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Comparative Genomics of the Transportome of Ten *Treponema* species

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Abstract

Treponema is a diverse bacterial genus, the species of which can be pathogenic, symbiotic, or free living. These treponemes can cause various diseases in humans and other animals, such as periodontal disease, bovine digital dermatitis and animal skin lesions. However, the most important and well-studied disease of treponemes that affects humans is 'syphilis'. This disease is caused by *Treponema pallidum* subspecie *pallidum* with 11-12 million new cases around the globe on an annual basis. In this study we analyze the transportome of ten *Treponema* species, with emphasis on the types of encoded transport proteins and their substrates. Of the ten species examined, two (*T. primitia* and *T. azonutricium*) reside as symbionts in the guts of termites; six (*T. pallidum, T. paraluiscuniculi, T. pedis, T. denticola, T. putidum* and *T. brennaborense*) are pathogens of either humans or animals, and *T. caldarium* and *T. succinifaciens* are avirulent species, the former being thermophilic. All ten species have a repertoire of transport proteins that assists them in residing in their respective ecological niches. For instance, oral pathogens use transport proteins that take up nutrients uniquely present in their ecosystem; they also encode multiple multidrug/macromolecule exporters that protect against antimicrobials and aid in biofilm formation. Proteins of termite gut symbionts convert cellulose into other sugars that can be metabolized by the host. As often observed for pathogens and symbionts, several of these treponemes have reduced genome sizes, and their small genomes correlate with their dependencies on the host. Overall, the transportomes of *T. pallidum* and other pathogens have a conglomerate of parasitic lifestyle-assisting proteins. For example, a *T. pallidum* repeat protein (TprK) mediates immune evasion; outer membrane proteins (OMPs) allow nutrient uptake and end product export, and several ABC transporters catalyze sugar uptake, considered pivotal to parasitic lifestyles. Taken together, the results of this study yield new information that may help open new avenues of treponeme research.

Keywords

Treponema; diverse lifestyles; pathogens; symbionts; free living; transport proteins

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1. Introduction

Treponema is a diverse genus of the divergent bacterial phylum Spirochaetes, the members of which are distantly related to other bacteria, both Gram-negative and Gram-positive types [1]. It is believed that its members have undergone extensive horizontal gene transfer not only with other bacteria, but also with archaea and possibly eukaryotes [2, 3]. Species of *Treponema* include human and animal pathogens, arthropod intestinal commensals, extremophiles and saprophytes [4]. Initially, treponemes were considered to be obligate anaerobes, but some are now known to be microaerophiles [5]. Species of *Treponema* include causative agents of a variety of diseases such as venereal and endemic syphilis, yaws, pinta, periodontal disease, and digital dermatitis [6, 7].

Venereal syphilis, caused by *Treponema pallidum* subsp. *pallidum* (Tpal), occurs with over 10 million new cases reported globally every year [8]. Its incidence is also on the rise in the United States. For details see Peeling et al and Trivedi et al [9, 10]. Syphilis can clinically manifest with genital sores, genital warts and skin rashes, sometimes associated with bone, cardiovascular and neurological damage. It can also result in miscarriage, stillbirths and/or congenital syphilis, and it triples the risk of HIV transmission [11, 12].

Other pathogens of *Treponema*, such as *T. denticola* (Tde), *T. putidum* (Tpu) *T. pedis* (Tpe), *T. brennaborense* (Tbr), and *T. paraluiscuniculi* (Tpar), utilize varying mechanisms of infection [4]. Orally residing Tde and Tpu cause periodontal diseases in humans, giving rise to polymicrobial infections in adults with the potential to evolve into severe forms [13, 14]. The two animal pathogens, Tpe and Tbr, infect various species of animals, typically targeting skin, promoting dermatitis, rash, and ulcers with the probability of evolving into deep necrotic lesions that can result in bone deformities [15, 16]. Another animal pathogen is Tpar, which is a sexually transmitted pathogen like Tpal; however, this treponeme causes rabbit venereal syphilis and apparently does not infect humans. These pathogens often exhibit discernible stages of disease progression [17]. Non-oral pathogens of *Treponema* give rise to treponematoses; etiological agents include other subspecies of *T. pallidum* and *T. carateum* [18], which were not analyzed in this study due to high genomic identity with an included species or lack of a complete published genome sequence.

Non-pathogenic treponemes are represented by *T. primitia* (Tpr), *T. azotonutricium* (Taz), *T. succinifaciens* (Tsu), and *T. caldarium* (Tca). The former two occur as symbionts in termite guts where the metabolomes and secretomes of the two types of organisms, the host and the bacteria, complement each other, providing and consuming essential metabolites for all [19]. Tsu is an avirulent spirochete isolated from pig colon, named for its production of succinic acid [20]. Finally, the only thermophile in this study, Tca, was isolated from cyanobacterial mat samples, collected at a freshwater hot spring [21].

In this communication we use and capitalize the first letter from the genus (T) followed by the first two letters from the species designations in lower-case as abbreviations to refer to these species as noted above, with two exceptions to clarify identical abbreviations (Tpal and Tpar). Table 1 provides the species names, abbreviations and basic information about the ten species of *Treponema* examined in this study.

Treponema species possess many morphological similarities to other well-known spirochetes such as species of *Leptospira* and *Borrelia* [22]. These Gram-negative bacteria are unique in the locations of their flagella, present in the periplasmic space, running the length of the bacterium, and playing a pivotal role in their corkscrew motility [23]. However, Tpal does not have the characteristic corkscrew motility that the other treponemes possess.

Treponema have diacylglycerol-containing glycolipids that resemble lipoteichoic acids of Gram-positive bacteria, which play a role in the interaction with animal host receptors to generate the symptoms associated with the aforementioned diseases [24]. Treponemes lack lipopolysaccharide (LPS), and instead, lipoproteins induce immune responses in the host. The syphilis causing Tpal has a flat-wave shape [25], while other treponemes have helical shapes [26]. Their shapes and embedded flagella enable the treponemes to penetrate tissues and vascular barriers, thus assisting in the dissemination of the microbe [27, 28].

The present research is in continuation of our work on pathogenic spirochaetes. Previously, Buyuktimkin et al. (2017) analyzed the transport proteins of three *Leptospira* strains including *L. interrograns, L. borgpetersenii* and *L. biflexa* [29]. They found minor differences among the transportomes of the three spirochetes; however, despite these small differences in the individual sets of transport proteins, the proteins played significant pathological and physiological roles [29]. The goal of the present research was to analyze the transport proteins of ten *Treponema* strains, and to check for similarities and differences among the pathogenic and non-pathogenic strains. We had a particular interest in the transportome of Tpal due to 1) its small genome size, 2) its versatility to persist as a highly effective parasite in humans, and 3) its limited repertoire of recognized outer membrane proteins (OMPs) in comparison to *E. coli* [30, 31]. How does this treponeme utilize a minimal OMP repertoire to accommodate its parasitic lifestyle? Transportome analyses should help shed light on this issue and others.

2. Materials and Methods

2.1 Genome-BLAST (G-BLAST) searches of the *Treponema* proteomes

The FASTA formatted protein-coding sequences of *T. denticola* ATCC 35405 [32], *T. pallidum* str. Sea 81-4 [33], *T. pedis* str. TA 4 [34], *T. primitia* ZAS-2 [35], T *azotonutricium* ZAS-*9* [35], *T. paraluiscuniculi* Cuniculi A [36], *T. succinifaciens* DSM 2489 [37], *T. brennaborense* DSM 12168 (unpublished), *T. caldarium* DSM *7334* (unpublished), *T. putidum* OMZ *758* [38], were used as the starting sequence data to be inputted into the G-Blast program. Genomes were selected on the basis of the draft qualities and completenesses of their sequenced genomes, as well the pathogenic potential of these strains in either humans or animals. Non-pathogens, symbionts and a thermophile were included in the analyses. The ten proteomes were screened for homologs of all proteins contained in the Transporter Classification Database (www.tcdb.org in September 2018 using the program G-BLAST [39]. This program is designed to retrieve information for both the genome query and TC top hit sequences, TC numbers, numbers of amino acyl residues (aas), numbers of predicted TMSs using the HMMTop 2.0 program, both query and hit protein e-values, regions with sequence similarity, and regions of TMS overlap between the query and the hit proteins. For prediction of the numbers of TMSs, G-BLAST uses the Web-based

Hydropathy, Amphipathicity, and Topology (WHAT) program, which aligns the plots of hydrophobicity and amphipathicity throughout the length of the protein [40,41]. Proteins lacking TMSs were not omitted, since multicomponent systems often possess soluble components that can be transport protein constituents.

2.2 Examination of distant transport protein homologues of *Treponema*

For G-BLAST searches, we initially used an arbitrary e-value cut off of 0.0001. Manual examination of the remaining proteins (having e-values of >0.0001) was done using topological data to determine if the proteins were either true homologues or false positives. As two proteins displaying homology in hydrophilic regions can give small e-values, it was necessary to manually examine the regions of overlap to prevent the selection of proteins that had good scores, but were not actually homologous in their transmembrane domains. The hydropathy profiles generated by the WHAT program were used to estimate whether the program had missed a TMS or predicted a TMS in an incorrect region. By using the AveHAS program, confirmation of predicted proteins with several homologues was accomplished [41]. Proteins that had moderate e-values, between 0.0001 and e^{-8}, were potential but not certain homologs, and hence, they were examined more closely using the aforementioned steps.

2.3 Identification of substrates transported

Authentic transport protein homologues were assigned substrates according to TCDB hit entries. For TC entries of unknown function, the genome context of the encoding genes was considered, especially if the encoding genes were within multicistronic operons. The scientific literature was also used to deduce functions.

2.4 Occurrence of multicomponent systems

Our analysis identified various multicomponent transport systems in the ten *Treponema* genomes. This identification was primarily based on the presence of the transmembrane (TM) protein of the systems; however, in some instances, other constituents were found. If the TM protein(s) was/were identified, the transport system was considered to be present.

3. Results

3. Transport Protein Subclasses

The Transporter Classification (TC) system includes an extensive list of transport proteins, many of which are characterized both structurally and functionally. In TCDB, transporters are organized into five well-defined classes (1–5) and two less well-defined classes (8–9). The five well-defined classes are (1) channels (2) secondary carriers (3) primary active transporters, (4) group translocators and (5) transmembrane electron flow carriers. The latter two classes include (8) auxiliary transport proteins and (9) transporters or putative transporters of unknown function or mechanism of transport.

Ten *Treponema* genomes were analyzed for the occurrence of transporters using G-BLAST [39] and TCDB as noted in the Methods section. The complete results are described in the

Supporting information section (S1 Table), whereas Table 2 gives an overview of the subclass distributions of the transporters from each organism.

3.1. Channel Proteins

TC subclass 1.A in TCDB (www.tcdb.org) includes α-type channels except for holins, which are included in subclass 1.E. These transporters range in number from 6 to 16 in the treponemes analyzed, with Tpal having the smallest number of such channel proteins. All *Treponema* species examined possess at least one homolog of the Mot family (TC#1.A.30.1), which is responsible for energizing the flagellar motor complex [42, 43]. In addition, two of the non-pathogenic treponemes (Taz and Tpr) possess at least one homolog of the TolQR system (TC#1.A.30.2.9), responsible for energizing the assembly of the outer membrane, and resulting in increased stability of this structure [44].

A family of α-type channels found in eight of the ten treponemes analyzed is the Mg^{2+} Transporter-E (MgtE) Family (TC#1.A.26); only Tbr and Tsu lack such transporters. These proteins are responsible for the uptake of Mg^{2+} and sometimes other inorganic divalent cations [45]. Of note, only non-pathogenic treponemes encode a member of the Camphor Resistance family (TC#1.A.43); these proteins are responsible for abating toxic fluoride anion accumulation [46].

Thirteen families of α-type channels are represented in the pan-transportome examined. However, homologues of only two families are present in all ten strains. One of these families is the H^+- or Na^+-translocating Bacterial Flagellar Motor (Mot) Family (TC#1.A.30.1), while the other is the Cation Channel-forming Heat Shock Protein (Hsp70) Family (TC#1.A.33). The homologues of the Hsp70 family may have numerous functions in the treponemes. Primarily, these proteins assist in the folding and assembly of newly synthesized proteins [47]. However, their channel-forming functions have been demonstrated only in eukaryotes [48], and assignment of this function to a prokaryotic Hsp70 protein is premature.

The syphilis-causing treponemes lack transporters belonging to the Epithelial Chloride Channel (E-ClC) family (TC#1.A.13) while almost all other treponemes possess at least two homologues of these proteins. Interestingly, these channels are characterized only in animals, whereas bacterial homologs are functionally indeterminate. These proteins may prove to exhibit chloride channel activities comparable to those found in eukaryotes.

3.2. β-type Porins

TC subclass 1.B includes outer membrane porins, mostly β-types. Although a few of them, especially outer membrane porins of Actinobacteria, have transmembrane α-helical structures, they are nevertheless included in subclass 1.B. This is also true of a few spirochete outer membrane porins that may also contain α-structures. Porins are of particular interest as they are potential cell surface antigens that can be used for vaccine production and serve as potential drug targets [49, 50]. A range of 15 to 27 of these porins was found in the *Treponema* ssp. Both Taz and Tpr possess the most, with 27, correlating with higher amounts of β-type porins in non-pathogens. While this correlation with porins and symbionts is highly provocative, the distribution of these β-type porins is diverse, and

assignment of any major role in the lifestyle of the organisms is currently speculative. Since these proteins localize to the outer membrane, usually via β-strands instead of α-helices, those containing zero or only one predicted α-helical TMSs were included in our study.

The only four families of β-type porins in which homologs are represented in all treponemes examined are the OmpA-OmpF Porin (OOP) family, the Outer Membrane Protein Insertion Porin (Bam Complex (OmpIP) family, the Outer Membrane Lipopolysaccharide Export Porin (LPS-EP) family, and the *Treponema* Porin (T-Por) family (TC#1.B.6, 1.B.33, 1.B.42, and B.45, respectively). The members of these families in well characterized bacteria serve functions of non-specific pores, outer membrane porin insertion, and LPS export [51, 52]. It should be noted that subclass 1.B. includes outer membrane pore-forming proteins, regardless of what they transport.

Further interesting facets of (β-type) porin-types include the high number in Tpal (23) representing 22.1% of the overall recognized transportome (see Table 2). Homologues of the *Treponema* Porin Major Surface Protein (TP-MSP) family (TC#1.B.38) are present in the pathogens Tpal, Tpar, Tpe, and Tde, and also in the non-pathogens Tbr and Tpu. However, the numbers of these proteins is much higher in Tpal, Tpar and Tpe as compared to the other strains. A transport protein of interest is the *Treponema* repeat protein K (TrpK) (TC#1.B.38.1.2) that is present in only three of the pathogenic treponemes (Tpal, Tpar and Tpe). TrpK of T. *pallidum* has been hypothesized to play an important role in immune evasion, thus enabling the pathogen to persist in the host [53]. In our analysis we also found homologues of this protein in Tpar and Tpe; it is likely that the role of the proteins in these two strains is similar to that in Tpal, thus allowing these treponemes to survive in the host.

Other porin families may be present, but they were ultimately excluded from our analyses, due to homology to proteins with repetitious elements of undetermined function. These families were the Corynebacterial Porin A (PorA) family, the OMS28 Porin (Oms28P) family, and the Poly Acetyl Glucosamine Porin (PgaA) family (TC#1.B.34, 1.B.52 and 1.B.55, respectively).

3.3. Pore-forming toxins

TC subclass 1.C includes pore-forming toxins (PFTs). The PFTs observed in all ten *Treponema* strains range in number from 3-6 per organism. Most notably, all *Treponema* species examined possess at least one homolog of a hemolysin, Hly III, which has been shown to exhibit pore-forming activity (TC#1.C.113.1.1) [54, 55]. Various families of pore-forming toxins are represented in these spirochaetes, but homologs belonging to 1.C.39 and 1.C.109 of the Membrane Attack Complex/Perforin family and the Bacterial Hemolysin A family, respectively, are absent in the syphilis-causing treponemes (Tpal and Tpar). All ten species encode proteins of the HlyC Family of Hemolysins (1.C.126). Interestingly, the HlyC Family includes putative hemolysins, increasing the total number of proteins to consider for *Treponema* pathogenesis, despite similar representation in all ten species, and despite the lack of biochemical evidence for the presence of hemolysins in Treponemes.

The unexpected observation of hemolysins should not be overlooked, as hemolysins have been shown to strongly induce pro-inflammatory cytokines in *Leptospira* spirochetes [56]. It

should be noted that toxins with zero or one predicted transmembrane α-helix 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 [57].

3.4. Holins

TC subclass 1.E consists of holins, “hole-formers” often involved in autolysin secretion and cell death. Both symbionts (Taz and Tpr) and other strains including Tde, Tbr, and Tsu possess one member of a Holin family, TC#1.E.14 or 1.E.49. Although holins have a variety of proposed functions in prokaryotes, including roles in cell lysis and biofilm formation [58], it is unlikely that the presence of these holins directly promotes pathogenicity in these treponemes.

3.5. Secondary Carriers

The second largest number of transport proteins for all ten species is found in TC subclass 2.A, secondary carriers. The numbers of these transporters ranges from 15 to 48, with Tpal and Tbr possessing the least and most, respectively. The syphilis-causing pathogens, Tpal and Tpar, encode significantly fewer secondary carriers, being most notably deficient in transporters of the Major Facilitator Superfamily (MFS, TC#2.A.1). This relative deficiency of secondary carriers may help to distinguish between obligate pathogens and those that are capable of surviving in the external environment.

The MFS is the largest global group of secondary active transporters present in nature, with a wide range of substrates [59]. Eight of the treponemes examined have multiple MFS transporters, the two exceptions being Tpal and Tpar as mentioned previously. The other pathogenic treponemes (Tpe, Tde and Tbr) that possess these transporters encode fewer than the non-pathogenic species. This may be explained by the relatively broad range of substrates experienced by bacteria capable of life outside an animal as compared with those that live only intracellularly in a specific range of hosts. Of note, Tde and Tpu encode multiple paired matches of MFS porters, including two macrolide efflux pumps (TC#2.A.1.21.1 and TC#2.A.1.62.2).

The oral pathogens, Tde and Tpu, and the avirulent thermophile Tca encode members of the MFS Glycoside-Pentoside-Hexuronide (GPH) family (TC#2.A.2). These transporters catalyze uptake primarily of glycosides rather than free sugars, likely to be particularly important for nutrient acquisition in the oral cavity [60]. Similarly, homologues of the non-MFS Amino Acid-Polyamine-organocation (APC) Family (TC#2.A.3) are exclusive to the oral pathogens (Tde and Tpu). These transport proteins have been shown to catalyze uptake of amino acids and their derivatives [61].

Secondary carriers of the Cation Diffusion Facilitator (CDF) Family (TC#2.A.4) are present in all ten treponemes; these proteins primarily catalyze the efflux from cells, but occasionally uptake of heavy metals into vesicles and organelles. Members of the Zinc (Zn^{2+})-Iron (Fe^{2+}) Permease (ZIP) Family (TC#2.A.5) and the Metal Ion (Mn^{2+}-iron) Transporter (NRAMP) Family (TC#2.A.55) catalyze heavy metal uptake, and are found in fewer *Treponema* species.

Representing the second largest superfamily of secondary carriers found in *Treponema*, the Resistance Nodulation Division (RND) Superfamily (TC#2.A.6) primarily consists of transporters with unknown substrate specificity. Only the SecD and SecF proteins [62], present in single copy in all ten organisms, fall outside of this designation. 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) [63]. It is likely that the RND transporters of unknown substrate specificity catalyze the efflux of heavy metals or antimicrobials [64]. The Drug/Metabolite Transporter (DMT) Superfamily (TC#2.A.7) is the third largest superfamily of secondary carriers represented in these spirochetes. The range found extends from three to nine, with the fewest in Tpal, Tpar, and Tpe. Like many of those found in the RND Superfamily, 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.

The largest superfamily of secondary carriers found in these treponemes is the Multidrug/Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily (TC#2.A.66) with representatives in all ten species. Proteins in this family usually use cationic (Na^+) antiport to drive the efflux of their substrates, multiple drugs and polysaccharide precursors. Tbr possesses the most with twenty, while Tpal and Tpar have the fewest with two each. A predominance of the MOP superfamily members is unusual for most other bacteria studied [65, 29, 66].

Additional families found in all ten treponemes include the Membrane Protein Insertase (YidC/Alb3/Oxa1) Family, the Dicarboxylate/Amino Acid:Cation (Na^+ or H^+) Symporter (DAACS) Family, the Trk (K^+) Family, the TRAP-T (organic compounds) Family, the PNaS (phosphate) Family, and the MPE (murein precursor exporter) Family (TC#2.A.9, 2.A.23, 2.A.38, 2.A.56, 2.A.58, and 2.A.103, respectively). The substrates of these families are diverse and likely satisfy the general needs for these substrates by the *Treponema* species. Table S1 reveals the presence of secondary carriers belonging to many other families, and most of them are well represented in eight of the treponemes, with the noticeable exceptions of Tpal and Tpar, the venereal disease pathogens. These families will not be discussed further here.

3.6. Primary Active Transporters

TC subclass 3.A are pyrophosphate hydrolysis-driven primary active transporters, usually multi-component systems. The ATP-binding Cassette (ABC) Superfamily is the best-represented family and transports the greatest variety of substrates of any other family in the subclass. The range of these transport proteins extends from 33 to 157, constituting around half of all the transport proteins in Tde, Tpu, Tpe, Taz, and Tpr. The variety and wealth of the transport proteins found in this subclass demonstrates robust uptake and efflux capabilities [67], clearly suggesting that they play important roles in treponemes. The large role of ABC transport implicates a major role for ATP in energization and metabolism in these organisms [68] and suggests that these organisms use fermentative mechanisms preferentially over oxidative mechanisms to generate energy. Although class 3 primary active transporters represent the largest groups of transport *proteins*, the number of *systems*

is actually smaller than the total number of secondary carriers which are usually single component systems.

The six species, Tpal, Tpar, Tde, Tpu, Tpe, and Tsu, possess relatively few transport proteins of ABC sugar uptake systems (TC#3.A.1.1), with an average of 3.2 per species compared to an average of 39 in the others. Transport proteins for sugars and nucleosides (TC#3.A.1.2), however, are more balanced in their distributions (average of 8 compared to an average of 14). Proteins of ABC uptake systems primarily for amino acids (TC#3.A.1.3 and 3.A.1.4) are not as robustly represented, but are most prevalent in Taz, Tpr, and Tca. As for ABC uptake systems specific for amino acids, Tpal and Tpar have no representatives of peptide uptake systems (TC#3.A.1.5) whereas Tde, Tpu, Tpe, Taz, Tpr have at least two, each consisting of 5 proteins. Further analyses of ABC uptake systems reveal the continued deficiency of these transporters in the syphilis-causing Tpal and Tpar as well as the avirulent Tsu. The other treponemes examined possess considerable numbers of these uptake systems, which transport substrates such as inorganic anions, polyamines, siderophores, organic anions, and vitamins. ABC efflux systems, those designated with TC#3.A.1.100 - 3.A.1.199, are notably deficient in Tpal and Tpar, with both possessing only six of these proteins for drug, lipoprotein, and polysaccharide efflux. Tde, Tpu, and Tpe, meanwhile, possess 65, 53, and 60 of these transport proteins, respectively, with significant but reduced presence in the remaining treponemes. The presence of high numbers of ABC efflux systems in Tde is consistent with the results of Seshadri et al. [32], who observed a total of 47 different ABC efflux proteins in Tde.

All treponemes possess V-type ATPases (TC#3.A.2), for energy interconversion, but these homologues are notably lacking in Tde. It has been suggested that this ATPase is nonfunctional in Tde [32]. The two oral pathogens, Tde and Tpu, have homologues of the ABC bacteriocin exporter (TC#3.A.1.111.9). These secreted bacteriocins in oral pathogens may have a wide range of effects, possibly acting as competitive tools against other micro flora in the oral cavity; they may also play a role in signaling inside biofilms [32].

Subclasses 3.B and 3.D, decarboxylation-driven transporters and oxidoreduction-driven transporters, are also present as TC class 3 primary active transporters in *Treponema* species. All ten treponemes examined possess at least one Na^+-transporting carboxylic acid decarboxylase family member (TC#3.B.1), coupling decarboxylation to sodium ion extrusion [69]. Subclass 3.D, represented primarily by the Putative Ion (H^+ or Na^+)-translocating NADH:Ferredoxin Oxidoreductase Family (TC#3.D.6), is not consistently found in the treponemes, with Tpal and Tpar notably deficient in this subclass of transporters. All other treponemes examined possess multiple constituents of this family, suggesting a role of these ion-pumping electron carriers in energy generation.

3.7. Possible Group Translocators

TC subclass 4.A includes bacterial phosphoenolpyruvate:sugar phosphotransferase systems (PTS), divided into families, primarily based on phylogeny, which correlates with sugar substrate specificities. While all treponemes possess homologs of the energy-coupling and regulatory proteins of the PTS, most PTS permeases are absent in treponemes [70]. Only Taz, Tpr, and Tca encode PTS permeases and thus have the ability to transport sugars using

these systems. The presence of the PTS in termite gut symbionts indicates the potential of these organisms to convert cellulose and other plant polysaccharides into phosphorylated glucose/fructose/cellobiose that can be catabolized via glycolysis.

All treponemes examined encode at least one member of the Proposed Fatty Acid Group Translocation (FAT) family (TC#4.C.1) in TC subclass 4.C of Acyl CoA Ligase-coupled transporters. These transporters have been linked to fatty acid uptake. While their Acyl CoA Ligase activities have been confirmed [103], their direct roles in transport have been less certain [71].

Representatives in TC Subclass 4.D are probably group translocating glycosyl transferases. Members of this subclass have demonstrated exopolysaccharide synthesis activities that are believed to be coupled to polysaccharide secretion [72]. Exopolysaccharides have been associated with biofilm formation, especially in oral pathogenesis [73]. Most notably, however, oral pathogens Tde and Tpu possess no homologs of proteins in this subclass. Tde does congregate in biofilms with other oral pathogens [74], so this finding likely suggests a separate mechanism of polysaccharide secretion, possibly via a Type I (ABC-type) secretion system or a member of the MOP superfamily. As noted above, all ten species contain members of the Multidrug/Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily (TC#2.A.66), with various multidrug efflux pumps of the Multi-Antimicrobial Extrusion (MATE) Family (TC#2.A.66.1). The symbionts and free-living treponemes possess homologs of the Putative Vectorial Glycosyl Polymerization (VGP) family (TC#4.D.1), possibly mediating protection in harsh environments.

3.8. Transmembrane Electron Carriers

Subclass 5.A includes electron carriers that transfer electron pairs from one side of the membrane to the other, thereby influencing cellular energetics. Four *Treponema* species examined (Tde, Tpu, Tpe, Tsu) do not possess a constituent of this subclass, while the remainder possess only one or two homologs of the Disulfide Bond Oxidoreductase D (DsbD) family (TC#5.A.1). These proteins catalyze cysteinyl residue oxidation in periplasmic proteins, but they may also influence the magnitude of the proton motive force. Their distribution among treponemes suggests that they do not contribute to pathogenicity.

3.9. Auxiliary Transport Proteins

Subclass 8.A includes auxiliary proteins with one component in Tbr belonging to the MPA1-C family (TC#8.A.3), associated with exopolysaccharide export, and three treponemes encoding a stomatin-like protein that may help with localization and insertion of proteins into the outer membrane (TC#8.A.21).

3.10. Poorly Characterized Transporters

TC subclass 9.A in TCDB contains known transport proteins whose biochemical mechanisms of transport are unknown. All ten treponemes possess homologs of the Fanciful K^+ Uptake-B (FkuB) family (TC#9.A.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. The range of transporters in this subclass found in the treponemes examined is 9 to 27, with no noticeable correlation to the *Treponema* lifestyle. Most of the 'unknown' substrate specificities among putative transporters are due to members of this subclass. Table 2 gives an overview of the transport proteins found in the ten *Treponema* genomes based on TC subclass.

3.11 Differences in transported substrates between pathogenic and non-pathogenic strains

To gain a better understanding of the contribution of transport systems to pathogenicity, the substrate specificities of the transport systems of both pathogenic and non-pathogenic species were predicted and are shown in Table 3. Overall, there are similarities among the ten strains with regards to the substrates transported; however, significant differences are evident. Results in Table 3 show that the numbers of transport proteins with unknown functions are the lowest in the two syphilis-causing treponemes (Tpal and Tpar). Interestingly, the numbers of these transport proteins differ among pathogens versus non-pathogens (6-47 versus 20-34) with no obvious pattern. However, the non-pathogenic strains contain more protein/peptide transporters (both uptake and export) than the pathogenic strains (22-55 versus 14-36). Both syphilis-causing strains had the least (14 and 15) of such transport proteins among all ten treponemes. The frequency of protein/peptide exporters in the non-pathogenic strains is surprising as these transporters sometimes assist in the secretion of virulence factors in other bacteria [75]. In terms of siderophore transport proteins, the oral pathogen Tde and the animal pathogen Tpe have the most with 20 and 19 respectively, while the range in the non-pathogenic treponemes is 1-9. The two syphilis-causing treponemes do not appear to encode siderophore transporters. It has been established that Tpal, cannot synthesize siderophores, and it lacks a TonB ortholog to energize transport across the outer membrane [6]. However, it can extract iron from transferrin and lactoferrin, which has been demonstrated in vitro [76]. With regards to drug exporters, again, the oral pathogen Tde has the most with 64 such proteins, while Tpal and Tpar have the least with only 4 each. This observation may be useful in designing inhibitors of multi-drug-resistance (MDR) pumps in the latter organisms.

Various numbers of vitamin transport proteins (3-8) are found in all ten treponemes; of interest is the 4-component Riboflavin uptake transporter, RfuABCD (TC#3.A.1.2.28), whose components are present in all ten species. Riboflavin is a precursor of FMN and FAD coenzymes, which are mediators of oxidative metabolism and other physiological processes [77]. Thus, riboflavin is central to the metabolic fitness of an organism. Prior to this study it had been shown that only pathogenic spirochetes possess this riboflavin transporter, but as revealed by the present study, the non-pathogenic treponemes seem to possess homologues as well. Interestingly, the dental pathogen, Tpu, has the smallest number of polysaccharide transporters, while the termite gut symbiont Tpr has the most with 19. This suggests that the substrate exopolysaccharides play protective roles in a wide range of *in vitro* environments.

3.12. Transport proteins that contribute to pathogenicity of the treponemes

Both pathogenic and non-pathogenic strains encode various pore-forming toxins (PFTs) as is evident in Table 4. The oral pathogen Tpu has two channel-forming colicins (TC# 1.C.1.1.2

and 1.C.1.2.2) that target other bacteria, while Tsu (non-pathogenic) has one (TC#1.C.1.2.3); these channel-forming colicins are absent in the remaining eight strains. Of the ten strains, only Tpu has a homologue (TC# 1.C.39.4.6) of the Membrane Attack Complex Family (MACPF). Similarly, only the animal pathogen Tpe has a homologue of the cytolytic RTX-toxin (TC#1.C.11.1.5). This PFT might play a role in the pathogenesis of Tpe, as it can cause ulcers at various body sites (oral cavity, ears, shoulders) in different animal species [34]. All strains contain various hemolysins, while a *Serratia* type PFT (TC#1.C.75.1.7) is also found in both pathogens and non-pathogens. Interestingly, the syphilis-causing duo only has three PFTs each. With regards to iron transporters, both pathogenic and non-pathogenic treponemes have a few in common, but the non-pathogenic strains have slightly more iron-siderophore uptake systems than the pathogens as shown in Table 5. Three OMRs are unique to the termite gut symbionts (Tpr and Taz) (Table 5). These proteins may catalyze the uptake of Fe^{3+}-enterobactin (TC#1.B.14.1.3; TC#1.B.14.3.6) and Fe^{3+}-catecholate (TC#1.B.14.1.4) thus assisting these two strains in their mutualistic lifestyle in the gut of termites.

Type III secretion systems (T3SS) are found in many Gram-negative bacteria. This unique virulence secretion mechanism enables the organism to transfer its effector proteins across the bacterial and host membranes in one energy-coupled step [78]. Components of the T3SS are present in all ten strains (Table 6). Not surprisingly, all ten treponemes have homologues of the flagellar export system (TC#3.A.6.2.1). It has been suggested that in addition to the export of flagellar subunits, these systems may export virulence factors [79, 80]. Also, components of the general secretory pathway (TC#3.A.5.1.1) are present in all the treponemes analyzed as expected. This system is universally present an all organisms so far examined. Type VI secretory systems (T6SS) have been shown to be major virulence factors in Gram-negative bacteria, but recent studies have shown that they also play roles in the regulation of bacterial interactions and competition [81, 82]. The pathogenic strains possess more recognized components of the T6SSs, thus suggesting that these systems are more complete in the pathogens as compared to the non-pathogenic treponemes.

3.13. Interesting facets of the transportome of *T. pallidum* str. Sea 81-4

So far, we have presented the results as a comparative analysis of all ten *Treponema* genomes. However, in this section we shall discuss interesting facets of the transportome of *T. pallidum* str. Sea 81-4 and how its small genome facilitates its parasitic lifestyle inside the human host. Minor descriptions of proteins that may have the potential to assist Tpal as a parasitic bacterium are given in Table 7, while Figure 1 gives a concise view of the transportome of Tpal.

To gain an understanding of immune evasion by Tpal and understand which transport proteins play a role in its occurrence, it is necessary to understand the pathogenesis of syphilis, which is described in much detail in the review by Radolf et al. 2016 [6]. In brief, it has been hypothesized that a *Treponema pallidum* repeat protein (TprK) (TC# 1.B.38.1.2) plays a pivotal role in escaping the binding of antibodies, thus contributing to the immune evasion potential of Tpal. Other potential OMPs found in Tpal include a repeat protein TprEb (TC# 1.B.38.1.3), the outer membrane bipartite porin TprC/TprD (TC# 1.B.38.1.5) and the outer membrane trimeric porin Tprl (TC#1.B.38.1.6). It has been hypothesized that

these OMPs, in conjunction with each other, can form large non-selective channels, which could help Tpal meet its nutritional requirements in the host [6]. Tpal has homologues of characterized methyl-accepting chemotaxis proteins (TC#9.B.305.1.27). These proteins can be involved in a wide range of physiological processes including biofilm formation, regulation of flagellar biosynthesis, toxin production and sensory transduction. The sensors may bind exogenously derived ligands in the periplasm [83].

In our analysis, we found ABC transporters specific for various sugars encoded within the Tpal genome. Hexoses are crucial sources of energy to assist in the parasitic lifestyle of the organism [84]. Tpal seems to possess a complete three-component methyl galactoside transporter (TC# 3.A.1.2.3). This system may be utilized for high affinity uptake of glucose/galactose across the cytoplasmic membrane. Another complete ABC system found is PnrABCDE, which is a five-component purine nucleoside permease (TC# 3.A.1.2.10). This transport system catalyzes uptake of nucleosides (adenosine, guanosine, inosine and xanthosine). Parasitic organisms need to replicate rapidly during initial host colonization, requiring purine and pyrimidine bases for the synthesis of DNA and RNA [84]. However, Tpal behaves contrary to this initial replicative rapidity and exhibits a rather sluggish replication rate (~30h) [85]. Overall, Tpal has limited capacity for the *de novo* synthesis of purine bases owing to its small genome size, and this system (PnrABCDE) has been hypothesized to be pivotal for the replicative needs of the organism [86].

Past studies have indicated that Tpal requires three transition metals, zinc, iron and manganese, to perform important structural and catalytic functions [52]. In this study, we found that the organism seems to possess a complete system for the uptake of manganese (Mn^{2+}), zinc (Zn^{2+}) and possibly iron (Fe^{2+}): TroABCD (TC#3.A.1.15.8). Tpal also has a complete zinc-specific uptake system, one constituent of which matched TC#3.A.1.15.5 as the top hit, while two constituents matched TC#3.A.1.15.11 as the best hits.

We found that Tpal encodes two putative transport proteins of the Fatty Acid Translocation (FAT) Family (TC#4.C.1). One is a homologue of the long chain fatty acid (LCFAS) synthase of *E. coli* (TC# 4.C.1.1.4); this protein catalyzes the esterification, concomitant with transport, of exogenous long-chain fatty acids into metabolically active CoA thioesters for subsequent degradation or incorporation into phospholipids [87, 88]. A second homologue is a carnitine/crotonobetaine CoA synthase (TC#4.C.1.1.6), a protein predicted to be involved in carnitine metabolism. It has been established that carnitine plays roles in bacterial metabolism: i) it may be used as a final electron acceptor in anaerobes, and ii) it may assist in the survival of bacteria within and outside the host [89]. To date, none of the spirochaetes have been shown to utilize carnitine in their metabolism, but Tpal has been predicted to possess a carnitine transporter [90, 91]. Our study also supports this prediction, which may promote survival of the bacteria under different environmental conditions, and it may assist in the establishment of infections in the host.

Amino acids serve a plethora of important biological functions in bacteria including protein synthesis and the provision of energy, carbon and nitrogen. However, the parasitic Tpal lacks the necessary biosynthetic machinery for the synthesis of many amino acids [92], and instead relies on the uptake of free amino acids and peptides from the external environment

[90]. In the Tpal genome, we found evidence for eight amino acid transport proteins as shown in Table 2. One of these transport proteins is a homologue of the probable glycine/alanine/asparagine/glutamine (AgcS) uptake porter (TC#2.A.25.1.9) of *Pseudomonas aeruginosa*. In addition, a complete three-component system of an L-histidine uptake porter (MetlQN) (TC# 3.A.1.24.5) was found.

4. Discussion

Treponemes are fascinating adaptive organisms as revealed by their occurrence in a wide range of hosts and external environments [4]. Normally, these organisms exist as a part of the normal flora of humans and animals; however, some species are pathogenic to one or another of these hosts, and some are pathogenic to more than one animal host [93]. In addition, some species occur in a variety of aquatic habitats [94]. The most significant pathogen of the *Treponema* genus is *T. pallidum*, which is the causative agent of syphilis. This disease may be transmitted horizontally by sexual contact or vertically via hematogenous dissemination across the placental wall [95]. Each year, there are 11-12 million new cases of syphilis in human adults [93], while the estimated number of cases of congenital syphilis range between 700,000 and 1,500,000. Syphilis may additionally result in miscarriage or stillbirth [96]. Currently, no vaccine is available for syphilis, and different antibiotic regimes, including penicillin and macrolides, may be opted for treatment. For more insight into antibiotic resistance in treponemes see the review of Stamm, 2015 [93].

The transportome analyses reported in this study unveiled interesting details concerning the physiological and metabolic capabilities of the ten treponemes studied. All strains encode transport proteins that provide the organism with the metabolic repertoire required to thrive in their respective ecological niches. For instance, the oral pathogen Tde encodes the most iron-siderophore transport proteins among all ten strains. Iron (Fe) is known to be essential for biofilm formation, as it regulates surface motility while stabilizing the polysaccharide matrix [97]. During limited availability of iron, certain physiological changes limit biofilm formation by affecting interactions with other oral residents [98]. The high numbers of siderophore transport proteins point to the importance of iron acquisition for biofilm formation by Tde. The other oral pathogen, Tpu, also contains a considerable number (14) of these iron-siderophore transport proteins.

In the case of the two termite gut symbionts (Taz and Tpr), various transport proteins were identified that support the two organisms in their symbiotic lifestyles. Both treponeme symbionts have ammonia transporters (TC#1.A.11.2.3 in Tpr and TC#1.A.11.2.7 in Taz) that were absent in the remaining eight strains. It has been hypothesized that in a symbiotic relationship, one of the major nitrogen metabolites transported to the symbiont by the host is ammonia [99]. This nitrogenous compound may be utilized by the two treponemes for the synthesis of amino acids, and these amino acids may be translocated back to the host via different amino acid efflux transporters. Interestingly, both symbionts have members of the Branched Chain Amino Acid Exporter (LIV-E) Family (TC#2.A.78), which supports the above-mentioned hypothesis of an amino acid-efflux mechanism in these two treponemes.

The parasitic properties of Tpal are of particular interest (see Results reported in section 3.13 for interesting facets of Tpal). Ever since its discovery in 1906, progressive research on Tpal has been slow due to uncontrolled circumstances. One was the difficulty in the *in-vitro* cultivation of the syphilis-causing organism [76]. However, genomic studies have helped in understanding the mechanisms that Tpal employs to gain nutritional benefits from its host [100]. From our analysis, we hypothesize that despite its small genome size (~1.1 Mbp), Tpal has a finely tuned transportome (9% of the total proteome) at its disposal. This transportome must be an efficient mining tool that gathers essential nutrients from its host. Also, these transport proteins must enable Tpal to adapt to a diverse range of microenvironments and stresses in the human host.

Treponemes have the key attributes of motility and chemotaxis [101]. The embedded flagella permitting corkscrew-like motility favors their dissemination and survival in their respective environments. Transport proteins identified in this study, including a flagellar protein export system, chemotaxis proteins, and flagellar motor energizers; all play roles in survival and virulence of the organisms [102].

The transportome of an organism usually represents about 10% of the overall proteome. However, in our study this percentage was less than 10% for four strains (Tsu 7.9%, Tpal 9%, Tpr 9.5% and Taz 9.5%). The transportome analysis revealed key aspects of pathogenesis, parasitism and symbiosis in the ten strains. The presence of various virulence factors such as PFTs, iron uptake systems and protein secretion systems in all ten treponemes is surprising, as the non-pathogens may have yet undisclosed pathogenic potential. Also, the non-pathogens contain more secondary carriers as compared to the pathogens (See Results section 3.12), which indicates their enhanced ability to live in a wide range of environments as compared to the host-restricted pathogens. The findings reported in this communication improve our understanding of the pathogenesis of different *Treponema* species and encourage further comparative studies of more such species.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- *Treponema* species can be pathogenic, symbiotic or free living, two causing syphilis.
- Ten species were analyzed for their genome-encoded complement of transport proteins.
- Transporter types correlate with the means of energy production and ecological niche.
- Widely divergent transport capabilities have evolved, correlating with organismal life styles.
- Identified transporters help explain why and how some became pathogenic or symbiotic.

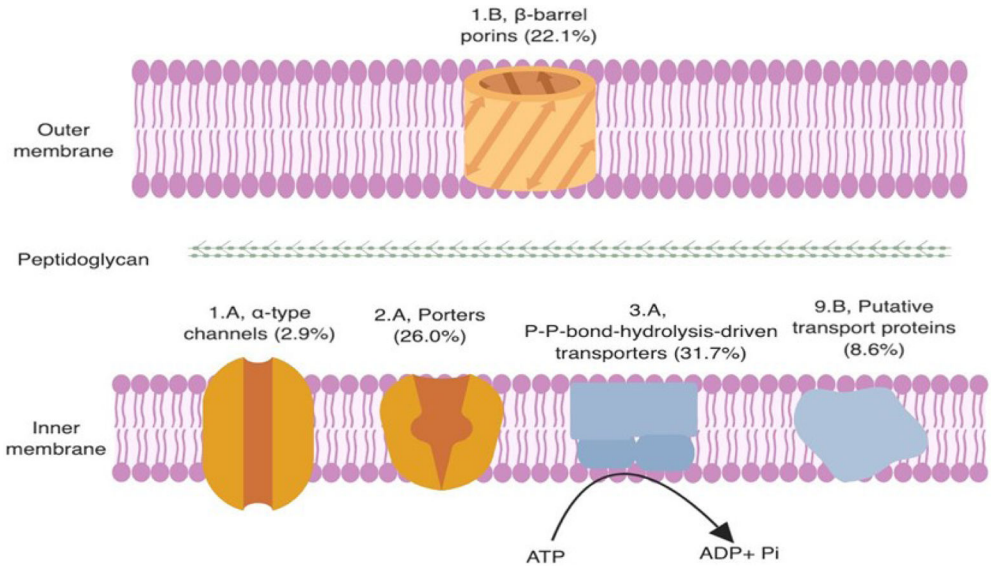

Figure 1. The transportome of Tpal in a nutshell.

The most well represented TC subclasses are shown in terms of pecentages of proteins in the overall transportome. TC 3.A has the highest representation (31.7%), with the ABC superfamily (TC#3.A.1) being the best represented family. However, secondary carriers (2.A) comprise 26.0% of the transporters, and since these systems are single component systems while ABC systems are multi-component systems, the number of secondary carriers actually excede the number of primary active transport systems. TC subclass 1.B also makes up a significant fraction (22.1%) of the transportome. In this subclass, members of the *Treponema* Porin Major Surface Protein (TP-MSP) Family (TC#1.B.38) are present in large numbers (see Table 2). The TC subclass (1.A; α-type channels) only represents 2.9% of the Tpal transportome.

Table 1.

Overview of the ten *Treponema* species included in this study.

Organismal genus	Species and strain	Abbreviations	Genome accession no.	Genome size (Mbp)	Total encoded proteins	Total transport proteins	Pathological condition
Treponema	*denticola* ATCC 35405	Tde	NC_002967.9	2.84	2767	293	Periodontal disease
	pallidum str. Sea 81-4	Tpal	NZ_CP003679. 1	1.14	960	89	Human syphilis
	pedis str. TA 4	Tpe	NC_022099.1	2.89	2473	294	Animal skin lesions
	primitia ZAS-2	Tpr	NC_015578.1	4.06	3450	329	Symbiont in termite gut
	azotonutricium ZAS-*9*	Taz	NC_015577.1	3.86	3345	320	Symbiont in termite gut
	paraluiscuniculi Cuniculi A	Tpar	NC_015714.1	1.13	976	101	Rabbit venereal syphilis
	succinifaciens DSM 2489	Tsu	NC_015385.1	2.73	2502	198	Avirulent
	brennaborense DSM 12168	Tbr	NC_015500.1	3.06	2609	284	Bovine digital dermatitis
	caldarium DSM *7334*	Tca	NC_015732.1	3.24	2828	291	Avirulent, thermophile
	putidum OMZ *758*	Tpu	NZ_CP009228 .1	2.08	2268	254	Periodontal disease

Table 2.

Overview of the *Treponema* transport protein numbers (left) and percentages (right) based on TC subclass.

TC subclass and description	Tde	Tpal	Tpe	Tpr	Taz	Tsu	Tpar	Tbr	Tca	Tpu	Tde	Tpal	Tpe	Tpr	Taz	Tsu	Tpar	Tbr	Tca	Tpu
1.A, α-type channels	9	3	7	11	11	10	3	10	10	7	3.1	2.9	2.4	3.3	3.4	5.1	3.0	3.5	3.4	2.8
1.B, β-barrel porins	16	23	15	27	27	20	15	19	18	15	5.5	22.1	5.1	8.2	8.4	10.1	15.0	6.7	6.2	5.9
1.C, Pore-forming toxins	4	3	5	5	6	4	3	4	5	5	1.3	2.8	1.6	1.5	1.8	2.0	3.0	1.4	1.7	1.9
1.E, Holins	1	0	0	1	1	1	0	1	0	0	0.3	0.0	0.0	0.3	0.3	0.5	0.0	0.4	0.0	0.0
2.A, Porters (uniporters, symporters, antiporters)	76	27	81	85	77	68	26	88	83	71	25.9	26.0	27.5	25.8	24.2	34.3	26.0	31.1	28.5	28.0
3.A, P-P-bond-hydrolysis-driven transporters	149	33	145	155	157	62	35	118	126	120	50.9	31.7	49.2	47.1	49.1	31.3	35.0	41.7	43.3	47.2
3.B, Decarboxylation-driven transporters	1	1	1	1	2	2	1	2	1	1	0.3	1.0	0.3	0.3	0.6	1.0	1.0	0.7	0.3	0.4
3.D, Oxidoreduction-driven transporters	5	0	5	6	4	4	0	4	14	7	1.7	0.0	1.7	1.8	1.3	2.0	0.0	1.4	4.8	2.8
4.B, Nicotinamide ribonucleoside uptake transporters	0	0	0	3	1	0	0	0	2	0	0.0	0.0	0.0	0.9	0.3	0.0	0.0	0.0	0.7	0.0
4.C, Acyl-CoA ligase-coupled transporters	3	1	3	4	5	2	2	3	3	2	1.0	1.0	1.0	1.2	1.6	1.0	2.0	1.1	1.0	0.6
4.D, Polysaccharide synthase exporters	0	0	0	2	4	2	0	3	2	0	0.0	0.0	0.0	0.6	1.3	1.0	0.0	1.1	0.7	0.0
5.A, Transmembrane two-electron transfer carriers	0	1	0	2	2	0	1	1	1	0	0.0	1.0	0.0	0.6	0.6	0.0	1.0	0.4	0.3	0.0
8.A, Auxiliary transport proteins	0	0	0	1	1	0	0	1	2	0	0.0	0.0	0.0	0.3	0.3	0.0	0.0	0.4	0.7	0.0
9.A, Recognized transporters of unknown biochemical mechanism	5	3	6	4	3	6	5	4	2	4	1.7	2.8	2.0	1.2	0.9	3.0	5.0	1.4	0.6	1.7
9.B, Putative transport proteins	24	9	27	22	19	17	9	25	22	22	8.3	8.7	9.2	6.9	5.9	8.7	9.0	8.7	7.8	8.7
Total	293	104	295	329	320	198	100	283	291	254	100	100	100	100	100	100	100	100	100	100

Table 3.

Overview of transport proteins in the ten examined strains of treponemes on the basis of predicted substrate specificities.

		T. denticola	**T. pallidum**	**T. pedis**	**T. primitia**	**T. azotonutricium**	**T. paraluiscuniculi**	**T. succinifaciens**	**T. brennaborense**	**T. caldaria**	**T. putidum**
Count Category	Inorganic ions	42	18	48	60	51	19	38	46	61	40
	Organic ions	138	30	142	150	170	30	81	146	141	112
	Macromolecules	52	25	49	81	52	26	43	48	43	43
	Unknown	47	7	44	23	31	6	20	31	34	45
	Non-specific	14	9	11	15	16	20	16	13	12	14
	Total	293	89	294	329	320	101	198	284	291	254
		T. denticola	**T. pallidum**	**T. pedis**	**T. primitia**	**T. azotonutricium**	**T. paraluiscuniculi**	**T. succinifaciens**	**T. brennaborense**	**T. caldaria**	**T. putidum**
Percent Category	Inorganic ions	14.3	20.2	16.3	18.2	15.9	18.8	19.2	16.2	21	15.7
	Organic ions	47.1	33.7	48.3	45.6	53.1	29.7	40.9	51.4	48.5	44.1
	Macromolecule	17.7	28.1	16.7	24.6	16.3	25.7	21.7	16.9	14.8	16.9
	Unknown	16.0	7.9	15.0	7.0	9.7	5.9	10.1	10.9	11.7	17.7
	Non-specific	4.9	10.1	3.7	4.6	5.0	19.7	8.1	4.6	4.0	5.6
	Total	100	100	100	100	100	100	100	100	100	100
		T. denticola	**T. pallidum**	**T. pedis**	**T. primitia**	**T. azotonutricium**	**T. paraluiscuniculi**	**T. succinifaciens**	**T. brennaborense**	**T. caldaria**	**T. putidum**
Count Subcategory	Inorganic cations	30	16	33	39	31	17	27	34	44	30
	Inorganic anions	12	1	15	19	18	1	11	11	16	10
	Electrons	0	1	0	2	2	1	0	1	1	0
	Sugars	7	3	8	25	55	3	16	37	54	8
	Sugar derivatives	5	2	5	14	23	2	3	18	18	4
	Carboxylates	7	3	9	21	14	3	10	16	10	6
	Amines	1	3	2	5	5	3	3	4	3	1
	Amino acids	20	8	23	31	22	8	17	18	18	16
	Siderophores	20	0	19	9	5	0	1	7	3	14
	Drugs	64	4	61	29	28	4	16	30	17	53
	Non-carboxylate organic anions	2	0	2	4	7	0	1	3	3	1

		T. denticola	T. pallidum	T. pedis	T. primitia	T. azotonutricium	T. paraluiscuniculi	T. succinifaciens	T. brennaborense	T. caldaria	T. putidum
	Nucleotides/nucleosides	5	4	7	6	7	4	6	8	7	5
	Vitamins	7	3	6	6	5	3	8	5	8	4
	Proteins/peptides	36	14	35	55	30	15	22	21	27	33
	Polysaccharides	7	6	8	19	13	6	13	20	9	4
	Lipids	7	3	4	5	6	3	3	4	5	5
	DNA	2	2	2	2	2	2	5	3	2	1
	Unknown	47	7	44	23	31	6	20	31	34	45
	Non-specific	14	9	11	15	16	20	16	13	12	14
	Total	293	89	294	329	320	101	198	284	291	254
		T. denticola	**T. pallidum**	**T. pedis**	**T. primitia**	**T. azotonutricium**	**T. paraluiscuniculi**	**T. succinifaciens**	**T. brennaborense**	**T. caldaria**	**T. putidum**
Percent Subcategory	Inorganic cations	10.2	18	11.2	11.9	9.7	16.8	13.6	12	15.1	11.8
	Inorganic anions	4.1	1.1	5.1	5.8	5.6	1.0	5.6	3.9	5.5	3.9
	Electrons	0	1.1	0	0.6	0.6	1.0	0	0.4	0.3	0
	Sugars	2.4	3.4	2.7	7.6	17.2	3.0	8.1	13	18.6	3.1
	Sugar derivatives	1.7	2.2	1.7	4.3	7.2	2.0	1.5	6.3	6.2	1.6
	Carboxylates	2.4	3.4	3.1	6.4	4.4	3.0	5.1	5.6	3.4	2.4
	Amines	0.3	3.4	0.7	1.5	1.6	3.0	1.5	1.4	1	0.4
	Amino acids	6.8	9.0	7.8	9.4	6.9	7.9	8.6	6.3	6.2	6.3
	Siderophores	6.8	0	6.5	2.7	1.6	0	0.5	2.5	1.0	5.5
	Drugs	21.8	4.5	20.7	8.8	8.8	4.0	8.1	10.6	5.8	20.9
	Non-carboxylic organic anions	0.7	0	0.7	1.2	2.2	0	0.5	1.1	1.0	0.4
	Nucleotides/nucleosides	1.7	4.5	2.4	1.8	2.2	4.0	3.0	2.8	2.4	2.0
	Vitamins	2.4	3.4	2	1.8	1.6	3.0	4.0	1.8	2.7	1.6
	Proteins/peptides	12.3	15.7	11.9	16.7	9.4	14.9	11.1	7.4	9.3	13
	Polysaccharides	2.4	6.7	2.7	5.8	4.1	5.9	6.6	7	3.1	1.6
	Lipids	2.4	3.4	1.4	1.5	1.9	3	1.5	1.4	1.7	2.0
	DNA	0.8	2.2	0.7	0.6	0.6	2	2.5	1.1	0.7	0.4
	Unknown	16.0	7.9	15.0	7.0	9.4	5.7	10.1	10.9	11.7	17.7

	T. denticola	T. pallidum	T. pedis	T. primitia	T. azotonutricium	T. paraluiscuniculi	T. succinifaciens	T. brennaborense	T. caldaria	T. putidum
Non-specific	4.8	10.1	3.7	4.6	5	19.8	8.1	4.5	4.3	5.4
Total	100	100	100	100	100	100	100	100	100	100

Table 4.

Occurrence of toxins in the ten *Treponema* species. Transporters marked in purple are pathogen-specific; those marked in green are specific to non-pathogens, while those in orange are present in both pathogens and non-pathogens.

TCID	Family	Function	Tde	Tpal	Tpe	Tpr	Taz	Tsu	Tpar	Tbr	Tca	Tpu
1.C.1.1.2	Colicin	Channel formation	0	0	0	0	0	0	0	0	0	1
1.C.1.2.2	Colicin	Channel formation	0	0	0	0	0	0	0	0	0	1
1.C.1.2.3	Colicin	Channel formation	0	0	0	0	0	1	0	0	0	0
1.C.11.1.5	RTX-toxin	Pore formation/cytolysis	0	0	1	0	0	0	0	0	0	0
1.C.39.4.6	MACPF	Pore formation	0	0	0	0	0	0	0	0	0	1
1.C.75.1.7	S-PFT	Haemagglutination	1	0	1	1	1	0	0	0	0	1
1.C.82.1.1	HP2-20	Pore formation	1	1	1	1	1	1	1	1	1	0
1.C.109.1.1	B-Hemolysin A	Pore formation	0	0	0	0	1	0	0	0	0	0
1.C.109.1.2	B-Hemolysin A	Pore formation	0	0	0	0	0	0	0	1	0	0
1.C.109.1.6	B-Hemolysin A	Pore formation	0	0	0	1	0	0	0	1	0	0
1.C.113.1.1	Hly III	Lysis	1	0	1	0	1	1	0	1	1	1
1.C.113.1.10	Hly III	Lysis	0	1	0	1	0	0	1	0	0	0
1.C.126.1.1	Hemolysin C	Lysis	1	1	1	1	1	1	1	1	1	1
1.C.126.1.6	Hemolysin	Lysis	0	0	0	1	0	0	0	0	1	0

Table 5.

Occurrence of iron and iron-siderophore transport proteins in ten *Treponema* species. Proteins marked in purple are pathogen-specific; those marked in green are specific to non-pathogens, and those in orange are present in both pathogens and non-pathogens. Polarity of transport is indicated in columns 3 and 4 where an X indicates that the transport process is uptake or efflux.

TCID	Substrate Transported	Uptake	Efflux	The	Tpal	Tpe	Tpr	Taz	Tsu	Tpar	Tbr	Tca	Tpu
1.B.14.1.3	Fe^{3+}-enterobactin	X		0	0	0	1	0	0	0	0	0	0
1.B.14.1.4	Fe^{3+}- catecholate	X		0	0	0	0	1	0	0	0	0	0
1.B.14.3.6	Fe^{3+}-enterobactin	X		0	0	0	1	0	0	0	0	0	0
2.A.4.7.1	Fe^{2+}		X	0	0	0	1	1	0	0	0	0	0
2.A.4.7.4	Fe^{2+}		X	0	0	0	0	0	0	0	0	1	0
2.A.4.7.9	Fe^{2+}		X	0	0	0	0	0	0	0	1	0	0
2.A.108.2.10	Fe^{2+}	X		0	0	0	0	1	0	0	0	0	0
3.A.1.5.2	Heme	X		3	0	3	0	0	0	0	0	0	2
3.A.1.9.1	Fe^{3+}	X		2	0	2	0	0	0	0	0	0	1
3.A.1.14.1	Fe^{3+}-citrate	X		0	0	0	0	0	0	0	0	1	0
3.A.1.14.2	Fe^{3+}-enterobactin	X		1	0	0	0	0	1	0	0	0	0
3.A.1.14.3	Fe^{3+}-hydroxamate	X		0	0	0	0	0	0	0	1	0	0
3.A.1.14.4	Fe^{3+}-chrysobactine	X		0	0	0	1	0	0	0	0	0	0
3.A.1.14.7	Fe^{3+}-hydroxamate	X		1	0	2	0	0	0	0	0	0	1
3.A.1.14.10	Heme	X		2	0	2	0	0	0	0	0	0	1
3.A.1.14.14	Fe^{3+}, Fe^{3+}-heme, Fe^{3+}-ferrichrome	X		1	0	0	0	0	0	0	0	0	0
3.A.1.14.15	Fe^{3+}-bacillibactin	X		0	0	2	0	0	0	0	0	0	0
3.A.1.14.17	Heme	X		1	0	3	0	0	0	0	0	0	0
3.A.1.14.18	Heme	X		0	0	1	1	0	0	0	0	0	0
3.A.1.14.19	Heme	X		0	0	0	1	0	1	0	0	0	0
3.A.1.14.20	Heme	X		0	0	1	0	0	0	0	0	0	0
3.A.1.15.6	Fe^{2+}	X		0	0	0	0	0	1	0	0	0	0
3.A.1.15.8	Fe^{3+}	X		4	4	4	0	0	0	4	1	0	4
3.A.1.15.12	Fe^{2+}	X		0	1	1	0	0	0	0	0	0	0

TCID	Substrate Transported	Uptake	Efflux	The	Tpal	Tpe	Tpr	Taz	Tsu	Tpar	Tbr	Tca	Tpu
3.A.1.20.1	Fe^{3+}	X		0	0	0	1	0	0	0	0	0	0
3.A.1.20.3	Fe^{3+}	X		1	0	0	1	0	0	0	1	0	0
3.A.1.21.1	Fe^{3+}-yersiniabactin	X		3	0	0	0	0	0	0	0	0	2
3.A.1.21.2	Fe^{3+}-carboxymycobactin	X		0	0	0	0	0	0	0	0	1	0
9.A.8.1.4	Fe^{2+}	X		0	0	0	0	0	1	0	0	0	0
9.A.8.1.9	Fe^{2+}	X		1	0	0	1	1	0	0	0	0	0
9.A.8.1.14	Fe^{2+}	X		0	0	0	0	0	0	0	1	0	0

Table 6.

Occurrence of secretion system components in ten *Treponema* species with numbers of transport proteins in both pathogenic and non-pathogenic strains. Transporters marked in purple are pathogen-specific; those marked in green are specific to non-pathogens, and those in orange are present in both pathogens and non-pathogens.

			Number of constituent proteins									
Family	**TCID**	**Function**	**Tde**	**Tpal**	**Tpe**	**Tpr**	**Taz**	**Tsu**	**Tpar**	**Tbr**	**Tca**	**Tpu**
T1SS	3.A.1.105.7	Drug exporter (3 components)	3	0	0	0	0	0	0	0	0	3
	3.A.1.105.16	Unknown exporter (3 components)	0	0	0	2	0	0	0	0	0	0
	3.A.1.121.4	Drug (fluoroquinolones) exporter (2 components)	2	1	1	4	2	1	1	0	1	3
	3.A.1.122.1	Drug (macrolides) exporter (2 components)	0	0	0	0	0	1	1	1	1	5
	3.A.1.122.2	Peptide (antimicrobials) exporter (4 components)	2	1	1	2	0	0	2	5	2	2
	3.A.1.122.12	Drug (arthrofactin) exporter (2 components)	0	1	1	0	0	0	1	0	0	0
	3.A.1.122.14	Unknown exporter (2 components)	1	0	0	1	0	0	0	1	1	2
	3.A.1.122.16	Drug (macrolides) exporter (2 components)	1	0	0	1	1	0	0	0	0	1
	3.A.1.125.1	Protein (lipoproteins) exporter (3 components)	1	2	2	2	3	1	2	2	1	3
	3.A.1.125.5	Unknown exporter (2 components)	1	1	1	0	0	1	1	1	1	1
	3.A.1.132.3	Unknown exporter (2 components)	1	0	0	2	0	3	0	3	1	1
	3.A.1.132.6	Unknown exporter (2 components)	1	0	0	0	0	0	0	2	0	1
T2SS (GSP)	3.A.5.1.1	SEC-SRP complex (8 components)	6	5	5	5	6	6	5	7	6	6
T3SS	3.A.6.1.1	Type III protein secretion complex (22 components)	3	1	1	2	1	4	1	0	1	2
	3.A.6.1.2	Type III secretion complex (10 components	6	8	8	7	0	0	0	1	1	0
T4SS	3.A.7.7.1	Conjugal DNA protein transfer system or ViRB (15 components)	1	1	1	1	1	2	1	1	1	1
	3.A.7.11.1	Type IV betaproteobacterial DNA secretion system (20 components)	1	1	1	1	1	1	1	1	1	1
	3.A.7.13.2	Conjugal DNA protein transfer system or VIRB (12 components)	2	3	3	2	0	1	2	0	1	1
	3.A.7.14.2	Type IV (conjugal DNA-protein transfer) (11 components)	0	0	0	2	0	0	0	0	0	2
	3.A.7.19.1	Conjugal DNA protein transfer system or VIRB (19 components)	1	1	1	3	0	6	1	2	3	2
T6SS	3.A.23.1.1	T6SS VasA-L (14 components)	4	2	2	6	6	3	2	3	10	5
	3.A.23.6.1	T6SS (21 components)	3	1	1	3	3	6	1	3	3	4

Table 7.

Transport proteins that may play roles in the parasitic lifestyle of *Treponema pallidum* sea 81-4.[1]

Transport protein name	TCID	Uniport ID	Proposed function
Treponema repeat protein K (TprK)	1.B.38.1.2	Q84AM6	Plays a role in immune evasion and persistence
Glycine/alanine/asparagine/glutamine (AgcS) uptake porter	2.A.25.1.9	W0WFC6	Uptake of amino acids
Galactose/glucose (methyl galactoside) porter (3 components)	3.A.1.2.3	P0AAG8, P0AEE5, P23200	Transport of glucose and galactose
Purine nucleoside permease (PnrABCDE) (5 components)	3.A.1.2.10	083340-43, P29724	Uptake of nucleosides; assists in replication
Manganese (Mn2+), zinc (Zn2+) and possibly iron (Fe2+) uptake porter: TroABCD	3.A.1.15.8	P96116-119	Transport of metal ions
L-Histidine uptake porter MetlQN (3-components)	3.A.1.24.5	Q9HT68, Q9HT69, Q9HT70	Uptake of amino acids
Carnitine/crotonobetaine CoA synthase	4.C.1.1.6	P31552	May assist in the survival of Tpal in the host

[1]This list is not exhaustive, and includes those proteins that we presume to assist Tpal to thrive as a parasitic bacterium; future research will certainly expand the number of these transport proteins.