8F853	2	0	Q04WT9	1	6.3E-96	B0SFX6	2	4.7E-84
							Q8F179	1	9.7E-142				B0SHQ6	0	0
							Q8F1N0	0	1.5E-125						
		1.B.14.8.2	Q2M5P4	0	Siderophores (PDTC)		Q8F406	0	4.6E-33	Q051R2	1	3.4E-32	B0SEN4	1	3.4E-31
		1.B.14.10.2	Q1DCQ0	0	Siderophore					Q04WQ2	0	2.2E-104	B0SHY1	0	3.6E-98
													B0SIS4	0	3.8E-84
		1.B.14.12.3	Q1DFM0	1	Siderophore		Q8F194	1	10.0E-90				B0SHR4	1	5.0E-95

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
1.B.16	Short Chain Amide and Urea Porin (SAP) Family														
		1.B.16.2.2	B0S9R9	1	Putative Amines, amides, polyamines (Amides, urea)								B0S9R9	1	0
1.B.17	Outer Membrane Factor (OMF) Family														
		1.B.17.1.2	P23598	1	Nonselective					Q04XP6	3	5.5E-12			
		1.B.17.2.2	P13509	0	Cation (Co^{2+}, Zn^{2+}, Cd^{2+})		Q8CVE8	1	3.1E-08				B0SC27	0	6.6E-10
		1.B.17.2.6	Q1D4Q0	1	Multidrug								B0SAW8	1	0.0002
		1.B.17.3.8	B3PY75	0	Putative Lipids (LPS)								B0SFY9	0	1.3E-13
1.B.22	Outer Bacterial Membrane Secretin (Secretin) Family														
		1.B.22.1.1	P15644	1	Proteins		Q8F3M6	3	9.2E-56	Q050Z9	2	1.3E-43	B0S8Q2	0	2.0E-56
1.B.33	Outer Membrane Protein Insertion Porin (Bam Complex) (OmpIP) Family														
		1.B.33.1.3	P0A943	1	Proteins (OMPs)								B0SHN0	1	3.4E-24
		1.B.33.2.3	C9LSC3	1	Proteins (OMPs)		Q8F3T2	1	2.1E-22	Q051K2	1	8.9E-23			
		1.B.33.4.1	Q8F605	1	Proteins (OMPs)		Q8F602	1	0	Q04ZY7	1	0	B0SH04	1	6.4E-169
							Q8F605	1	0	Q04ZY5	1	0	B0SC13	2	2.8E-145
							Q8F611	1	2.0E-106				B0SIP7	1	9.0E-100
1.B.37	Leptospira Porin OmpL1 (LP-OmpL1) Family														
		1.B.37.1.1	Q48546	1	Nonselective		G1UB30	1	5.2E-168	Q04YG8	3	3.2E-160	B0SDB5	2	4.3E-64
1.B.42	Outer Membrane Lipopolysaccharide Export Porin (LPS-EP) Family														
		1.B.42.1.2	P0ADC6	6	Lipids (LPS)		Q8F8T0	7	7.8E-06	Q054H5	7	6.1E-06	B0SBZ1	6	1.1E-06
		1.B.42.1.2	P0A9V1	0	Lipids (LPS)		Q8F3J7	0	3.6E-64	Q050X4	0	8.3E-68	B0S8M6	1	5.1E-70
		1.B.42.1.10	Q72SC6	1	Lipids (LPS)		Q8F398	1	0	Q050C8	2	0	B0S8V6	0	0
1.B.46	Outer Membrane LolAB Lipoprotein Insertion Apparatus (LolAB) Family														
		1.B.46.1.1	P61316	1	Lipids (Lipoprotein)								B0SF28	0	9.5E-05
		1.B.46.1.4	P57067	1	Lipids (Lipoprotein)		Q8F712	1	8.9E-05	Q04ZC8	1	9.4E-05			

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
1.B.48	Curli Fiber Subunit, CsgA, Porin, CsgG (CsgG) Family														
		1.B.48.1.7	Q8EXT1	2	Proteins					Q04WV0	2	6.4E-69			
		1.B.48.1.10	B0SDG2	1	Proteins								B0SDG2	1	8.6E-143
		1.B.48.3.4	Q8F8C2	4	Proteins		Q8F8C2	4	0	Q054B0	4	0	B0SEU3	1	7.3E-88
		1.B.48.5.1	Q87R31	1	Proteins								B0SB90	1	2.6E-10
1.B.55	Poly Acetyl Glucosamine Porin (PgaA) Family														
		1.B.55.3.2	B0SB44	0	Polysaccharides		Q8F3Y2	1	3.5E-85	Q051C9	1	5.3E-79	B0SB44	0	0
		1.B.55.3.3	E8V4M1	0	Polysaccharides		Q8F8T7	1	2.0E-12	Q054N3	1	2.0E-11			
1.B.66	Putative Beta-Barrel Porin-2 (BBP2) Family														
		1.B.66.1.4	M6CZ76	1	Unknown		Q8F4S2	1	9.2E-107				B0SEV8	1	1.9E-147
		1.B.66.1.7	B0SC23	1	Unknown		Q8F022	1	1.6E-99	Q055M1	1	5.5E-100	B0SC23	1	0
1.C Pore-Forming Toxins															
1.C.11	Pore-forming RTX Toxin (RTX-toxin) Family														
		1.C.11.1.7	Q7VH79	3	Cation Selective Pore	This family is likely methyl-accepting chemotaxis proteins	Q8F2F8	1	9.7E-18	Q051P8	3	6.4E-25	B0SEM8	2	8.6E-23
							Q8F6V9	2	8.8E-16	Q04ZQ4	2	2.8E-17	B0SDX0	2	6.6E-18
							Q8F882	2	9.5E-15	Q04X92	4	4.1E-17	B0SEW1	2	1.1E-13
							Q8F880	2	1.4E-14	Q054E9	2	1.7E-13	B0SIH4	2	9.4E-15
													B0SIH5	2	2.5E-14
1.C.67	SphH Hemolysin (SphH) Family														
		1.C.67.1.1	O34095	1	Nonselective		Q8CVE3	0	0	Q04XS2	1	8.08E-169			
							P59116	0	1.1E-157	Q04YC9	2	1.9E-151			
							Q8CVD9	0	7.3E-156						
							P59115	0	2.2E-143						
1.C.82	Pore-forming Amphipathic Helical Peptide HP(2-20) (HP2-20) Family														
		1.C.82.1.1	Q9ZK21	0	Nonselective		Q8F0R9	0	5.2E-53	Q054E5	0	1.8E-49	B0SAG4	0	2.9E-49

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
1.C.109	Bacterial Hemolysin A (B-Hemolysin A) Family														
		1.C.109.1.4	I6YB99	0	Nonselective								B0S9J1	0	2.8E-36
		1.C.109.1.5	J8IXD8	0	Nonselective		Q8F969	0	2.5E-44	Q054H0	2	4.5E-45			
1.E Holins															
1.E.40	Mycobacterial 4 TMS Phage Holin (MP4 Holin) Family														
		1.E.40.3.6	Q8F0F1	4	Proteins (Murein Hydrolase)		Q8F0F1	4	2.4E-66	Q053Z9	4	5.6E-47			
2.A Porters (Uniporters, Antiporters, Symporters)															
2.A.1	Major Facilitator Superfamily (MFS)														
		2.A.1.2.39	Q5JAK9	12	Multidrug (Tetracycline)		Q8F6D6	12	2.6E-75	Q053S3	12	2.3E-73			
		2.A.1.2.73	Q9D2V8	14	Multidrug (Tetracycline)		Q8F4V6	12	2.3E-37	Q050K3	12	4.1E-38	B0SC87	12	1.5E-37
		2.A.1.2.82	Q8EXM1	12	Multidrug					Q04WQ1	12	0	B0SCL6	12	6.4E-84
		2.A.1.2.84	Q9F632	12	Sugars & polyols (Arabinose)					Q04WK9	12	3.5E-66	B0SH56	12	1.5E-78
													B0SBF2	12	2.4E-53
		2.A.1.3.31	A5H8A5	16	Multidrug (Fluoroquinolone)		Q8F0F4	14	6.8E-113						
		2.A.1.4.3	P08194	12	Sugars & polyols (Glycerol-3-Phosphate)		Q8EZ20	12	2.5E-89	Q04XL4	12	7.7E-90	B0SG38	11	2.5E-89
		2.A.1.8.12	O82811	12	Anion (Nitrate, nitrite)								B0SEP4	13	2.6E-79
		2.A.1.21.3	O31137	12	Multidrug (Tetracycline)								B0SGD2	12	3.5E-06
													B0S9M0	12	0.0001
		2.A.1.24.3	A9WGR7	12	Unknown								B0SCK8	12	5.1E-23
		2.A.1.28.5	B0SL69	12	Unknown								B0SCU1	12	0
		2.A.1.38.2	Q0E7C5	12	Siderophores (Enterobactin)		Q8F767	12	1.9E-18	Q053Z1	12	2.1E-21	B0SAQ5	12	6.8E-21
		2.A.1.59.1	A6UVW2	12	Unknown								B0SEM9	12	0.0002
		2.A.1.59.2	B2JBG5	12	Unknown								B0SCE0	11	2.2E-55
		2.A.1.66.2	Q8F7L4	12	Putative Carboxylates (4-hydroxybenzoate)		Q8F7L4	12	0				B0SFG6	12	3.6E-160
		2.A.1.81.1	D5AKT2	12	Cation (Cu^{2+})								B0SAR3	12	5.0E-47

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
2.A.2	Glycoside-Pentoside-Hexuronide (GPH):Cation Symporter Family														
		2.A.2.3.1	P0CE45	11	Sugars & polyols (Melibiose)								B0SDC6	12	1.6E-16
		2.A.2.3.7	Q0HIQ0	12	Sugars & polyols (Melibiose)								B0SC42	11	9.6E-36
2.A.3	Amino Acid-Polyamine-Organocation (APC) Family														
		2.A.3.4.7	Q9KZF1	12	Amino acids								B0S9W8	12	2.6E-28
		2.A.3.5.1	Q53148	12	Amines, amides, polyamines (Ethanolamine)								B0SFI5	12	4.0E-54
		2.A.3.8.17	P45539	12	Amino acids								B0SBF7	12	2.4E-36
		2.A.3.8.28	K1M2K1	12	Amino acids (Large, neutral)		Q8F8N1	11	9.8E-41	Q04Z40	12	4.6E-41			
2.A.4	Cation Diffusion Facilitator (CDF) Family														
		2.A.4.1.3	O07084	5	Cation (Co^{2+}, Zn^{2+}, Cd^{2+})		Q8F433	7	6.3E-57	Q051E7	7	3.0E-56	B0S9G2	6	1.4E-41
					Cation (Co^{2+}, Zn^{2+}, Cd^{2+})		Q8EZ48	6	3.3E-44	Q04XQ6	5	9.9E-42	B0SAQ8	5	4.1E-39
2.A.5	Zinc (Zn^{2+})-Iron (Fe^{2+}) Permease (ZIP) Family														
		2.A.5.5.2	Q8N1S5	8	Cation (Zn^{2+})								B0SIK7	8	2.4E-43
2.A.6	Resistance-Nodulation-Cell Division (RND) Family														
		2.A.6.1.5	Q88RT6	12	Cation (Co^{2+}, Zn^{2+}, Cd^{2+})					Q052Z7	12	0	B0SG51	12	0
													B0SFV0	12	0
													B0SHS1	12	0
													B0SGJ5	12	0
		2.A.6.1.5	Q88RT4	1	Cation (Co^{2+}, Zn^{2+}, Cd^{2+})					Q050M6	1	4.5E-11	B0SGJ7	1	2.2E-10
		2.A.6.1.6	Q1LCD7	1	Cation (Co^{2+}, Zn^{2+}, Cd^{2+})					Q050M7	1	2.7E-17			
		2.A.6.1.6	Q1LCD8	12	Cation (Co^{2+}, Zn^{2+}, Cd^{2+})		Q8F4S4	12	0	Q050M9	12	0	B0SC24	12	0
							Q8F4S3	12	0						

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
		2.A.6.1.7	Q1DDM8	1	Cation (Co^{2+}, Zn^{2+}, Cd^{2+})		Q8F664	1	2.4E-32	Q04ZF3	1	1.1E-30	B0SFV3	1	1.1E-36
		2.A.6.1.8	Q1DDM4	12	Cation (Co^{2+}, Zn^{2+}, Cd^{2+})								B0SIF6	12	0
		2.A.6.1.11	Q1CVN2	1	Cation (Co^{2+}, Zn^{2+}, Cd^{2+})					Q04ZF2	1	8.4E-06	B0SFV2	1	3.9E-10
		2.A.6.1.16	B8GZE8	1	Cation (Co^{2+}, Zn^{2+}, Cd^{2+})								B0SFY8	1	1.8E-09
		2.A.6.1.16	B8GZE9	12	Cation (Co^{2+}, Zn^{2+}, Cd^{2+})		Q8F5X3	12	0						
							Q8F8H3	12	0						
		2.A.6.2.14	B2FLY4	12	Multidrug		Q8EZW1	11	2.8E-07						
		2.A.6.2.21	Q51487	1	Multidrug								B0SHU2	1	7.9E-39
		2.A.6.2.22	Q8RTE4	12	Multidrug								B0SHU1	12	0
		2.A.6.2.30	Q9K2Y1	1	Multidrug		Q8CVE2	1	1.4E-14						
		2.A.6.2.32	Q9HWH5	1	Multidrug					Q053J6	1	7.7E-19			
		2.A.6.2.44	Q2FD82	1	Multidrug		Q8EZW4	1	1.0E-25				B0SHU0	1	5.0E-36
		2.A.6.3.1	P25197	12	Multidrug		Q8EZK7	14	6.8E-161	Q04XP5	12	1.1E-146			
		2.A.6.3.3	E8PBU7	11	Multidrug		Q8F8K5	10	1.1E-175	Q04XP1	11	7.6E-175	B0SI76	11	3.3E-174
							Q8EZC8	14	7.4E-147						
		2.A.6.3.4	Q1DEX6	9	Multidrug								B0SDF4	11	3.5E-79
		2.A.6.3.6	Q8CX78	12	Multidrug		Q8EZW3	12	1.1E-137	Q053K1	13	6.7E-130	B0SHH0	11	1.3E-101
							Q8EZI2	11	3.2E-104	Q054Q7	11	5.3E-106	B0SAD8	13	1.0E-96
							Q8F3G5	13	3.7E-103				B0SEE5	12	1.1E-26
		2.A.6.4.3	Q5SKE6	11	Proteins	Annotated as SecD/ SecF	Q8F706	7	9.4E-59	Q04ZD5	7	5.3E-59	B0SGE3	6	3.9E-53
							Q8F705	6	1.3E-33	Q04ZD6	6	9.0E-31	B0SGE4	6	9.2E-32
		2.A.6.7.3	Q1CY80	12	Unknown		Q8CXT6	11	1.2E-117	Q04Z67	12	1.1E-100			
2.A.7	Drug/Metabolite Transporter (DMT) Superfamily														
		2.A.7.1.4	P69937	4	Amines, amides, polyamines (Quaternary ammonium)					Q054M0	3	4.4E-14	B0SBU5	4	1.2E-15
		2.A.7.3.26	P0ABT9	10	Putative Amino Acid								B0SI87	10	1.4E-09
		2.A.7.3.36	O29740	10	Putative Amino Acid		Q8F8P1	10	1.9E-11	Q04Y54	10	1.9E-12	B0SA83	10	6.4E-12
		2.A.7.3.47	Q9UYQ7	10	Putative Amino Acid		Q8F1L2	9	1.4E-17	Q04XU1	10	3.8E-18	B0SDC0	10	2.2E-34
		2.A.7.3.58	B0SDB7	10	Putative Amino Acid								B0SDB7	10	5.4E-165

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
		2.A.7.23.1	P42243	10	Putative Amino Acid (Tryptophan)		Q8F6G7	10	1.1E-27	Q053U1	10	3.8E-26	B0SIH0	10	8.7E-33
		2.A.7.26.4	A9T501	4	Unknown								B0SBU1	4	9.1E-24
		2.A.7.33.5	U2UX39	4	Unknown								B0SFC0	4	0.0005
		2.A.7.34.2	B0RV55	4	Unknown								B0SCR7	4	2.1E-28
		2.A.7.34.5	Q11YS2	4	Unknown					Q052T3	4	2.9E-25			
		2.A.7.34.6	G0ENB6	4	Unknown		Q8F5V6	3	3.5E-22						
2.A.9	Cytochrome Oxidase Biogenesis (Oxa1) Family														
		2.A.9.2.1	Q8LBP4	6	Proteins								B0SAE6	5	2.1E-36
		2.A.9.3.1	P25714	3	Proteins		P97041	5	4.2E-34	Q04XE2	5	1.2E-32			
2.A.17	Proton-dependent Oligopeptide Transporter (POT/PTR) Family														
		2.A.17.1.4	P75742	14	Peptides (Dipeptide/Tripeptide)		Q8F3I9	12	1.6E-61						
		2.A.17.1.6	Q5M4H8	14	Peptides (Dipeptide/Tripeptide)					Q051N8	11	2.5E-67	B0S9V2	11	1.1E-54
2.A.19	Ca^{2+}:Cation Antiporter (CaCA) Family														
		2.A.19.5.3	Q57556	10	Cation (Ca^{2+})								B0SCN8	10	1.2E-30
2.A.21	Solute:Sodium Symporter (SSS) Family														
		2.A.21.3.2	P96169	14	Sugars & polyols (Glucose)								B0SA99	14	2.2E-48
		2.A.21.3.8	A1S2A8	15	Sugars & polyols (Glucose)		Q8F864	15	3.6E-36	Q04YE1	15	5.7E-37	B0S8V1	15	8.2E-16
		2.A.21.5.1	Q92911	13	Carboxylates (Monocarboxylate)		Q8F4A8	13	1.6E-29	Q051V1	13	3.5E-31	B0SF07	13	1.2E-21
		2.A.21.8.3	B0S9U4	13	Amines, amides, polyamines (Choline)								B0S9U4	13	0
2.A.22	Neurotransmitter:Sodium Symporter (NSS) Family														
		2.A.22.4.2	O67854	12	Amino acids		Q8F511	12	6.6E-92	Q050H6	12	3.5E-89	B0S8U1	12	1.7E-90
2.A.23	Dicarboxylate/ Amino Acid: Cation Symporter (DAACS) Family														
		2.A.23.1.5	O59010	9	Amino acid (Leucine)		Q8F9X1	9	1.4E-55	Q04X74	9	1.0E-53	B0SHY9	9	7.2E-50

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
2.A.25	Alanine or Glycine:Cation Symporter (AGCS) Family														
		2.A.25.1.6	B0SM05	12	Amino acid (Alanine)		Q8F2Z5	12	0	Q050F6	12	0	B0SDH7	12	0
2.A.28	Bile Acid:Na^+ Symporter (BASS) Family														
		2.A.28.1.2	Q12908	9	Organoanion (Bile Acid)								B0S9L2	9	2.4E-38
		2.A.28.2.2	E0D3H5	9	Carboxylates (Pyruvate)								B0SIE8	10	5.8E-53
		2.A.28.2.4	Q9K0A9	10	Organoanion		Q8CXS2	9	1.2E-32	Q053T9	9	9.5E-31	B0S9M3	9	1.5E-29
							Q8CXT7	8	4.1E-28						
2.A.33	NhaA Na^+:H^+ Antiporter (NhaA) Family														
		2.A.33.1.1	P13738	10	Cation (Na^+, H^+)								B0SBD5	10	1.9E-87
2.A.35	NhaC Na^+:H^+ Antiporter (NhaC) Family														
		2.A.35.1.1	P27611	12	Cation (Na^+, H^+)					Q04ZN5	10	1.2E-80			
		2.A.35.1.2	P54571	12	Cation (Na^+, H^+)		Q8F621	7	1.7E-47						
		2.A.35.1.6	O07553	12	Cation (Na^+, H^+)								B0SDV2	12	8.5E-56
2.A.36	Monovalent Cation:Proton Antiporter-1 (CPA1) Family														
		2.A.36.6.3	Q87KV8	13	Cation (K^+, H^+)		Q8EZ70	11	5.7E-75						
		2.A.36.6.4	Q0ZAH6	13	Cation (K^+, H^+)								B0SBF6	9	1.2E-71
2.A.37	Monovalent Cation:Proton Antiporter-2 (CPA2) Family														
		2.A.37.1.1	P03819	12	Cation		Q8EYB5	13	2.3E-133	Q056M0	13	8.2E-131	B0SB61	13	6.1E-125
		2.A.37.1.3	Q0ZAH7	13	Cation		Q8EYC0	13	2.9E-35	Q056J5	13	2.5E-30			
		2.A.37.1.8	Q8VYR9	13	Cation								B0SA59	13	1.1E-29
		2.A.37.4.2	Q9SUQ7	13	Cation		Q8F5W8	15	9.5E-71				B0S9J4	13	1.8E-85
2.A.38	K^+ Transporter (Trk) Family														
		2.A.38.4.3	O32081	9	Cation (K^+)		Q8EZ85	14	2.7E-68	Q04Y32	16	8.7E-65	B0S9Q8	14	3.5E-63
													B0SF50	9	2.4E-40
		2.A.38.4.4	P39760	1	Cation (K^+)		Q8EZ84	1	1.9E-26						
		2.A.38.4.6	G8V398	12	Cation (K^+)		Q8F982	11	4.5E-44	Q04Y02	9	2.7E-46			

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
2.A.47	Divalent Anion:Na^+ Symporter (DASS) Family														
		2.A.47.1.13	Q65NC0	14	Anion (Sulfate)		Q8F5L4	15	9.1E-45	Q052K5	15	1.1E-43			
		2.A.47.5.1	Q58086	13	Anion (Sulfate)								B0SA86	14	8.1E-40
2.A.49	Chloride Carrier/Channel (ClC) Family														
		2.A.49.4.1	Q57753	10	Anion (Cl^-)								B0SCJ7	11	1.7E-35
													B0SCW6	10	1.1E-19
		2.A.49.5.4	A8AGW0	11	Anion (Cl^-)		Q8EYK3	11	1.3E-30	Q055Z2	12	7.7E-31			
		2.A.49.8.2	M2XWR6	10	Anion (Cl^-)		Q8F243	11	5.0E-30						
2.A.50	Glycerol Uptake (GUP) Family														
		2.A.50.2.1	P39580	12	Amino Acid (D-Alanine)		Q8F3B8	10	1.7E-24	Q050T2	11	8.1E-24	B0S9E5	11	1.5E-26
							Q8F3V9	12	7.5E-23	Q051I3	12	2.2E-23	B0S9T1	12	2.6E-25
							Q8F3Z3	13	1.1E-22	Q051C2	13	8.8E-23	B0SCF8	11	9.2E-24
							Q8F2K0	13	1.9E-22	Q051V7	11	1.2E-22	B0SG46	11	1.5E-23
							Q8F4B7	11	5.1E-22	Q04ZV0	13	1.5E-22	B0SEG5	11	2.6E-22
							Q8F6E3	12	2.3E-20	Q053P8	11	7.8E-20	B0SDB0	11	4.9E-22
							Q8F6U7	11	3.8E-18	Q04ZC3	11	6.5E-19	B0SBZ5	11	2.9E-21
						putative alanyl teichoic acid synthesis protein, DltB (may transport activated alanine across the membrane)				Q04WR7	13	4.2E-26	B0SAS0	11	3.5E-21
										Q04WR5	13	1.5E-22	B0SFH7	11	2.4E-19
										Q04WG6	11	2.0E-22	B0SIQ2	12	2.1E-24
													B0SIN3	13	1.3E-22
2.A.51	Chromate Ion Transporter (CHR) Family														
		2.A.51.1.3	P14285	11	Anion (Chromate)								B0SAX7	11	2.8E-24
2.A.53	Sulfate Permease (SulP) Family														
		2.A.53.3.8	Q8F8H7	10	Anion (Bicarbonate)		Q8F8H7	10	0	Q04YH8	10	0	B0S8R9	11	2.4E-144
													B0SFZ1	14	5.6E-74
		2.A.53.5.1	Q9SL95	11	Anion (Molybdate)								B0SEN7	9	1.1E-47
2.A.55	Metal Ion (Mn^{2+}-iron) Transporter (Nramp) Family														
		2.A.55.3.4	Q93JK1	11	Cation (Mn^{2+})								B0SDV1	11	1.0E-10

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
2.A.56	Tripartite ATP-independent Periplasmic Transporter (TRAP-T) Family														
		2.A.56.1.1	O07838	13	Carboxylates (dicarboxylate)								B0SGW8	13	3.6E-80
		2.A.56.1.9	R4JTF7	3	Carboxylates (dicarboxylate)								B0SGX0	1	8.4E-32
2.A.58	Phosphate:Na^+ Symporter (PNaS) Family														
		2.A.58.2.2	M7AKZ4	8	Anion (Phosphate)								B0SGH9	8	1.0E-67
2.A.59	Arsenical Resistance-3 (ACR3) Family														
		2.A.59.1.2	P45946	10	Anion (Arsenite)								B0SEX9	10	1.7E-123
2.A.64	Twin Arginine Targeting (Tat) Family														
		2.A.64.1.1	P69423	5	Proteins								B0SEC5	5	1.5E-24
		2.A.64.2.1	Q9SJV5	6	Proteins		Q8F4W3	6	3.6E-22						
		2.A.64.2.1	Q9LKU2	1	Proteins		Q8F4W2	1	1.2E-05						
		2.A.64.2.2	Q9RJ69	6	Proteins					Q050J6	6	1.4E-21			
2.A.66	Multidrug/Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily														
		2.A.66.1.4	P28303	13	Multidrug		Q8F9X2	11	2.5E-39	Q04X75	11	3.0E-34			
		2.A.66.1.28	Q9WZS2	12	Multidrug		Q8EZ91	12	7.8E-30	Q04Y29	12	1.7E-29	B0SHP5	12	3.7E-36
													B0S9R1	12	1.3E-27
		2.A.66.2.21	Q8F225	12	Lipids (O-antigen)		Q8F225	12	0	Q053H1	12	0	B0SGP9	12	1.3E-113
		2.A.66.4.1	P37169	14	Peptides & conjugates (Peptidoglycan)		Q8F5E7	13	1.2E-51	Q052F8	13	3.4E-55	B0SBW5	13	1.2E-54
		2.A.66.5.2	A4BUA1	12	Lipids (O-antigen)		Q8F5M3	12	5.6E-14	Q052M1	12	4.5E-13			
2.A.69	Auxin Efflux Carrier (AEC) Family														
		2.A.69.4.4	Q1DE54	10	Carboxylates (Malate)		Q8EZ06	9	3.6E-31	Q04XK9	9	4.9E-30	B0SA82	10	2.2E-46
2.A.83	Na^+-dependent Bicarbonate Transporter (SBT) Family														
		2.A.83.2.1	A9DHE6	10	Anion (Bicarbonate)		Q8EYE6	10	2.0E-49	Q056K2	9	2.2E-52	B0SFZ3	10	5.7E-56
													B0SIP5	10	3.1E-61

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
2.A.86	Autoinducer-2 Exporter (AI-2E) Family														
		2.A.86.1.8	O32095	8	Sugars & Polyols (AI-2)		Q8F3P6	7	4.0E-11	Q051N2	7	1.1E-10			
		2.A.86.1.11	F8FLY5	7	Sugars & Polyols (AI-2)		Q8F6G8	8	2.9E-25	Q053U3	8	6.1E-27	B0SDC1	8	4.0E-23
		2.A.86.2.3	Q3IS50	7	Sugars & Polyols (AI-2)								B0S9U1	7	1.3E-11
2.A.88	Vitamin Uptake Porter (VUT or ECF) Family														
		2.A.88.8.5	B2FPS5	6	Multidrug		Q8F440	6	3.2E-38	Q051B8	6	2.8E-36	B0S9G8	6	1.1E-40
2.A.102	4-Toluene Sulfonate Uptake Permease (TSUP) Family														
		2.A.102.4.5	C7D7I4	9	Anion (Sulfite)								B0SAD4	7	1.4E-12
2.A.109	Tellurium Ion Resistance (TerC) Family														
		2.A.109.1.2	F0N2E7	9	Anion (Te^{2-})					Q04WT2	9	6.4E-73			
		2.A.109.2.2	Q7URC1	7	Anion (Te^{2-})								B0SGK5	7	1.9E-52
		2.A.109.2.3	L5DDD7	7	Anion (Te^{2-})		Q8F6U0	7	1.0E-53	Q053Q6	7	4.3E-52			
2.A.111	Na^+/H^+ Antiporter-E (NhaE) Family														
		2.A.111.1.1	Q8F285	12	Cation (Na^+, H^+)		Q8F285	12	0	Q053F5	13	0	B0SCS6	12	0
2.A.114	Putative Peptide Transporter Carbon Starvation CstA (CstA) Family														
		2.A.114.1.8	L0DJM9	15	Peptides		B0SAH8	15	7.8E-38	Q054Z2	15	1.2E-45			
2.A.115	Novobiocin Exporter (NbcE) Family														
		2.A.115.1.6	E0HYB9	3	Multidrug		Q8F8F1	3	2.8E-38	Q04YI7	3	1.1E-39			
2.A.121	Sulfate Transporter (CysZ) Family														
		2.A.121.4.6	Q8EZV9	5	Putative Anion (Sulfate)		Q8EZV9	5	5.4E-110						
2.A.123	Sweet; PQ-loop; Saliva; MtN3 (Sweet) Family														
		2.A.123.2.4	Q8F4F7	3	Sugars & Polyols		Q8F4F7	3	8.7E-51	Q051Z4	3	1.5E-38	B0SHL1	3	3.9E-23
2.C Ion-Gradient-Driven Energizers															
2.C.1	TonB Family of Auxiliary Proteins for Energization of Outer Membrane Receptor (OMR)-mediated Active Transport														
		2.C.1.2.1	P0A855	1	Macromolecules (Proteins)		Q8CVE4	1	1.1E-05	Q053X8	1	3.8E-07	B0SDV7	1	3.6E-05

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
3.A P-P-Bond-Hydrolysis-Driven Transport															
3.A.1	ATP-binding Cassette (ABC) Superfamily														
		3.A.1.3.10	P0AEM9	1	Amino Acids		Q8F6X5	1	2.1E-12				B0S8Z2	1	2.2E-11
		3.A.1.3.15	P54535	1	Amino Acids		Q8EY81	1	3.2E-09	Q056P9	1	2.3E-09			
		3.A.1.5.15	Q9X270	6	Sugars & Polyols		Q8F8C3	6	9.7E-12						
		3.A.1.5.18	O51310	6	Peptides (Oligopeptide)								B0SEU4	6	3.5E-11
		3.A.1.5.19	Q2FZR7	6	Peptides (Oligopeptide)		Q8F8C4	7	7.7E-21	Q054B2	6	7.5E-20	B0SEU5	5	9.1E-15
		3.A.1.5.20	P42061	1	Peptides (Oligopeptide)		Q8F004	1	3.5E-17	Q055J5	1	6.7E-17			
		3.A.1.5.21	P33915	6	Peptides (Oligopeptide)								B0SAT7	6	4.0E-71
		3.A.1.5.21	P0AFU1	6	Peptides (Oligopeptide)								B0SAT6	6	1.8E-67
		3.A.1.5.21	P33913	1	Peptides (Oligopeptide)					Q04Z53	1	8.9E-44			
		3.A.1.5.24	Q7CQ74	6	Peptides (Oligopeptide)		Q8EYI6	6	5.1E-71	Q056G9	6	1.3E-71			
		3.A.1.5.24	Q8ZNJ9	6	Peptides (Oligopeptide)		Q8EYI5	6	6.8E-70	Q056H0	6	1.4E-70			
		3.A.1.5.24	Q8ZNK0	1	Peptides (Oligopeptide)		Q8F1P5	1	4.9E-44				B0SCA2	1	3.4E-45
		3.A.1.6.1	P16701	6	Anion (Sulfate)					Q04ZE1	6	1.0E-59	B0SF81	6	3.7E-55
		3.A.1.6.1	P0AEB0	6	Anion (Sulfate)		Q8F6Z2	6	3.3E-71	Q04ZE2	6	1.8E-73	B0SF80	6	9.77E-71
		3.A.1.6.1	P0AG78	1	Anion (Sulfate)		Q8CVE5	1	5.9E-124	Q04ZW6	1	1.2E-123	B0SF82	1	1.7E-103
					Anion (Sulfate)		Q8CVF2	1	2.0E-115	Q04ZE0	1	1.2E-114			
		3.A.1.6.7	Q8RVC7	6	Anion (Sulfate)		Q8F6Z3	6	1.9E-59						
		3.A.1.7.5	O51233	1	Anion (Phosphate)								B0SGL2	1	6.4E-25
		3.A.1.11.1	P0AFK6	6	Amines, amides, polyamines (Putrescine/Spermidine)								B0SE22	6	2.1E-26
		3.A.1.11.2	P31135	6	Amines, amides, polyamines (Putrescine/Spermidine)								B0SE23	6	1.4E-24
		3.A.1.11.7	Q97Q45	1	Amines, amides, polyamines (Putrescine/Spermidine)								B0SE25	1	3.5E-11
		3.A.1.14.5	Q56991	1	Siderophores								B0SIS1	1	1.4E-48
		3.A.1.14.18	Q32AY2	9	Siderophores								B0SIS0	10	7.0E-62
		3.A.1.14.18	Q32AY3	1	Siderophores		Q8F5F7	1	6.9E-22						
		3.A.1.15.6	Q9RNI8	7	Cation (Zn^{2+})								B0SFQ6	9	1.0E-09
		3.A.1.15.13	B2IWS9	1	Cation (Zn^{2+})								B0SFQ4	1	1.6E-18
		3.A.1.17.8	A7NH44	1	Vitamins (Thiamine)								B0SBG6	1	2.9E-13

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
		3.A.1.27.1	A4PCH7	6	Lipids								B0SD60	7	2.6E-49
													B0S9U7	6	2.3E-31
		3.A.1.27.1	A4PCH8	1	Lipids								B0SD61	3	7.6E-47
		3.A.1.27.3	P64606	5	Lipids		Q8F4I4	6	1.7E-30	Q052B7	6	2.2E-30			
		3.A.1.27.4	Q0SFA1	1	Lipids		Q8F4I5	1	3.2E-55						
		3.A.1.27.5	P63357	1	Lipids					Q052B8	1	1.3E-52			
		3.A.1.103.3	Q50862	7	Lipids (O-Antigen)								B0SAK2	6	5.6E-22
													B0SDY3	6	1.2E-08
		3.A.1.103.3	Q50863	1	Lipids (O-Antigen)								B0SDY4	1	4.5E-49
		3.A.1.103.4	Q1D3I2	7	Lipids (O-Antigen)		Q8F654	6	1.5E-08	Q04ZK4	6	7.5E-08			
		3.A.1.105.11	G0Q3D4	6	Multidrug		Q8EYC7	6	3.8E-17	Q056I8	6	7.3E-16			
		3.A.1.105.12	F8D412	6	Multidrug		Q8F4C8	6	1.8E-38	Q051W8	6	1.2E-39	B0S9Y7	6	4.7E-41
							Q8F7H5	7	6.0E-31	Q04YR2	7	1.6E-30	B0SE97	6	1.4E-33
		3.A.1.106.1	P60752	5	Lipids		Q8F066	4	6.5E-96	Q056B7	5	8.7E-99			
		3.A.1.106.2	Q2G2M9	5	Lipids		Q8F003	4	1.1E-98	Q055J6	5	2.0E-99	B0S962	5	1.5E-95
							Q8F9P1	8	4.4E-77	Q04XC8	8	2.7E-76	B0SDE6	7	3.4E-95
		3.A.1.109.1	P08716	8	Proteins								B0SAW5	8	1.0E-78
		3.A.1.109.2	P18770	6	Proteins								B0SAW7	4	2.2E-79
		3.A.1.110.10	C1DS84	6	Proteins								B0SA62	3	1.3E-19
		3.A.1.111.2	P33116	5	Peptides (Subtilin)								B0SBU9	5	8.2E-72
		3.A.1.117.3	O06967	6	Multidrug					Q052Q3	5	8.5E-36	B0S8Z9	6	1.1E-92
		3.A.1.122.12	A0ZUB1	5	Multidrug								B0SFY7	3	2.8E-111
		3.A.1.125.1	P75958	4	Lipids (Lipoprotein)		Q8F9C2	4	4.8E-17	Q055T6	4	1.5E-18	B0SDK0	4	3.6E-18
							Q8F6L9	4	3.0E-15	Q054T3	11	1.4E-07	B0SDJ2	4	4.5E-13
							Q8EZY9	11	6.1E-07						
		3.A.1.125.2	Q7D911	10	Lipids (Lipoprotein)		Q8F1Z5	10	1.8E-30	Q053J3	10	9.1E-34	B0SCV3	10	1.7E-18
		3.A.1.132.1	Q93LN0	2	Polysaccharides (Exopolysaccharide)		Q8F3E2	2	2.9E-21	Q050V6	5	9.1E-19			
		3.A.1.132.2	Q52899	1	Polysaccharides (Exopolysaccharide)								B0SAY7	2	5.5E-26
		3.A.1.132.2	O07330	8	Polysaccharides (Exopolysaccharide)								B0SAY5	8	3.9E-12
		3.A.1.132.3	A0L4L0	1	Polysaccharides (Exopolysaccharide)					Q051L8	1	1.0E-54			
		3.A.1.132.4	Q2SDB0	8	Polysaccharides (Exopolysaccharide)					Q050V7	6	1.2E-38	B0SBK3	6	6.4E-17
										Q056F5	6	1.2E-18			
		3.A.1.201.12	B2WTH9	13	Multidrug		Q8F5R7	7	3.4E-64						
		3.A.1.203.8	Q6NLC1	5	Organoanion (Fatty Acids)								B0SCH9	5	4.3E-67

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
3.A.2	H^+- or Na^+-translocating F-type, V-type and A-type ATPase (F-ATPase) Superfamily														
		3.A.2.1.2	P21903	6	Cation (H^+)		Q8F2I8	6	1.4E-07	Q04ZT9	7	1.4E-05			
		3.A.2.1.6	F8L1Z5	1	Cation (H^+)		Q8F2J0	1	6.9E-13	Q04ZU1	1	4.5E-12	B0SDA1	1	7.5E-09
		3.A.2.1.6	F8L1Z6	2	Cation (H^+)		Q8F2I9	2	2.0E-10	Q04ZU0	2	2.5E-10	B0SDA0	2	1.3E-07
		3.A.2.1.6	F8L1Z7	6	Cation (H^+)								B0SD99	7	3.0E-11
3.A.3	P-type ATPase (P-ATPase) Superfamily														
		3.A.3.1.5	B0SMV3	11	Cation (Na^+, K^+)								B0SEB6	11	0
		3.A.3.4.2	Q72RN5	8	Cation (Mg^{2+})		Q8F426	7	0						
					Cation (Mg^{2+})		Q8F427	2	5.2E-114						
		3.A.3.5.4	Q9ZHC7	9	Cation (Cu^{2+})								B0SBE3	7	0
		3.A.3.5.23	Q72N56	8	Cation (Cu^{2+})		Q8F8G3	8	0	Q04YI2	10	0	B0SEA5	8	0
		3.A.3.6.10	O32219	9	Cation (Co^{2+}, Zn^{2+}, Cd^{2+})								B0SGJ3	8	1.5E-157
		3.A.3.7.1	P03959	12	Cation (K^+)		Q8F1M1	10	2.1E-150						
		3.A.3.7.2	Q1M606	7	Cation (K^+)		P59219	7	0						
		3.A.3.7.2	Q1M607	1	Cation (K^+)		Q8F1M2	1	1.4E-31						
		3.A.3.27.2	B0STR2	9	Cation (Cu^{2+})								B0SI48	9	0
		3.A.3.30.3	B0SLF7	10	Cation (Ca^{2+})					Q050U1	9	0	B0SCY0	10	0
3.A.5	General Secretory Pathway (Sec) Family														
		3.A.5.1.1	P0AG96	3	Proteins		Q8F0R6	1	6.7E-06	Q054E8	1	7.1E-06	B0SAG7	1	5.0E-06
		3.A.5.1.1	P0AG99	3	Proteins		Q8F5I6	2	7.8E-08	Q052H9	2	8.2E-09	B0SB23	2	2.0E-09
		3.A.5.2.2	P0A5Z2	10	Proteins		G1UB19	10	2.3E-94	Q055C4	10	1.0E-94	B0SA27	10	4.4E-93
		3.A.5.7.2	Q8U4B5	6	Proteins		Q8F705	6	1.7E-06						
3.A.6	Type III (Virulence-related) Secretory Pathway (IIISP) Family														
		3.A.6.2.1	P54700	5	Proteins		Q8F300	4	3.9E-51	Q050G0	5	1.4E-51	B0SDH3	5	2.7E-50
		3.A.6.2.1	P54702	6	Proteins		Q8F302	6	1.2E-20	Q050G2	6	2.0E-20	B0SDH1	6	1.2E-18
		3.A.6.2.1	P40727	4	Proteins		Q8F303	7	1.5E-36	Q050G3	4	7.1E-38	B0SDH0	5	2.2E-44
		3.A.6.2.1	P15928	2	Proteins		Q8F320	2	3.7E-32	Q052W7	2	2.6E-32	B0SCT6	2	7.3E-34
		3.A.6.2.1	P40729	7	Proteins		Q8F304	7	1.2E-122	Q050G4	7	6.2E-123	B0SDG9	9	1.9E-107
		3.A.6.2.1	P0A1L5	2	Proteins		Q8F301	2	6.3E-10	Q050G1	2	4.7E-10	B0SDH2	2	7.0E-09

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
3.A.10	H^+, Na^+-translocating Pyrophosphatase (M^+-PPase) Family														
		3.A.10.1.1	P31414	16	Anion (Pyrophosphate)								B0S8X5	16	0
		3.A.10.1.16	O80384	16	Anion (Pyrophosphate)		Q8F641	16	0	Q04ZM0	16	0			
3.A.11	Bacterial Competence-related DNA Transformation Transporter (DNA-T) Family														
		3.A.11.1.1	P39694	1	Nucleic acids (DNA)		Q8F2J7	1	1.1E-04	Q04ZU7	1	0.006	B0SDA7	1	0.001
		3.A.11.1.1	P39695	12	Nucleic acids (DNA)		Q8F8Z2	11	9.2E-11				B0SF25	13	1.7e-09
		3.A.11.1.3	Q8VRL2	10	Nucleic acids (DNA)								B0SGF0	6	4.2E-06
3.A.12	Septal DNA Translocator (S-DNA-T) Family														
		3.A.12.1.3	K8ITE0	4	Nucleic acids (DNA)		Q8F1W7	4	0	Q04ZC6	4	0	B0SGD5	4	0
3.A.15	Outer Membrane Protein Secreting Main Terminal Branch (MTB) Family														
		3.A.15.1.1	P15643	1	Proteins		Q8F3M5	2	2.4E-05	Q050Z8	2	2.8E-05			
		3.A.15.1.1	P15750	1	Proteins		Q8F3N3	2	7.4E-06	Q051A6	2	0.0003	B0S8Q9	1	3.8E-05
		3.A.15.2.1	Q8VRL3	4	Proteins		Q8F3M8	5	8.8E-66	Q051A1	5	1.4E-66	B0S8Q4	6	1.6E-67
		3.A.15.3.1	O68433	6	Proteins		Q8F6L1	6	1.0E-07	Q04ZR7	6	8.6E-06			
		3.A.15.3.1	Q9XD71	1	Proteins		Q8F3M9	1	6.7E-19	Q051A2	1	2.9E-19	B0S8Q5	1	5.3E-14
3.B Decarboxylation-Drive Transporters															
3.B.1	Na+-transporting Carboxylic Acid Decarboxylase (NaT-DC) Family														
		3.B.1.1.2	Q57079	1	Cation (Na^+)		Q8F3H0	2	1.2E-78	Q051Q3	2	1.3E-77	B0SHH5	3	1.1E-80
													B0SBL8	1	7.2E-77
		3.B.1.1.5	Q9V0A4	3	Cation (Na^+)		Q8EZP9	3	1.1E-73						
3.D Oxidoreduction-Driven Transporters															
3.D.1	H+ or Na+-translocating NADH Dehydrogenase (NDH) Family														
		3.D.1.1.1	P0AFE0	5	Cation (H^+)		Q8F7Q6	5	4.7E-10						
		3.D.1.2.1	P29919	3	Cation (H^+)		Q8F7P9	3	4.8E-18	Q04YA4	3	1.2E-17	B0SFT4	3	5.93E-15
		3.D.1.2.1	P29923	3	Cation (H^+)					Q04YB2	3	2.5E-07	B0SFU2	3	2.3E-16
		3.D.1.2.1	P29924	16	Cation (H^+)		Q8F7Q8	19	2.0E-120	Q04YB3	19	8.1E-122	B0SFU3	19	3.8E-114
		3.D.1.2.1	P29925	14	Cation (H^+)					Q04YB4	15	7.5E-84	B0SFU4	15	4.0E-104
		3.D.1.2.1	P29926	15	Cation (H^+)								B0SFU5	14	1.3E-51
		3.D.1.3.1	Q56218	1	Cation (H^+)		Q8F7Q0	1	3.6E-47	Q04YA5	1	1.4E-47	B0SFT5	1	3.0E-47
		3.D.1.3.1	Q56222	1	Cation (H^+)		Q8F7Q4	1	1.4E-119	Q04YA9	1	2.7E-122	B0SFT9	1	7.8E-117
		3.D.1.3.1	Q56226	3	Cation (H^+)		Q8F7Q7	3	2.6E-09						

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
		3.D.1.5.1	Q746T2	8	Cation (H^+)		Q8F7Q5	8	1.3E-84	Q04YB0	8	2.0E-83	B0SFU0	8	6.4E-82
		3.D.1.5.1	Q746T5	5	Cation (H^+)					Q04YB1	5	3.5E-09			
		3.D.1.5.1	Q746T8	13	Cation (H^+)		Q8F7Q9	9	8.1E-66						
		3.D.1.5.1	Q746T9	13	Cation (H^+)		Q8F7R0	15	7.1E-48	Q04YB5	13	8.4E-49			
		3.D.1.5.1	Q74GA5	2	Cation (H^+)		Q8F7Q2	2	4.1E-98	Q04YA7	2	4.3E-98	B0SFT7	1	5.6E-97
		3.D.1.6.2	P23710	1	Cation (H^+)					Q04YA6	1	5.7E-28			
		3.D.1.8.1	Q95695	5	Cation (H^+)								B0SFU1	5	3.1E-11
		3.D.1.9.3	Q8F7D6	2	Cation (H^+)		Q8F7D6	2	0	Q055A3	1	0			
		3.D.1.9.3	Q8EYE0	12	Cation (H^+)		Q8EYE0	12	0	Q056K7	12	0	B0SCU8	12	6.4E-92
		3.D.1.9.3	Q8EYE1	6	Cation (H^+)		Q8EYE1	6	5.0E-112	Q056K6	6	4.8E-100	B0SCU9	7	1.0E-35
		3.D.1.9.3	Q8EYE2	8	Cation (H^+)		Q8EYE2	8	2.0E-169	Q056K5	8	1.8E-158	B0SCV0	8	4.4E-63
		3.D.1.9.3	Q8EYE3	14	Cation (H^+)		Q8EYE3	14	0	Q056K4	16	0	B0SCV1	16	3.9E-82
3.D.2	Proton-translocating Transhydrogenase (PTH) Family														
		3.D.2.1.1	P07001	5	Cation (H^+)					Q04X89	3	8.5E-90			
		3.D.2.2.1	P0C186	1	Cation (H^+)		Q8F9Y5	1	4.4E-66				B0SH36	1	6.7E-83
		3.D.2.2.1	P0C187	3	Cation (H^+)		Q8F963	3	1.2E-19	Q055N0	3	1.2E-19	B0S9T4	3	2.6E-21
		3.D.2.2.1	P0C188	10	Cation (H^+)		Q8F962	10	3.8E-115	Q055N1	9	1.3E-115	B0S9T5	10	2.3E-122
3.D.3	Proton-translocating Quinol:Cytochrome c Reductase (QCR) Family														
		3.D.3.5.2	O23166	1	Cation (H^+)		Q8EXX9	1	9.5E-12	Q04Y80	1	1.9E-09	B0SDY6	1	1.4E-06
3.D.4	Proton-translocating Cytochrome Oxidase (COX) Family														
		3.D.4.3.3	H7F0T0	12	Cation (H^+)					Q050U7	12	5.1E-137	B0SI54	12	1.3E-140
		3.D.4.3.3	F8H841	1	Cation (H^+)					Q050U6	1	6.6E-52	B0SI53	1	3.9E-53
		3.D.4.3.3	D9IA45	2	Cation (H^+)					Q050U4	1	8.7E-18	B0SI51	1	1.3E-14
		3.D.4.4.1	P24012	5	Cation (H^+)								B0SE53	5	4.3E-08
		3.D.4.4.1	P12946	8	Cation (H^+)		Q8F9F7	8	2.1E-10	Q055W0	8	2.3E-11	B0SE57	8	5.7E-15
		3.D.4.4.3	P98005	19	Cation (H^+)		Q8F9F2	12	2.4E-117	Q055V6	12	2.7E-117	B0SE54	12	4.7E-117
		3.D.4.4.3	Q5SLI2	2	Cation (H^+)		Q8F9F3	2	2.7E-35	Q055V7	2	3.9E-33	B0SE55	2	4.8E-39
		3.D.4.5.1	P0ABJ3	5	Cation (H^+)		Q8F9F1	5	3.3E-14	Q055V5	5	8.2E-14			
		3.D.4.10.2	P98008	12	Cation (H^+)								B0SFG1	12	3.6E-104
		3.D.4.10.2	Q52527	1	Cation (H^+)								B0SFG2	1	9.7E-28
		3.D.4.11.1	P00414	6	Cation (H^+)								B0SFG3	5	4.7E-11

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
3.D.10	Prokaryotic Succinate Dehydrogenase (SDH) Family														
		3.D.10.1.1	Q65GF4	1	Cation (H^+)					Q04ZY8	1	1.1E-49	B0SBH3	1	2.0E-46
		3.D.10.1.2	Q8F4Z1	1	Cation (H^+)		Q8F4Z1	1	0	Q050R7	1	0	B0S8S2	1	0
													B0SGI9	1	0
		3.D.10.1.2	Q8F4Z0	5	Cation (H^+)		Q8F4Z0	5	0	Q050R8	5	8.3E-123	B0S8S1	5	1.1E-67
4.B Nicotinamide Ribonucleoside Uptake Transporters															
4.B.1	Nicotinamide Ribonucleoside (NR) Uptake Permease (PnuC) Family														
		4.B.1.1.1	P24520	7	Cofactors (Nicatinamide)								B0SIM2	7	3.0E-22
4.C Acyl CoA Ligase-Coupled Transporters															
4.C.1	Proposed Fatty Acid Group Translocation (FAT) Family														
		4.C.1.1.6	P31552	1	Organoanions (Free Fatty Acids)					Q056J9	1	6.3E-26	B0S8X7	1	6.7E-28
		4.C.1.1.7	Q42524	1	Organoanions (Free Fatty Acids)		Q8F6X6	2	1.7E-10						
4.D Polysaccharide Synthase/Exporters															
4.D.1	Putative Vectorial Glycosyl Polymerization (VGP) Family														
		4.D.1.1.2	Q54066	4	Polysaccharides		Q8F5R4	3	1.9E-05	Q053Y2	2	1.6E-10	B0SAH8	2	1.2E-08
4.D.2	COG0392; UPF0104 Putative Transporter (COG0392) Family														
		4.D.2.1.6	F2KQZ0	8	Polysaccharides		Q8F4R1	6	2.5E-13	Q050N1	5	2.1E-14	B0SAI1	4	3.7E-17
										Q04ZP9	5	1.1E-12			
4.D.3	Glycan Glucosyl Transferase (OpgH) Family														
		4.D.3.1.2	I7KBV6	7	Polysaccharides		Q8F8D0	5	1.2E-18	Q054B7	6	4.6E-19			
		4.D.3.1.3	E4PZP4	7	Polysaccharides								B0SEV0	6	2.0E-19
5.A Transmembrane 2-Electron Transfer Carriers															
5.A.1	Disulfide Bond Oxidoreductase D (DsbD) Family														
		5.A.1.1.1	P36655	9	Electrons, $2e^-$		Q8F6I5	8	1.5E-33	Q053B4	9	9.6E-29	B0SH98	8	2.8E-25
		5.A.1.6.2	M6C6L6	6	Electrons, $2e^-$					Q050T9	6	7.6E-31	B0SI46	6	3.7E-90
5.A.3	Prokaryotic Molybdopterin-containing Oxidoreductase (PMO) Family														
		5.A.3.3.3	Q9HR72	10	Electrons, $2e^-$		Q8F171	10	9.6E-11	Q04Z22	10	2.1E-10	B0SGB3	10	7.1E-10

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
8.A Auxiliary Transport Proteins															
8.A.1	Membrane Fusion Protein (MFP) Family														
		8.A.1.3.2	P11091	0	Nontransport Auxillary								B0SAW6	1	3.0E-16
8.A.21	Stomatin/Podocin/Band 7/SPFH (Stomatin) Family														
		8.A.21.2.1	O59180	3	Nontransport Auxillary		Q8F4G9	3	9.7E-32	Q052A4	3	2.6E-31	B0SHK1	2	3.1E-29
													B0SHK0	1	8.8E-26
9.A Recognized Transporters of Unknown Biochemical Mechanism															
9.A.8	Ferrous Iron Uptake (FeoB) Family														
		9.A.8.1.4	Q5XPH7	9	Cations (Fe^{2+})		Q8F332	10	0	Q052W0	10	0	B0SDS9	9	0
9.A.25	Por Protein Secretin System (PorSS) Family														
		9.A.25.1.1	Q5EGM5	1	Proteins		Q8F1L4	1	1.2E-11	Q04XL1	1	1.9E-06	B0S970	1	1.7E-07
9.A.40	HlyC/CorC (HCC) Family														
		9.A.40.2.2	Q0PBV6	4	Nonselective		Q8EZB8	4	1.1E-78	Q04Y13	4	7.8E-77	B0SCR4	4	1.9E-70
9.B Putative Transport Proteins															
9.B.8	DUF2157 (DUF2157) Family														
		9.B.8.2.1	Q8F4I9	12	Unknown		Q8F4I9	12	0	Q052C2	12	9.8E-153			
		9.B.8.7.1	B2U729	12	Unknown								B0SFF8	11	4.3E-18
9.B.14	Putative Heme Handling Protein (HHP) Family														
		9.B.14.1.2	P45403	15	Putative transporter		Q8F0Z0	15	6.6E-69				B0SCS1	15	4.7E-64
		9.B.14.1.3	P33927	15	Putative transporter					Q04YF7	15	3.0E-67			
		9.B.14.1.10	Q8F8J8	19	Putative transporter		Q8F8J8	19	0						
		9.B.14.2.5	I6ZP97	6	Putative transporter		Q8F0H8	6	4.3E-12	Q04YS7	6	5.3E-12	B0SA08	6	2.2E-12
9.B.18	SecDF-associated Single Transmembrane Protein, YajC (YajC) Family														
		9.B.18.1.1	P0ADZ7	1	Putative transporter		Q8F707	1	6.4E-13	Q04ZD3	1	1.6E-10	B0SGE1	1	6.4E-06
9.B.27	DedA or YdjX-Z (DedA) Family														
		9.B.27.2.3	P0ABP6	6	Putative transporter		Q8F8I2	6	7.9E-59	Q04YH4	6	7.7E-59	B0SGX3	4	1.1E-08
							Q8F3X4	4	1.8E-09	Q051H1	4	5.0E-09			
		9.B.27.2.5	D6GX19	5	Putative transporter		Q8F3P8	5	1.5E-14	Q051N0	4	1.2E-12	B0S9T9	4	3.2E-15
		9.B.27.3.3	B1J6T5	4	Putative transporter					Q04WJ5	4	1.7E-13			

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
9.B.29	Small 5 TMS Putative Permease (5PP) Family														
		9.B.29.1.1	Q8EYR1	5	Putative transporter		Q8EYR1	5	2.0E-114	Q056F8	5	6.2E-85			
		9.B.29.1.2	BOSJ50	4	Putative transporter								B0SBK6	4	1.7E-115
9.B.30	Hly III (Hly III) Family														
		9.B.30.1.1	P54176	7	Nonselective								B0SD02	6	5.5E-49
9.B.31	YqiH (YqiH) Family														
		9.B.31.1.2	Q45064	5	Unknown		P59247	5	7.0E-24	Q04ZS2	5	3.3E-21	B0SGF5	5	1.2E-24
9.B.34	Kinase/Phosphatase/Cyclic-GMP Synthase/Cyclic di-GMP Hydrolase (KPSH) Family														
		9.B.34.1.2	P0AAP1	5	Unknown		Q8F2R4	5	9.9E-21	Q053C7	5	2.5E-21	B0S9C7	6	4.9E-21
										Q04WY9	3	1.1E-24			
9.B.44	YiaA-YiaB (YiaAB) Family														
		9.B.44.1.1	P0ADJ8	4	Putative transporter								B0S9N1	4	2.0E-34
9.B.67	Putative Inorganic Carbon (HCO3-) Transporter/O-antigen Polymerase (ICT/OAP) Family														
		9.B.67.4.2	B0SEF1	9	Putative anion (Bicarbonate)		Q8F474	12	1.7E-85	Q051R9	12	1.2E-88	B0SEF1	9	0
							Q8F4F2	15	4.5E-37	Q051Z0	12	4.8E-36			
9.B.97	Acyltransferase-3/Putative Acetyl-CoA Transporter (ATAT) Family														
		9.B.97.1.6	Q8EY27	11	Unknown					Q04X27	11	4.9E-154			
		9.B.97.1.8	B0SI19	11	Unknown								B0SI19	11	0
		9.B.97.5.1	B9DIS8	11	Unknown		Q8F6C6	10	5.0E-27				B0SAM6	11	1.7E-35
							Q8F968	10	4.0E-16				B0SBG8	10	3.6E-18
													B0SAH4	12	2.4E-13
9.B.104	Rhomboid Protease (Rhomboid) Family														
		9.B.104.1.2	Q46G03	6	Putative Proteins Cleavage		Q8F9S0	7	5.5E-14	Q056S4	7	1.1E-12	B0SH91	7	3.6E-12
										Q054D3	6	2.2E-12			
		9.B.104.1.3	F0Z2G1	6	Putative Proteins Cleavage		Q8F8A8	6	7.0E-10						
		9.B.104.1.5	Q8F2A9	5	Putative Proteins Cleavage		Q8F2A9	5	0	Q053E1	5	9.8E-154	B0SDS2	5	1.8E-56
							Q8F6T2	7	4.5E-18	Q04ZF7	6	1.2E-15	B0SEG0	6	8.1E-20

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
9.B.105	Lead Resistance Fusion Protein (PbrBC) Family														
		9.B.105.1.1	Q58AJ7	10	Putative transporter		Q8F6H0	4	1.2E-05						
		9.B.105.1.6	Q8F9Y4	3	Putative transporter		Q8F9Y4	3	2.0E-98	Q04X88	3	3.5E-85	B0SH38	3	1.5E-40
		9.B.105.2.1	Q8F224	9	Putative transporter		Q8F224	9	0	Q053H2	9	5.5E-119	B0SGP8	9	1.7E-36
9.B.107	8 TMS Putative Permease (8-PP) Family														
		9.B.107.1.2	B0RDT1	8	Putative transporter								B0SG05	8	1.3E-23
9.B.114	Vancomycin-sensitivity protein (SanA) Family														
		9.B.114.1.1	P0AFY2	2	Unknown		Q8EYT9	1	5.3E-22	Q04XG5	1	2.3E-20	B0SA77	1	1.1E-18
9.B.115	Putative Integral Membrane Steroid 5alpha-reductase (SalphaR) Family														
		9.B.115.1.1	M2X3K0	7	Putative transporter								B0SIJ1	7	3.2E-15
		9.B.115.1.6	D7VS98	5	Putative transporter		Q8F3E0	6	9.0E-49	Q050V4	6	2.6E-48	B0SE74	6	9.9E-45
9.B.122	DUF3592 or PF12158 (DUF3592) Family														
		9.B.122.1.3	A5FB62	2	Unknown								B0SET0	2	0.0002
9.B.125	AmpE/CobD (AmpE/CobD) Family														
		9.B.125.2.1	E3GWE8	6	Unknown		Q8EXQ8	5	1.6E-44	Q04WM3	5	1.2E-43			
9.B.142	Integral membrane Glycosyltransferase family 39 (GT39) Family														
		9.B.142.2.3	G0LCP3	13	Putative transporter		Q8F7U9	11	7.6E-24	Q04Z16	11	5.7E-25	B0SGB0	13	3.7E-30
		9.B.142.2.6	H8KSM2	13	Putative transporter								B0SED3	10	1.2E-13
		9.B.142.3.10	P73520	11	Putative transporter								B0SAX1	11	2.1E-08
		9.B.142.4.3	Q8F4R2	11	Putative transporter		Q8F4R2	11	0	Q050N2	11	0	B0SAH9	11	1.8E-37
		9.B.142.8.1	H2CB90	13	Putative transporter								B0SBV4	9	2.2E-59
		9.B.142.11.4	G2LJT4	11	Putative transporter					Q050P2	10	9.7E-23			
										Q04Y78	9	5.7E-17			
		9.B.142.11.5	R4TKC5	13	Putative transporter		Q8F4Q7	9	2.1E-22				B0SCA0	9	2.5E-22
9.B.145	DUF389/PF04087 (DUF389) Family														
		9.B.145.1.2	A6DJZ4	6	Unknown		Q8F1W6	7	1.3E-23	Q054K9	7	9.0E-21	B0SE29	7	7.6E-24
9.B.146	Putative Undecaprenyl-phosphate N-Acetylglucosaminyl Transferase (MurG) Family														
		9.B.146.1.2	G2HLY8	11	Putative transporter		Q8F131	11	1.8E-16	Q053P4	11	3.3E-16	B0SCS9	11	9.2E-22
		9.B.146.1.3	Q8F4J3	10	Putative transporter		Q8F4J3	10	0	Q04Y84	10	0	B0S986	10	5.9E-142

Table 2. (*continued*)

Transporter Classification (TC)							*L. interrogans*			*L. borgpetersenii*			*L. biflexa*		
Family TC#	Family Name	Hit TCID	Hit Uniprot #	Hit TMS #	Substrate(s)	Comments	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value	Uniprot #	Query TMS #	E-value
9.B.147	10 TMS Integral Membrane Protein (10-IMP) Family														
		9.B.147.1.5	Q09CH6	10	Unknown		Q8F9W1	9	7.0E-56	Q04X64	9	7.1E-59			
		9.B.147.1.7	K0VHI2	10	Unknown								B0SBC3	8	3.5E-07
9.B.149	M50 Peptidase (M50-P) Family														
		9.B.149.1.9	O58089	8	Putative transporter		Q8F9X4	7	1.1E-34	Q04X77	8	6.8E-38	B0S939	8	7.7E-32
9.B.174	Two Tunnel Gated C-terminal Processing Protease (CTP) Family														
		9.B.174.1.1	O35002	1	Putative auxiliary protein		Q8CVF1	1	2.2E-59	Q053A3	1	2.3E-59	B0SG62	1	4.7E-54

1 Substrate class with specific substrate in parenthesis if known.
2 Represents Expect value (E) as x.xE-xx. Values represented are taken from GBlast results.

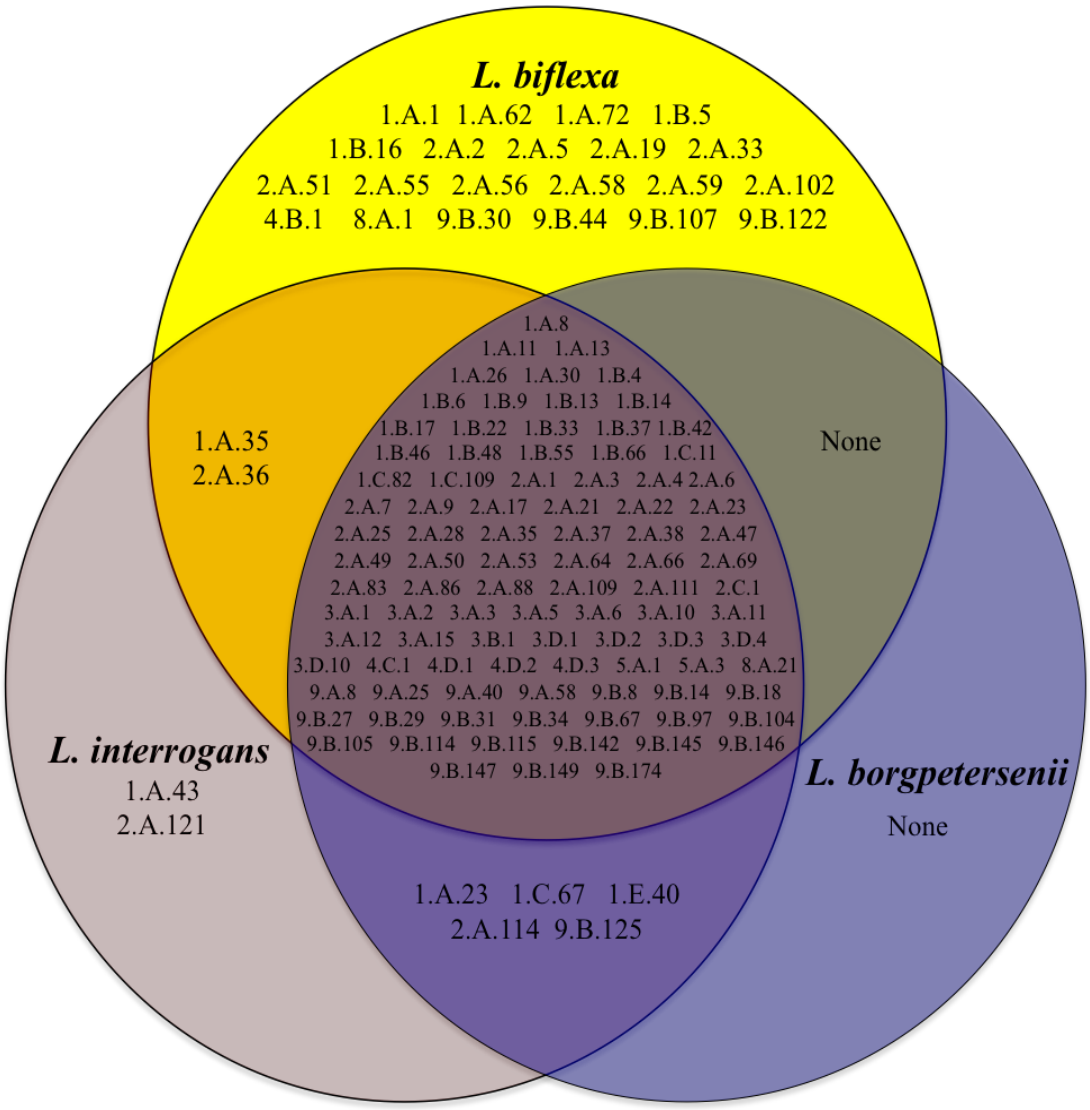

Figure 1. Representation of families unique to *L. interrogans*, *L. borgpetersenii*, *L. biflexa*, both *L. biflexa* and *L. interrogans*, both *L. biflexa* and *L. borgpetersenii*, and both *L. interrogans* and *L. borgpetersenii*. Families found in all three species are listed in central area.

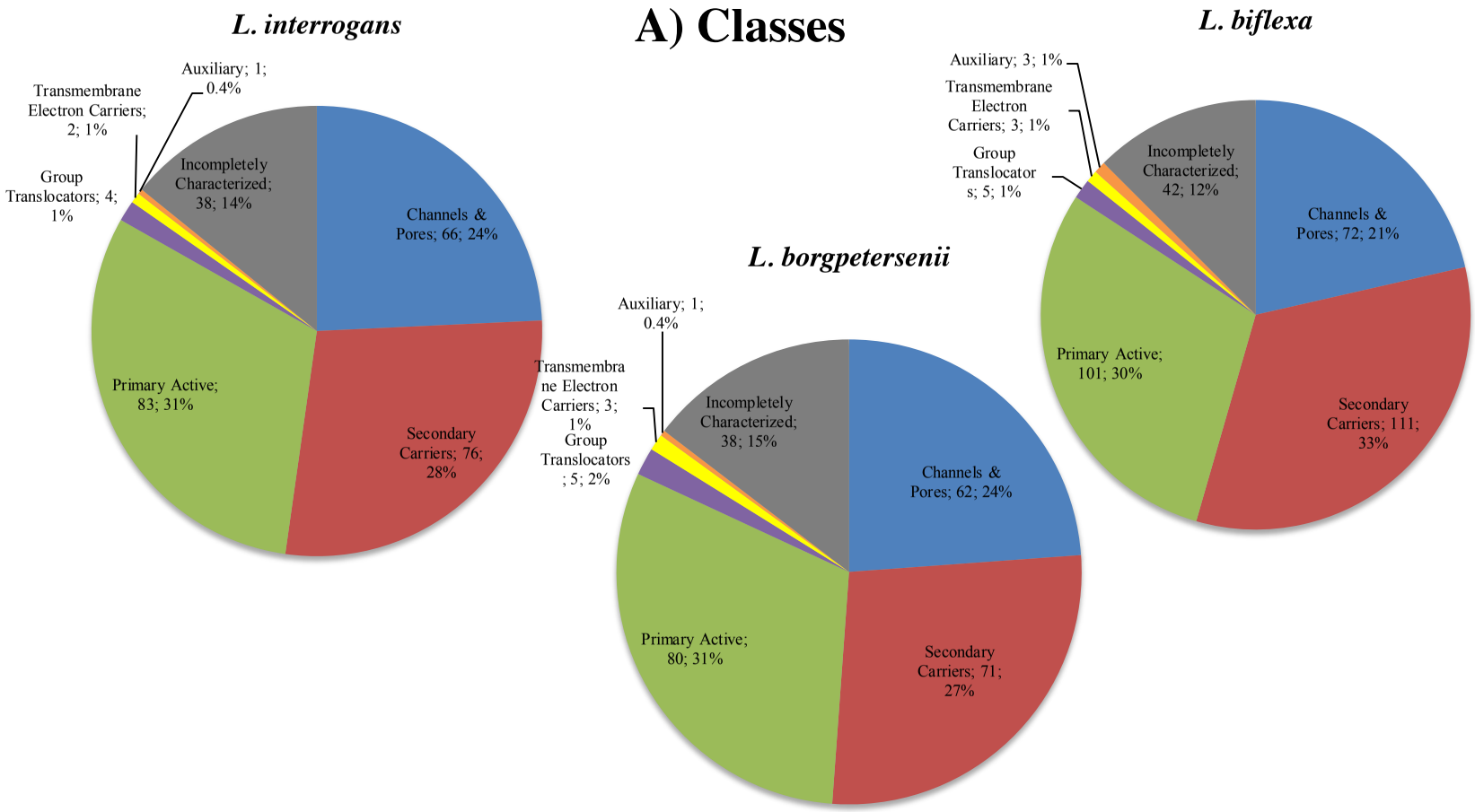

Figure 2. Distribution of transporters based on TC (A) classes and (B) subclasses in *L. interrogans*, *L. borgpetersenii*, and *L. biflexa*.

B) Subclasses

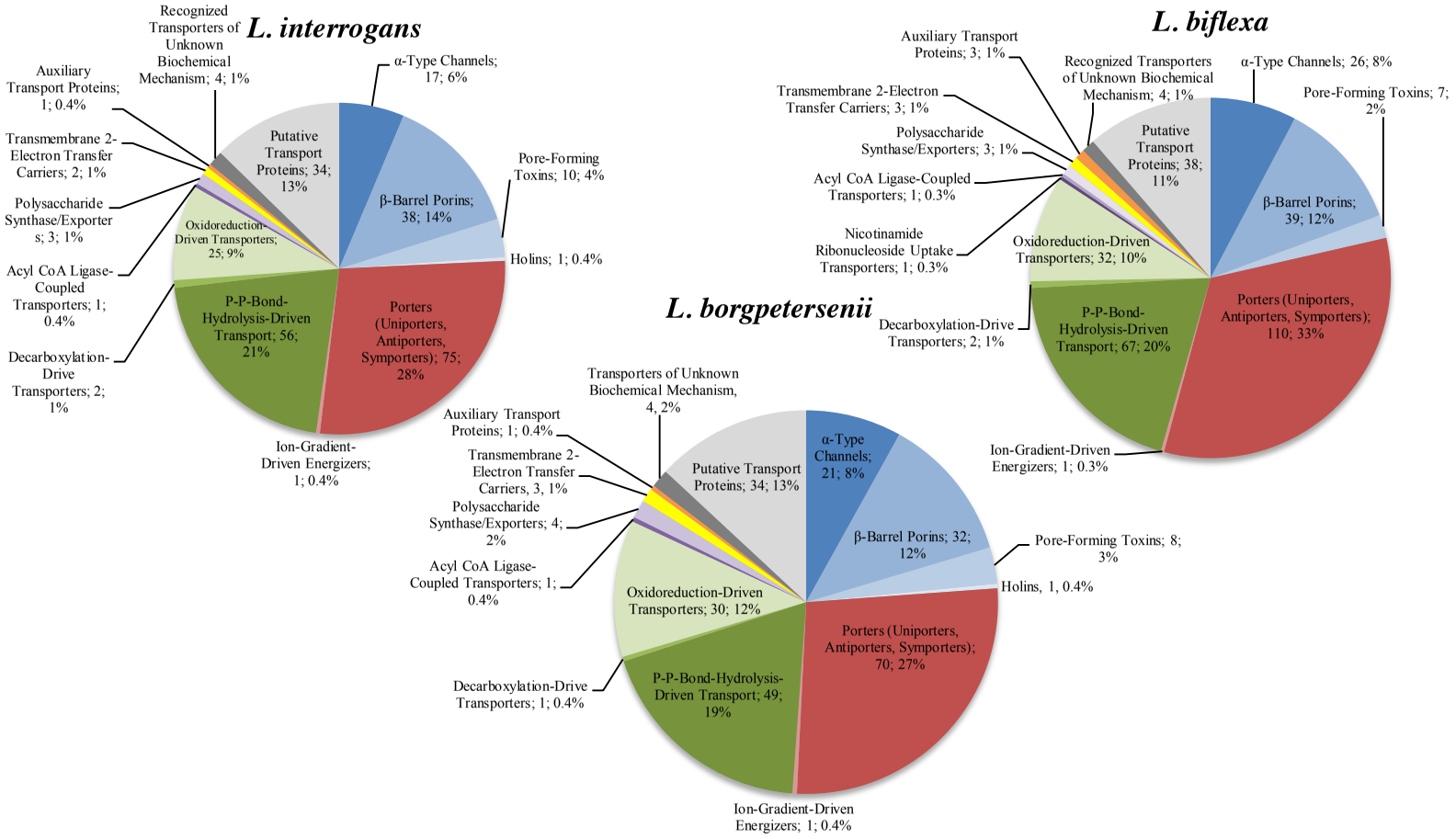

Figure 2. (*continued)* Distribution of transporters based on TC (A) classes and (B) subclasses in *L. interrogans*, *L. borgpetersenii*, and *L. biflexa*.

A) Category

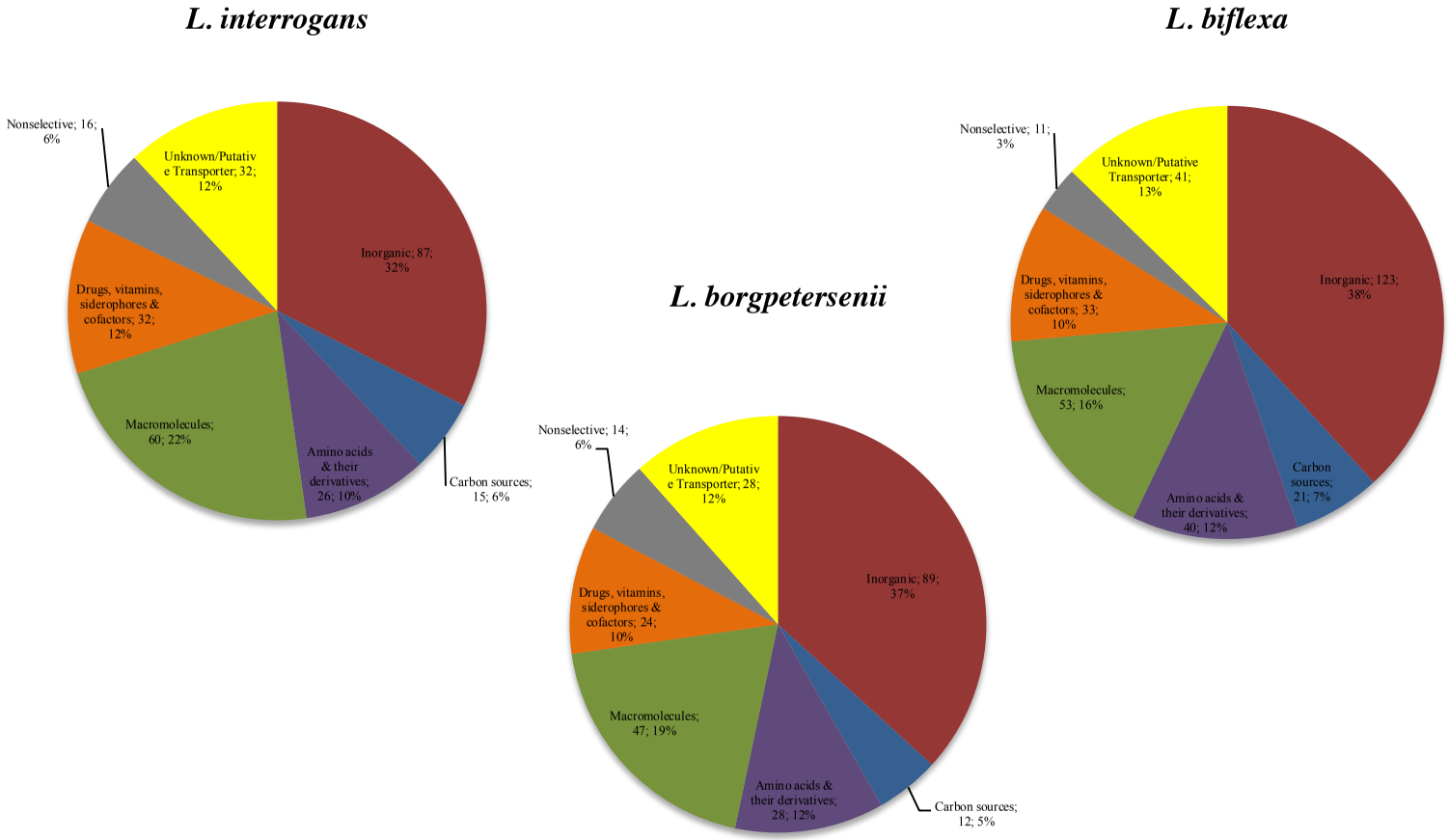

Figure 3. Distribution of transporters based on substrate (A) category and (B) subcategory in *L. interrogans*, *L. borgpetersenii*, and *L. biflexa*.

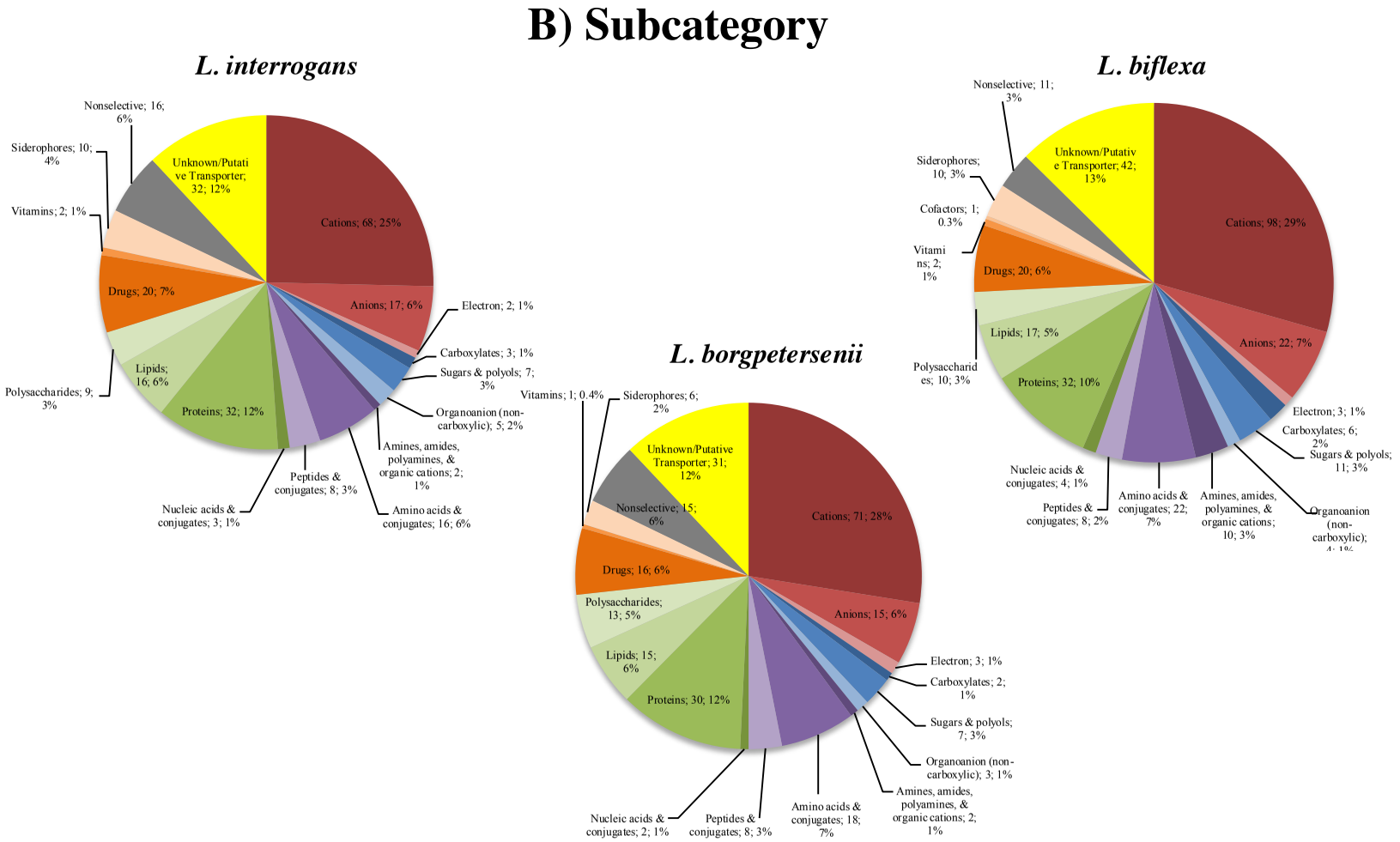

Figure 3. (*continued)* Distribution of transporters based on substrate (A) category and (B) subcategory in *L. interrogans*, *L. borgpetersenii*, and *L. biflexa*.