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Bioinformatic Analyses of Integral Membrane Transport Proteins Encoded Within the Genome of the Planctomycetes species, *Rhodopirellula baltica*

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Abstract

Rhodopirellula baltica (*R. baltica*) is a Planctomycete, known to have intracellular membranes. Because of its unusual cell structure and ecological significance, we have conducted comprehensive analyses of its transmembrane transport proteins. The complete proteome of *R. baltica* was screened against the Transporter Classification Database (TCDB) to identify recognizable integral membrane transport proteins. 342 proteins were identified with a high degree of confidence, and these fell into several different classes. *R. baltica* encodes in its genome channels (12%), secondary carriers (33%), and primary active transport proteins (41%) in addition to classes represented in smaller numbers. Relative to most non-marine bacteria, *R. baltica* possesses a larger number of sodium-dependent symporters but fewer proton-dependent symporters, and it has dimethylsulfoxide (DMSO) and trimethyl-amine-oxide (TMAO) reductases, consistent with its Na^+-rich marine environment. *R. baltica* also possesses a Na^+-translocating NADH:quinone dehydrogenase (Na^+-NDH), a Na^+ efflux decarboxylase, two Na^+-exporting ABC pumps, two Na^+-translocating F-type ATPases, two Na^+:H^+ antiporters and two K^+:H^+ antiporters. Flagellar motility probably depends on the sodium electrochemical gradient. Surprisingly, *R. baltica* also has a complete set of H^+-translocating electron transport complexes similar to those present in β-proteobacteria and eukaryotic mitochondria. The transport proteins identified proved to be typical of the bacterial domain with little or no indication of the presence of eukaryotic-type transporters. However, novel functionally uncharacterized multispanning membrane proteins were identified, some of which are found only in *Rhodopirellula* species, but others of which are widely distributed in bacteria. The analyses lead to predictions regarding the physiology, ecology and evolution of *R. baltica*.

Keywords

Marine ecology; Planctomycetes; transport proteins; sodium motive force; electron transport; cellular energization

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1. Introduction

Rhodopirellula baltica is an aerobic marine halotolerant Planctomycete, a member of a diverse genus of ecological importance with an unusual cell structure, including an intracellular membrane-enclosed nucleoid, the pirellulosome [1-6]. It and other Planctomycetes exhibit resistance to a number of traditionally used antibiotics and heavy metals, and they have the capacity to degrade and utilize a wide range of organic materials in their environments [7-9]. They lack peptidoglycan and instead display a proteinaceous cell wall, possibly with degradative enzymes anchored to the external surface [10-12]. These organisms produce a variety of secondary products such as pigments including carotenoids [13].

R. baltica belongs to the phylum *Planctomycetes*, a divergent phylum which some phylogenetic analyses claim to be deep-branching within the domain Bacteria [14, 15]. These organisms are most closely related to other members of the so-called PVC superphylum also encompassing the phyla Verrucomicrobia, Lentisphaerae, Chlamydiae and the uncultured OP3 phylum [16-18]. Cells of species in the phylum Planctomycetes are characteristically divided into two and sometimes three major compartments, and all species examined have at least two such compartments. There is a cytoplasmic membrane, defining the protoplast and cytoplasm within it, and this membrane is closely apposed to the cell wall. Such an arrangement is highly unusual for bacteria, and these organisms lack a peptidoglycan wall polymer characteristic of most walled bacteria of both Gram-positive and Gram-negative types. Although there are aspects of electron micrographs of sections of the wall in whole cells which might be interpreted by some as murein-like, e.g., electron dense and electron light stratified layers, the 10% SDS-resistant walls of several species appear to retain shape on isolation after SDS treatment in a manner typical of a classical peptidoglycan-containing murein sacculus [19-21].

The suggestion that a murein wall structure, including an outer membrane, somehow applies to Planctomycetes on the basis of some genomic data does not seem justified. However, homologs of Gram-negative bacterial wall synthesis enzymes are present [22], suggesting that the precursor of the *Planctomycetes* must have been a more typical Gram-negative bacterium. Without localization of the proteins detected bioinformatically, there is little convincing evidence for the presence of a typical Gram-negative bacterial envelope.

Homologs of outer membrane proteins present in *R. baltica* might serve different roles from those served in most Gram-negative bacteria. In all Planctomycetes examined, a single bilayer intracytoplasmic membrane (ICM) lies inside the cytoplasm and defines a region called the paryphoplasm lying between the cytoplasmic membrane (CM) and this ICM. The ICM displays no obvious continuity with the CM. The ICM surrounds a compartment termed the pirellulosome containing the ribosomes and nucleoid. In some species, a third compartment lies within the pirellulosome, defined by one membrane as in anaerobic anammox plantomycetes such as *Kuenenia stuttgartiensis*, or two closely apposed membranes forming a ‘nuclear envelope’ as in the case of aerobic *Gemmata obscuriglobus*. Thus, in an organism such as *Gemmata*, molecular transport must occur across not only the cytoplasmic membrane but also across up to three internal membrane bilayers and between up to three internal compartments. It should be noted, however, that *R. baltica* appears to have only two compartments delineated by two phospholipid bilayers, the CM and ICM.

Marine Planctomycetes include: *R. baltica*, *Blastopirellula marina*, many related unnamed isolates, and several about-to-be-named new species of this genus. *Planctomyces maris* and *P. brasiliensis* are hypersaline pond organisms. However, to date, no *Gemmata* or *Pirellula* strains have been isolated from marine habitats. Several researchers are currently working

on new marine isolates of Planctomycetes [8]. The studies reported here are likely to be relevant to the various marine Planctomycetes in addition to *R. baltica* (see Discussion).

As noted above, the cell structure of *R. baltica* shares with other Planctomycetes compartmentalization of the cell cytoplasm delineated by the CM and the ICM [16, 23]. The outer ribosome-free compartment is called the paryphoplasm and the nucleoid- and ribosome-containing compartment is enclosed by the ICM. Unlike most other Planctomycetes, *R. baltica* may also contain vesicle-like compartments containing ribosomes embedded within the outer ribosome-free compartment. The ribosome-free outer paryphoplasm does not appear to be a periplasm (that is, a region between the cell wall or outer membrane and the CM), since the CM is closely apposed to the cell wall and lines the outer perimeter of the paryphoplasm. Consistent with their unusual internal structure, cell walls in Planctomycetes are neither of the Gram-positive nor of the Gram-negative type in structure or composition. Instead they consist largely of proteins [20, 24]. In *R. baltica*, these proteins are unusual in composition, rich in cysteine and proline with unique planctomycete-specific YTV motifs [10].

Planctomycetes, including *R. baltica* and closely related species, associate with marine macroalgae and form epiphytic communities with intimate relationships involving nutritional exchange [25]. In fact, they dominate kelp surface biofilms with over 50% of the members of these bacterial communities being Planctomycetes species [26]. These organisms display a dimorphic life cycle with a motile free swimming stage and a sessile biofilm stage. At distinct stages in its life-cycle, *R. baltica* produces both holdfast material in the sessile stage and flagella in its motile ‘swarmer’ (or more accurately ‘swimmer’) stage. The transition between these two states is influenced by nitrogen availability; nitrogen limitation promotes the motile state while nitrogen excess promotes biofilm formation [27].

R. baltica is a model Planctomycete with biotechnologically promising features [6, 28]. It forms complex mixed biofilm communities with biodegradative and bioremediative capabilities [29], degrades sulfated polymeric carbohydrates produced by kelp [26] and can oxidize anthropogenic and algae-produced haloalkanes [30, 31]. This organism has genome-encoded enzymes for the synthesis of complex organic molecules that may have applications in the pharmaceutical field. These enzymes include polyketide synthases and enzymes for vitamin and amino acid biosynthesis [32]. *R. baltica* has been shown to exhibit a high degree of environmental adaptability [33] and contributes to biomass remineralization in natural environments. It has a complete complement of carbohydrate catabolic enzymes which provide primary sources of energy such as ATP for energizing transport [34].

R. baltica was the first Planctomycete to have its genome fully sequenced [32]. It has a circular chromosome of 7.15 Mbp with genes for heterolactic acid fermentation, the interconversion of C1 compounds [35], and an amazingly large number (110) of putative sulfatases, probably related to its association with macroalgae [26]. In addition to encoding a fragmentary peptidoglycan biosynthetic pathway, it has many typical Gram-negative bacterial enzyme systems such as those involved in lipopolysaccharide biosynthesis and flagellar motility. The availability of the fully sequenced genome of *R. baltica* immediately led to phylogenetic, proteomic and transcriptomic analyses, providing the first detailed molecular description of a Planctomycete [34, 36-40].

Transporters allow communication between the cytoplasm of a cell and the external medium and are responsible for the uptake of all nutrients and the export of secondary metabolites and end-products of metabolism. Further, the compartmentalized cell of *R. baltica* presumably requires the existence of transport proteins in both the CM and the ICM. It has

been proposed that this complex structure might relate to a common origin with eukaryotes that also display compartmentalization, but this proposal is highly speculative.

In this communication, we deduce the full complement of bioinformatically recognizable putative integral membrane transport proteins in *R. baltica*. Usually, about one quarter of all proteins in a living cell are integral membrane proteins, and about one quarter of these are transport proteins. In accordance with this trend, we find that the percentage of all proteins in *R. baltica* that were retrieved by the G-BLAST program as integral membrane transport proteins was 4.7%, only slightly lower than for other bacteria.

Examination of these transport proteins revealed that almost without exception, transporters present in *R. baltica* are typical of the bacterial domain with virtually none exhibiting greater similarity to those of eukaryotes. As might be expected of a marine organism, *R. baltica* possesses numerous primary mechanisms for extruding sodium and thus generating a sodium motive force (*smf*) which can then be used to drive nutrient uptake and toxic product export. We find evidence of transporters that may be involved in regulating communal life, and the presence of dual Na^+-translocating F-type ATPases as well as dual Na^+-pumping ABC-type pumps and probable dual Na^+:H^+ and K^+:H^+ antiporters, providing the potential for separate energization of distinct cellular compartments. These observations provide clues to the unique characteristics of the Planctomycetes that may explain their unusual physiological characteristics.

2. Methods

2.1 G-BLAST search of the R. baltica proteome

The proteome of *R. baltica* strain SH1 (hereafter called *R. baltica*) was screened for homologues of all proteins contained in the Transporter Classification Database (TCDB; www.tcdb.org) as of September, 2012 using G-BLAST [41]. G-BLAST is a tool to screen all proteins encoded within a genome against all protein entries in TCDB. The program retrieves information for both the query and top hit sequences, TC#, protein size in number of amino acid residues, number of TMSs, e-value for the query and hit proteins, regions of sequence similarity and regions of TMS overlap. FASTA-formatted protein sequences from the completed genome were used. Each putative open-reading frame was used as a query in the BLASTP software to search for homologous proteins in TCDB. The low complexity filter was not used as it is normally of value only for larger datasets including proteins with multiple repeat elements. In addition, each open reading frame (ORF) was scanned with the HMMTOP 2.0 program [42] to predict the number of putative transmembrane segments (TMSs). The Web-based Hydropathy, Amphipathicity and Topology (WHAT) program [43] was used with a window size of 19 residues and an angle of 100 degrees to display the hydropathy plot for individual proteins in order to resolve the differences in the numbers 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 value of 0.001 was used with the G-BLAST program so proteins retrieved with larger values (greater sequence divergence) were not recorded. 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 periplasmic, outer membrane and secreted proteins that are exported via the Sec translocase. The topological prediction programs often miss these TMSs, recording them to have zero TMSs. Consequently, the numbers retrieved were not reliable and were therefore not always recorded. For example, single TMS proteins such as extracytoplasmic solute binding receptors of ABC transport systems were often predicted to lack a TMS, and therefore these proteins were not included in our study of the integral membrane transport proteins.

2.2 Identification of distant transport protein homologs

We ran G-BLAST using two different e-value cutoff settings. For the first setting (0.001, described above), we obtained 863 proteins in *R. baltica* that were similar to TCDB proteins. TCDB contains many 0 TMS proteins, such as ATPase subunits of ABC transporters and various auxiliary subunits. Of the matches in *R. baltica*, we found that 312 proteins had 0 TMSs in the query, and 218 proteins had 0 TMSs in the TC-target protein. If a protein had 0 TMSs in either target or query, it was excluded. For the second run of G-BLAST with the more inclusive e-value cutoff (0.1), 2100 proteins were obtained. Of these, 928 had 0 TMS in the query and 499 had 0 TMS in the hit. Consequently, all such sequences were removed from the second dataset.

Proteins retrieved between the values of 0.001 and 0.1 were examined manually to determine the likelihood that these proteins were members of recognized transport protein families, or if they might comprise representatives of novel families of putative transport proteins. A total of 328 proteins were retrieved using the 0.001 to 0.1 cutoff, but only 21 proved to be recognizable transport proteins. These were incorporated into TCDB (see section 3.9). The 21 proteins were manually examined by conducting searches as follows. 1. TC-BLAST searches provided preliminary evidence for family assignment. 2. NCBI BLAST searches provided confirmation or refutation of family assignment based on the conserved domain database (CDD) and hits obtained with values to the query sequence of less than 1×10^{-7}. 3. Topological analyses revealed similarities and differences between the query sequence and members of the assigned family. 4. Proteins proving to represent new potential families were included in TC subclass 9.B.

Candidate proteins were subsequently examined in greater detail to estimate their substrate specificities. On the basis of the numbers and locations of TMSs as well as degrees of sequence similarity with entries of known function in TCDB, transport proteins were classified into families and subfamilies of homologous transporters according to the classification system presented in TCDB. Regions of sequence similarity were examined using the WHAT program which shows hydropathy plots to ensure that homology was in a transmembrane region of 3 or more TMSs and not in hydrophilic domains. Proteins encoded within single multi-cistronic operons were often identified in order to gain evidence for multicomponent systems and to help deduce probable functions. Operon analyses (genome context, a.k.a., synteny analyses) were performed for candidate proteins with assigned or unassigned transport functions as described in Castillo *et al*. (2010) and Reddy *et al*. (2012) [44, 45].

2.3 Functional predictions for uncharacterized proteins

Functional predictions were based on the top hits in TCDB regardless of organismal source of the hit protein. Reliability depended upon the e-value obtained as well as the family association. Thus, for families of uniform function, e.g., CaCA (TC# 2.A.19) or Trk (TC# 2.A.38), e-values could be high (greater than 1×10^{-8}), but for families of diverse function, e-values had to be smaller than 1×10^{-24} for reliable prediction. Confirmation was often possible using genome context analyses as described in the previous paragraph.

The substrate specificities of particular homologues identified in the sequenced genomes were sometimes predicted based on homology to functionally characterized proteins and their genomic context. Assignment to a family or subfamily within the TC system often allows prediction of substrate type with confidence [46-50]. When an expected transport protein constituent of a multi-component transport system could not be identified with BLASTP, tBLASTn was performed because such expected proteins are sometimes undetectable by BLASTP due to sequencing errors including frameshift mutations or

because of incorrect initiation codon assignment, sequence divergence, and/or pseudogene formation.

2.4 Use of the BOMP program for identification of putative beta barrel proteins

In addition to the putative beta barrel porins retrieved using G-BLAST (see above), five proteins were identified using the Beta-barrel integral Outer Membrane Proteins (BOMP program; http://services.cbu.uib.no/tools/bomp/handleForm; [51]) in a study independent of the G-BLAST-based search. For the BOMP experiment, we used the whole proteome of *R. baltica* as our starting point. Consequently, no protein could have been excluded prior to the BOMP analyses depending on their predicted topology.

2.5 Overview of programs used

Transport proteins thus obtained were systematically analyzed for unusual properties using published [41] and unpublished in-house software. Among the programs described by Reddy and Saier [41], used in this report, were the GSAT, Protocol1, Protocol2, TSSearch, SSearch and GBlast programs. Unpublished software was used to tabulate information according to TC# or other criteria such as substrate type. Unusual characteristics of the query sequences in *R. baltica* were identified based in part on topologies that differed from corresponding family members in TCDB as well as e-values obtained with G-BLAST. 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 actual protein sequence, but they can also be artifactual, 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.

3. Results

3.1 Distribution of transporters according to TC class and substrate specificity

Figures 1 A and B present pie charts showing the distribution of transporters according to TC class and subclass, respectively, while Tables S1A and S1B present the numerical values for the various classes and subclasses. 12% of the integral membrane transport proteins identified fall into TC class 1 (channels and pores). Primary active transporters are the dominant species, accounting for 41% of these proteins, while secondary carriers represent 33%. Group translocators and transmembrane electron carriers each comprise about 1% while auxiliary transport proteins comprise about 2%. Finally, 33 (10%) of the integral membrane transport proteins identified are of unknown mechanism of action (class 9; Figure 1 and Table S1). Of these, 15 proteins are established transporters while 18 are putative transporters.

As shown in Figure 1B, class 1 channel protein fall into two classes, α-type channels (subclass 1A) and β-barrel porins (subclass 1B). The former represent the vast majority of these channel proteins (10.5% of all the transport proteins identified, while the β-barrel porins represent a much smaller fraction, only 1.5% of the identified transporters). These values are to be contrasted with those of *E. coli* where only 3% of the total transporters were α-type channels while 8% were β-type porins (Tang and Saier, unpublished results). Thus, while *R. baltica* has a greater percentage of cytoplasmic membrane (or inner cytoplasmic membrane)-type channels, it appears to have a deficiency of outer membrane-type porins.

This conclusion was confirmed using the Beta-barrel Outer Membrane Protein (BOMP) program, a tool for prediction of beta-barrel integral outer membrane proteins (http://services.cbu.uib.no/tools/bomp/handleForm). We first analyzed the proteome of *R. baltica*;

the total number of proteins analyzed was 7325, of which 45 were putative integral ß-barrel outer membrane proteins according to BOMP. None of these were predicted with a confidence level corresponding to the top score of 5, and only 5 proteins reached a confidence level of 4. Of these, only two proteins matched a recognized β-barrel porin in subclass 1.B of TCDB, and only one (Q7UTG3) was predicted to have at least 16 strands using the "prodiv" software in TOPCONS. Known β-barrel porins have 8-22 transmembrane strands.

Of the 5 proteins we identified using BOMP with a score of 4, one of them (NP_863863.1) displayed an e-value of e-25 against 1.B.13.3.2. This particular protein was not identified in the G-BLAST because although it displayed an N-terminal hydrophobic peak, the HMMTOP program predicted 0 TMSs. The first one of the other candidates identified by BOMP has been entered manually into TCDB under 9.B.153.1.1. The second one was not entered because it could not be verified to be an actual porin. The third one was entered under 9.B.154.1.1. The fifth one was highly similar to 9.B.154.1.1 and was not entered separately.

Conducting the same search with *E. coli* O157:H7 str. Sakai, 16 proteins were retrieved with a score of 5, and 26 proteins were retrieved with a score of 4. This shows that *R. baltica* has a very sparse set of confidently predicted β-barrel porins.

In the closely related organism, *Rhodopirellula sp*. (BioProject ID: PRJNA193783), containing 7238 proteins, 79 were putative integral ß-barrel outer membrane proteins. However, only 10 of these were predicted by BOMP with a confidence level of 4 or 5. The proteins predicted at a confidence level of 3 or below did not have convincing BLAST hits against TCDB. Six of the 10 having a score of 4 or 5 exhibited similarity with known β-barrel porins. Therefore, it seems that a very limited set of outer membrane proteins may characterize *Rhodopirellula* species in general.

ATP-driven primary active transporters (TC subclass 3.A) comprise the largest fraction of primary active transporters in *R. baltica* (34%), while decarboxylation-driven porters (TC subclass 3.B) represent only 0.3%, and electron flow-driven systems (TC subclass 3.D) represent 7%. These numbers are similar to those for *E. coli* where these three percentages are 30%, 0.3% and 5% (Tang and Saier, unpublished results). However, group translocators are largely lacking in *R. baltica*, which lacks the sugar transporting phosphoenolpyruvate:sugar phosphotransferase systems (PTS; TC subclass 4.A [52]) and has only one percent of its transporters as polysaccharide exporters (TC subclass 4.D). These numbers contrast with *E. coli*, which has 5% of its transporters as constituents of the PTS and 2% of its porters as polysaccharide exporters. Transmembrane electron flow carriers (TC class 5) are also less prevalent in *R. baltica*, compared to *E. coli*, where the percentages are 1% and 4%, respectively. In this regard, it is interesting to note that a substantial fraction of these carriers in *E. coli* are concerned with periplasmic protein disulfide bridge formation.

Auxiliary transport proteins (TC class 8) represent 2% of all integral membrane proteins in *R. baltica* and 3% in *E. coli*. Finally, proteins of unknown mechanism of action (TC class 9), identified in these studies, represent only 3% in *R. baltica* but 12% in *E. coli* (Tang and Saier, unpublished results). It is possible that the actual percentages in *R. baltica* are greater since being a member of a poorly characterized phylum, it may possess transporters belonging to families that are still not recognized.

Based on homology as revealed using G-BLAST and recorded in Table 1, the largest percentage of *R. baltica* transporters act on inorganic molecules (37%), primarily cations (27%) and anions (6%). Surprisingly, the second largest group exhibits specificity for

macromolecules (28% for *R. baltica*; 17% and 16% for *E. coli* and *M. tuberculosis*, respectively; Figures 2A and 2B and Table S2A). The vast majority of these macromolecular transporters are specific for proteins (22%), while those specific for polysaccharides, lipids and nucleic acids each comprises only 1-3%. *R. baltica* has one member each of families TC# 4.D.1 and 4.D.2, both of which may function in polysaccharide export. However these putative group-translocating systems have not been well characterized in any organism, and consequently their transport functions cannot be assigned with confidence [53]. By contrast, *R. baltica* exports many different proteins to the cell surface and extracellular medium. This observation is in agreement with the fact that it has a proteinaceous cell wall and with previously published data (see Introduction).

Roughly 9% of the transporters exhibit specificity for each of the following categories: (1) carbon sources, (2) amino acids and their derivatives, and (3) drugs and related compounds (Figure 2A). The break-down of these substrate classes into subclasses is depicted in Figure 2B.

3.2 Topological characteristics of transport proteins in R. baltica

When all of the integral membrane transport proteins of *R. baltica* retrieved in the G-BLAST searches with a BLAST cutoff e-value of 0.001 were analyzed for topology, the plot shown in Figure 3A was obtained. This figure is based on the data presented in Table S3. It can be seen that the numbers of proteins with 1, 6 and 12 TMSs are more numerous that the other topological types. Channels (Figure 3B) show a different distribution where putative 1 and 3 TMS proteins are more numerous than the others, a distribution unusual for bacteria where 6 TMS channels are usually more numerous than 3 TMS channels. Almost all channel proteins have between 1 and 7 TMSs, only a few having 11 to 13 TMSs, as is true for other bacteria.

Secondary carriers (Figure 3C) show just the opposite distribution with a large majority having between 10 and 14 TMSs. All of the proteins predicted to have 1 TMS are auxiliary proteins (mostly of RND superfamily exporters; TC# 2.A.6) while the proteins predicted to have 3 TMSs are either auxiliary proteins or underestimated mis-predictions. Some of the proteins predicted to have 4, 5 or 6 TMSs are authentic secondary carriers. All but one of the proteins predicted to have 7 TMSs are correctly predicted and belong to the Autoinducer-2 Exporter (AI-2E) Family (TC# 2.A.86). Proteins predicted to have 8 or 9 TMSs belong to various families and are probably correctly predicted ± 1 or 2 TMSs.

Primary active transport proteins have tremendously varied topologies (Figure 3D). Almost all of the 1 TMS proteins are receptors for ABC uptake transporters. Those predicted to have 4 to 7 TMSs are likely to be half ABC transporters that function with two integral membrane proteins that comprise the transport pathway. Proteins with 8 or more TMSs belong to several families including the ABC functional superfamily [54], the P-type ATPase superfamily [55], and various cation-transporting electron carrier families (see below). In this graph, it is apparent that in contrast to secondary carriers (Figure 3C), 10 TMS proteins predominate over 12 TMS proteins. This is due to the fact that many ABC permeases and P-type ATPases exhibit ten TMSs. The four graphs shown in Figure 3 therefore illustrate the dramatic differences between topological distributions in these different functional types of transport systems.

3.3 Channel proteins in R. baltica

Figure 1 shows the distribution of integral membrane transport proteins in *R. baltica* based on transporter class as defined in TCDB. *R. baltica* encodes within its genome a surprisingly large percentage of channel proteins for a prokaryote. Thus, 41 such proteins correspond to

12% of all integral membrane transport proteins identified. Thirty six of these proteins proved to be α-type channels while only 5 proved to be β-type porins. The number of α-type channels is much larger in *R. baltica* (10%) than in *E. coli* or *M. tuberculosis* where the percentage is approximately 3% (F. Tang and M. Saier, unpublished results). Large proportions of α-type channel proteins are characteristic of eukaryotes, especially animals and ciliates (U. Kumar and M. Saier, manuscript in preparation).

Four proteins gave top TC hits with voltage-gated ion channel (VIC) family members (TC# 1.A.1), all derived from prokaryotes. The first of these (Q7ULF5) has 6 TMSs, including both the voltage sensor and the two TMS channel domain [56]. Its top hits in TCDB were 6 TMS homologues from Gram-negative bacteria, with scores of 1×10^{-18} to 1×10^{-19}, showing that these proteins are divergent in sequence. As *R. baltica* belongs to the *Planctomycetes*, a distinct phylum with only distant relationships to most Gram-negative bacteria, this is not surprising. The closest related phyla are those in the recently delineated PVC superphylum (see Introduction). Such relationships were originally based on 16S rRNA but have been confirmed with 23S rRNA and genome-derived phylogenetic and indel analyses [57].

The second *R. baltica* VIC family member has an N-terminal 2 TMS channel domain followed by a large full-length TrkA domain with a nucleotide-binding Rossmann-fold motif, which probably serves a regulatory function. This domain could be an ATP binding domain as observed for the TrkH protein in *E. coli* [58]. The third VIC family member is unusual, having 3 probable N-terminal TMSs; this large protein (809 aas) exhibits a partial Rossmann-fold domain, followed by at least one RyR/IP3 repeat domain found in eukaryotic ryanodine receptors (TC# 1.A.3) [59, 60]. Finally the fourth member of this family is a 6 TMS protein with a typical 4 TMS voltage sensor domain followed by the usual 2 TMS channel domain with centrally localized P-loop. This domain structure is characteristic of almost all voltage-sensitive potassium channels found ubiquitously in living organisms.

R. baltica has two MIP family (TC# 1.A.8) members as do *E. coli* and many other bacteria, one being an aquaporin similar to those found in bacteria and plants, and the other being a typical prokaryotic glycerol facilitator. It also has three Amt family (TC# 1.A.11) ammonium channels. These three homologues were most similar to a proteobacterial, a cyanobacterial and a euryarchaeal Amt in TCDB. A single *Gemmata* homologue proved to be 47%, 32% and 28% identical to the three *R. baltica* homologues. Two of the *R. baltica* proteins were of normal size, but one was a larger fused protein with a C-terminal sensor kinase domain, presumably involved in regulation.

Three members of the epithelial chloride channel family (E-ClC; TC# 1.A.13), many of which are calcium-activated, are found in *R. baltica*, all resembling bacterial homologues in TCDB. Additional potential members of this family may exist, but they gave G-BLAST matches of greater than 0.001, and therefore were not considered further. One member of the Testis-Enhanced Gene Transfer (TEGT) Family (TC# 1.A.14) is found in *R. baltica*. It most resembles a human member of this TC family (1×10^{-9}), thought to function as a calcium channel [61, 62]. This degree of channel diversity is unusual for a prokaryote.

R. baltica has 8 mechanosensitive channels, one of the MscL family and 7 of the MscS family. Of the latter, two proteins exhibit 12 TMSs, three probably have 4 TMSs and two display 3 TMSs. MscS family members have tremendously varied topologies although the structures of only two small members of this family, each having four TMSs, have been determined at high resolution by x-ray crystallography [63, 64]. All of these MscL and MscS homologues are likely to function in osmotic adaptation since all members of the MscL and MscS families studied to date are involved in this phenomenon [63]. While *R.*

baltica has 1 and 7 members of these two types of channels, *E. coli* has 1 and 6 members. Therefore, there is no reason to suspect that these proteins play a compartmentalized role in *R. baltica*, although this possibility cannot be excluded.

R. baltica has representation in three families of divalent cation transporters, two members of the MgtE magnesium transporter family, one member of the CorA or MIT broad specificity divalent cation transporter family and one member of the MCU magnesium channel family [65]. Each of these three families probably serves a dissimilar function, MgtE functioning as a specific magnesium uptake system [66], CorA functioning as a general divalent cation channel [67], and MCU functioning as a magnesium channel, acting specifically in the assembly of an F-type ATPase (TC# 3.A.2) [65, 68].

R. baltica has 11 proteins belonging to the H^+- or Na^+-translocating Bacterial Flagellar Motor/ExbBD Outer Membrane Transport Energizer (Mot-Exb) Superfamily. Two of these proteins correspond to sodium-driven flagellar motor proteins, MotA and MotB. These proteins probably drive flagellar rotation using the sodium electrochemical gradient [69]. In addition, 9 proteins belong to the TolQ/TolR subfamily [70]. None apparently belongs to the ExbB/ExbD subfamily. Five TolQ homologues and four TolR homologues were identified. Each pair of these proteins functions together as a unit, so the smaller number of TolR proteins retrieved probably resulted because the TolR proteins are shorter and less well conserved than the TolQ proteins. TolB was found and is annotated as such in *R. baltica*, but it is highly sequence divergent compared to the *E. coli* protein, while TolA and Pal, other constituents of the *E. coli* Tol complex, were not found, even with PSI-BLAST iterations. In δ-proteobacteria, a TolQR pair functions in adventurous gliding motility [71], and it is possible that motility is a function of the TolQR proteins in *R. baltica*. Our results clearly show that this organism possesses multiple TolQR proteins, but we do not feel we can predict their functions. It should be noted that TolQ/TolR homologues in Gram-negative bacteria have been implicated in other functions such as maintenance of outer membrane integrity [72]. These observations are of particular interest in view of the unusual envelope structure of the Planctomycetes. These proteins may function in a capacity that differs from that in more traditional bacteria such as *E. coli*.

Very few outer membrane proteins were identified in *R. baltica*. One outer membrane porin belongs to the FadL family (TC# 1.B.9), members of which normally function in the transport of hydrophobic substance such as fatty acids in *E. coli* [73, 74]. *R. baltica* also exhibits multiple components of the outer membrane protein insertion complex, the OmpIP or Bam family complex (TC# 1.B.33) [75]. The fact that additional outer membrane porins were not identified could be due to the extensive sequence divergence observed for this class of proteins. Porins from one phylum are frequently so divergent from those in other organisms that they cannot be recognized using simple BLAST searches [76]. Alternatively, *R. baltica* may have few traditional outer membrane porins due to its unusual envelope composition. In *R. baltica*, the ratio of recognized beta to alpha channels is 0.14, while that in *E. coli* is 2.67 (Tang and Saier, unpublished results). Thus, compared to *E. coli*, *R. baltica* has almost no outer membrane porins. The presence of these few porin genes does not prove that they are functional. The dearth of outer membrane proteins in *R. baltica* was confirmed by use of the β-barrel outer membrane protein (BOMP) program as described in section 3.1.

3.4 Secondary Carriers in R. baltica

The largest superfamily of secondary carriers found in nature is the Major Facilitator Superfamily (MFS; TC# 2.A.1) [44]. Surprisingly, *R. baltica* has relatively few (13) such carriers (Tables 1 and 2). In *R. baltica*, there are only two MFS sugar uptake porters, but 4 drug exporters are present. Other MFS families are in general represented by only a single member. For example there is just one member each of the NHS, ACS, AAHS, ADT,

UMF18, AAA and POT families (Table 2). A most surprising observation is that *R. baltica* has an ATP:ADP antiporter (AAA), normally found only in obligatory intracellular pathogens [77]. It might allow energy exchange in a biofilm setting since close symbiotic relationships can involve energy exchange [78]. Alternatively, it could allow energy transfer between intracellular compartments. Evidence for such a function has been postulated in plants, where AAA family homologs are present in chloroplast membranes [79].

It is also surprising that *R. baltica* has two members of the Lysophospholipid Transporter (LplT) Family since most bacteria possessing this system have only one [48]. In *E. coli*, this protein facilitates the export of phospholipids for the extracytoplasmic acylation of lipoproteins. The resultant lysophospholipid is then transported back into the cytoplasm for reacylation using the LplT carrier. Possibly the presence of two such homologs reflects the *R. baltica* compartmentalized cell structure with two types of membranes, the CM and the ICM. It is interesting to suggest that as in eukaryotes, lipid signaling might be a mechanism for intercompartmental signaling in *R. baltica* [80].

The APC superfamily is well represented with 18 members, more than observed for the MFS (Table S1 and Table 3) [81]. However, only 5 members are specific for amino acids and their derivatives. Nine of these proteins belong to the Solute:Sodium Symporter (SSS) Family, members of which use sodium symport rather than proton symport for substrate uptake [81, 82]. Most recognized uptake systems of both the MFS and other members of the APC superfamily use proton symport. The occurrence of many SSS transporters is in agreement with the sodium-rich marine environment of *R. baltica*. Of the SSS family members, five exhibit specificity for sugars, but surprisingly, 3 of these may be specific for sialic acid, a sugar normally found on cell surfaces in glycoproteins and glycolipids. Perhaps this reflects the biofilm associations of *R. baltica* with kelp which is rich in glycolipids [83] and glycoproteins [84, 85].

The RND Superfamily is overrepresented in *R. baltica* with 18 members, the largest number for any superfamily of secondary carriers. Ten of these carriers are predicted to be multidrug efflux pumps, 3 export heavy metals, 3 export lipooligosaccharides, one (SecDF) facilitates protein secretion via the general secretory pathway (Sec translocon; 3.A.5), and one is of unknown function [86].

The DMT superfamily (TC# 2.A.7) is underrepresented with only 5 members, two of which export drugs, two of which take up metabolites and one of which is of unknown function. Of the remaining secondary carriers, most belong to families with a single representative in *R. baltica*. Exceptions include the CPA1 monovalent cation:proton antiporter family (TC# 2.A. 36) [87] with 4 members, two that may be Na^+:H^+ antiporters and two that may be K^+:H^+ antipoters. Di- and tri-valent cations may be taken up by the four members of the Nramp family of heavy metal ion transporters (TC# 2.A.55) [87, 88]. The MOP superfamily (TC# 2.A.66) [89] is well represented with 5 members exporting drugs, one exporting lipids and two exporting polysaccharides. MOP family members, like SSS family members, usually use the *smf* rather than the *pmf* to drive transport. The AI-2E family (TC# 2.A.86) [90] has 6 members in *R. baltica*, all of unknown function. Members of this family are known to take up signaling molecules such as quorum sensing autoinducers; these proteins could play a role in biofilm formation and communal life. In general, the predicted substrate specificities of the secondary carriers are not unusual for a bacterium.

3.5 Primary active transporters

Forty two integral membrane proteins of the ABC superfamily were retrieved by the G-BLAST program, but five of these proved to be either extra-cytoplasmic receptors of uptake systems or auxiliary membrane fusion proteins of export systems [91]. Thirteen display 8 –

12 TMSs and are therefore probably full length transporters, but the remaining 24 proteins have between 4 and 6 TMSs, suggesting that they occur as dimeric structures. We therefore predict that *R. baltica* has 25 to 30 complete ABC transporters. Of these systems, there are about twice as many exporters as importers (Table 1). The uptake systems (Table 4) are specific for monosaccharides (4 proteins; 4 systems), peptides (2 proteins; 1 system), inorganic phosphate (2 proteins; 1 system), heavy metals (1 or 2 systems), anions (2 systems) and a hydrophobic substance, possibly a lipid (1 system). Of the efflux systems (Table 5), about one third of the ABC families represented in *R. baltica* are of topological type 1, one third are of type 2 and one third are of type 3 [54]. Of these exporters, 7 systems probably transport drugs, 5 export lipids, 4 – 5 export peptides or proteins, 1 or 2 systems export polysaccharides and 2 systems export Na^+. ABC-mediated Na^+ export provides a mechanism to generate a sodium motive force (*smf*) using ATP hydrolysis as the energy source. It is interesting to note that all of the ABC efflux systems resemble systems found in prokaryotes; not one more closely resembles those found in eukaryotes [92; see TCDB].

F-type ATPases interconvert chemical energy and chemiosmotic energy by transporting either protons or sodium ions in response to ATP synthesis or hydrolysis [93]. Each such system has five hydrophilic subunits and three hydrophobic subunits. The latter subunits are the a-subunit (6 TMSs), the b-subunit (1 TMS) and the c-subunit (2 TMSs). G-BLAST retrieved six integral membrane subunits, two a-subunits, two b-subunits and two c-subunits. The top hits in all cases were the proteins of the sodium-transporting F-type ATPase of *Propionigenium modestum* [94, 95]. This observation suggests that unlike most bacteria, *R. baltica* has two F-type ATPases, both of which pump Na^+ by hydrolyzing ATP or utilize the *smf* instead of the proton motive force (*pmf*) to synthesize ATP. This fact is in agreement with the observation noted above that *R. baltica* has few proton-dependent secondary carriers (MFS and DMT families) while having a large number of sodium-dependent carriers of the SSS and MOP families.

We compared the MFS (TC# 2.A.1), which is always proton-coupled, with the SSS (TC# 2.A.21), which is always sodium-coupled. Striking differences were observed between terrestrial bacterial genomes compared to *R. baltica* in their ratios of SSS (sodium-dependent transporters) to MFS (proton-dependent transporters): 9 SSS : 13 MFS in *R. baltica;* 2 SSS : 8 MFS in *Leptospira interogans*; 1 SSS : 10 MFS in *S. pneumoniae;* 0 SSS : 8 MFS in *Streptococcus pyogenes* and 4 SSS : ~100 MFS in *E. coli* depending on the strain. The average ratio (SSS/MFS) for the three terrestrial organisms with representation in both the SSS and the MFS families was 0.13 but was 0.69 for *R. baltica*. Additionally, all of these strains possess proton-dependent F-type ATPases rather than the sodium-dependent ATPases found in *R. baltica*. Constituents of V-type ATPases, important in vacuolar acidification in eukaryotes [96], were not identified in *R. baltica*. However, subunits of bacterial V-type ATPases, homologous to those present in *Enterococcus hirae* (3.A.2.2.2) were found in other species of *Rhodopirellula* (*R. europaea*, *R. maiorica* and *R. sallentina*).

We selected five sequenced marine bacteria: *Synechococcus* sp. strain WH8102 (BioProject PRJNA230), *Ruegeria pomeroyi* (formerly known as *Silicibacter pomeroyi*) (BioProject PRJNA57863), *Hahella chejuensis* (BioProject PRJNA58483), *Pseudoalteromonas haloplanktis* TAC125 (BioProject PRJNA15713) and *Vibrio vulnificus* YJ016 (BioProject PRJNA58007) for comparative studies. They contained 2517, 4252, 6773, 3486, and 5023 known proteins, respectively. Each protein set was blasted, using BLASTP with a cutoff of 1e-20, against TCDB on June 14, 2013. Only the top hit for each marine bacterial protein was recorded (using the command uniq). For each organism, the number of known proteins identified as SSS (TC# 2.A.21) or MFS (TC# 2.A.1) was recorded. In addition, the set of proteins in each organism recognizable as an F-type, V-type or A-type ATPase of the F-

ATPase Superfamily (TC# 3.A.2) were examined for matches to either H^+- or Na^+-translocating ATPases.

In *Synechococcus* sp., the SSS to MFS ratio was 0 SSS (TC# 2.A.21) : 2 MFS (TC# 2.A.1), a surprisingly low content of members of both families. In *Ruegeria pomeroyi*, the ratio was 7 SSS : 10 MFS, a ratio comparable to that of R. baltica. In *Hahella chejuensis*, the ratio was 16 SSS : 19 MFS, displaying a somewhat higher proportion of SSS family members compared to *R. baltica*. In *Pseudoalteromonas haloplanktis* TAC125, the corresponding ratio was 13 SSS : 12 MFS, higher than for *R. baltica*. In *Vibrio vulnificus* YJ016, the ratio was 13 SSS : 22 MFS, lower than for the other marine bacteria. These results show that while *R. baltica* has a high ratio of SSS : MFS, this ratio is also high in other marine bacteria. Of these five marine bacteria, only *Hahella chejuensis* and *Pseudoalteromonas haloplanktis* have SSS : MFS ratios higher than *R. baltica*. The average for the four organisms with representation in both the SSS and the MFS was 0.75 and that for *R. baltica* 0.69 showing that *R. baltica* has a ratio typical of other marine bacteria. All of the marine bacteria examined have only proton-dependent F-ATPases, except *Hahella chejuensis* which has both proton- and sodium-dependent F-ATPases. This shows that *R. baltica*'s possession of two sodium-pumping F-ATPases is unusual compared to the other marine bacteria examined.

R. baltica also has a sodium-exporting decarboxylase (TC# 3.B.1) [97, 98] that provides even more evidence that it uses the sodium currency rather than, or in addition to, the proton currency. The reason why *R. baltica* has two sodium-pumping F-type ATPases instead of one (very few have more than one) is a mystery. Perhaps it allows *R. baltica* to optimally produce energy in two different developmental stages (e.g., the motile stage and the sessile stage), in two different cell compartments, or under different environmental conditions (see Discussion).

R. baltica has three P-type ATPases [99]. The first belongs to family 5 (TC# 3.A.3.5), and therefore functions in the transport (probably export) of copper ions. The second belongs to family 25 (TC# 3.A.3.25), members of which are of unknown function, although a possibility is that these proteins transport iron, an essential element for synthesis of active electron flow carriers. The third belongs to family 27 (TC# 3.A.3.27), which has been reported to function in the insertion of copper into cytochrome oxidase and does not pump this ion across the membrane [100]. By virtue of these recognized and putative functions, we propose that the functions noted above for their homologues correspond to the functions of these transporters in *R. baltica*.

All living organisms so far examined possess a functional general secretory complex, the Sec translocon (TC# 3.A.5) [101], and *R. baltica* is no exception. This organism also has a complete twin arginine targeting (Tat) system (TC# 2.A.64). In addition, we identified multiple integral membrane protein constituents of several protein secretion systems. These include the type III protein secretion system (TC# 3.A.6) [102], the type IV protein secretion system (TC# 3.A.7) [103, 104] and the main terminal branch (MTB; type II) protein secretion system (TC# 3.A.15) [105]. A large number of integral membrane MTB constituents (52) are encoded within the *R. baltica* genome, suggesting that *R. baltica* has multiple such systems. Since MTB systems normally export proteins exclusively across the outer membranes of Gram-negative bacteria following secretion across the inner membrane by the general secretory pathway (Sec translocon; TC# 3.A.5), the presence of multiple systems suggests that these systems in *R. baltica* may exhibit specificity for their protein substrates. Just one protein of the type VI protein secretion system (TC# 3.A.23) [106] was identified, but since some of the constituents of these systems are homologous to

constituents of other protein secretion systems, it should not be concluded that *R. baltica* has a type VI secretion system.

Of these protein secretion systems, type IV secretion systems can also export DNA, thereby mediating conjugation. *R. baltica* also has a DNA-T competence system (TC# 3.A.11) [107] for the uptake of DNA during transformation and an ATP-dependent S-DNA-T septal DNA translocase (TC# 3.A.12) for pumping DNA across the bacterial cell division plane (in this species, through the bud neck during budding division) when septation is completed before DNA transfer [108]. Thus, *R. baltica* has a remarkable set of protein and DNA transport systems.

Subclass 3.D. in TCDB includes cation (H^+ and Na^+) translocating electron flow carriers. *R. baltica* includes proteins of TC families 3.D.1 (proton-translocating NADH dehydrogenase; NDH), TC# 3.D.2 (proton-translocating transhydrogenase; PTH) and TC# 3.D.4 (proton-translocating cytochrome oxidase). All of these complexes in *R. baltica* probably expel protons from the cytoplasm (most probably from the paryphoplasm across the cytoplasmic membrane but conceivably also from the riboplasm across the ICM), although the possibility of Na^+ extrusion by some of these systems cannot be excluded. Finally, *R. baltica* possesses a succinate dehydrogenese complex (SDH; TC# 3.D.10) which oxidizes succinate while reducing a quinone. The presence of this last system shows that *R. baltica* has the full complement of electron transport complexes found in mitochondria and α-proteobacteria [109].

3.6 Transmembrane electron flow carriers

R. baltica possesses 5 homologues of transmembrane electron flow carriers. Two of these belong to TC family 5.A.1 (DsbD), which function in periplasmic sulfhydryl oxidoreduction in *E. coli* [110]. Two others belong to TC family 5.A.3 (PMO), one which is probably a dimethylsulfoxide (DMSO) reductase while the other may be a trimethylamine N-oxide (TMAO) reductase [111]. The presence of both of these oxidoreductases is consistent with *R. baltica* living in a marine environment since these compounds are normally produced by marine animals [112], and these reductases are common in marine bacteria [113]. Additionally, *R. baltica* possesses a sodium-pumping NDH (TC# 3.D.5) [114, 115], found in relatively few bacteria. This provides even more evidence that this organism relies heavily on the *smf*. All of these observations are consistent with the conclusion that *R. baltica* generates and uses an *smf* as a primary source of chemiosmotic energy.

3.7 Auxiliary transport proteins

R. baltica has at least 3 Membrane Fusion Proteins (MFP; TC# 8.A.1) and 2 Cytoplasmic Membrane-Periplasmic Auxilliary-1 (MPA1; TC# 8.A.3) proteins [116-118]. The 3 MFPs may facilitate the efflux of aromatic acids and drugs while the two MPA1 proteins facilitate the export of complex carbohydrates via polysaccharide transporters of the PST family within the MOP superfamily (TC# 2.A.66) [89]. The last two auxiliary proteins belong to the stomatin family (TC# 8.A.21). One of these proteins is a stomatin homologue and the other is a protease. These two proteins, found ubiquitously in the three domains of life, may function together for the processing of integral membrane proteins and the activation of channels and transport proteins in eukaryotes where they have been studied most extensively. The fact that these proteins are found in *R. baltica* does not indicate a connection with eukaryotes as these proteins are common in bacteria [119, 120].

3.8 Putative transporters of unknown mode of action

Five families are represented in subclass 9.A, known transporters of unknown mechanism of action. The first of these five families is the FeoB family (TC# 9.A.8) of iron uptake

permeases with 1 member [121]. The second family is the MOM-IP family (TC# 9.A.24) [122] of the mitochondrial outer membrane insertion pathway (2 members). The third is the SdpAB family of peptide toxin secretion systems (TC# 9.A.3; 1 member) [123]. The fourth is the HCC family of probable divalent cation channels (TC# 9.A.40; 2 members) [124]. The last is the TadC family of pilus biogenesis proteins (TC# 9.A.47; 2 members) [125; see descriptions of these families and proteins in TCDB]. Constituents of the MOM-IP family are found in bacteria as well as mitochondria, and the presence of an SdpAB system, the peptide antibiotic-like killer factor exporter, suggests that *R. baltica* may participate in fratricide and cannibalism when living communally [126].

Twenty five proteins identified in *R. baltica* resemble members of subclass 9.B, putative transporters of unknown mechanism. Several of these are worthy of note. These families/systems include HHP [9.B.14; heme handling protein; required for heme production; 127], YajC [9.B.18; auxiliary protein for SecDF; 128], MgtC [9.B.20; possible Mg2+ uptake systems; 129], SdpI [9.B.32; SdpC immunity protein; see also the preceding paragraph concerning SdpAB; 123], CstA [9.B.59; carbon starvation protein; putative peptide transporter; 130], Pbr [9.B.105; lead resistance protein; 131], YhjD (TC# 9.B.126; putative lipid exporter) and WzyE (TC# 9.B.128; putative polysaccharide exporter). Most of the *R. baltica* proteins in subclasses 9.A and 9.B retrieved have prokaryotic homologues as top hits in NCBI BLAST searches, showing that these proteins are typical of transporters in the bacterial domain. Of the 33 proteins found in TC class 9, 14 hit TC entries from proteobacteria, 7 from euryarchaea, 5 from firmicutes, 3 from animals, and 1 each from species of bacteroidetes, actinobacteria, cyanobacteria, and plants. Thus only four of the 33 class 9 proteins in *R. baltica* most closely resembled eukaryotic homologues, and when these four *R. baltica* proteins were subjected to NCBI Blast searches, bacterial homologues were retrieved as top hits.

3.9 Putative Transporters with low to negligible sequence similarities to previously existing proteins in TCDB

In the analyses reported above, the *R. baltica* proteome was screened against all proteins included in TCDB as of December 2012, and hits with scores of 0.001 or less were recorded and analyzed. In this section, we report on proteins retrieved with scores between 0.001 and 0.1. 328 proteins were retrieved, and as expected, almost all of them were false positives. We were, however, able to identify 21 proteins which could either be shown to belong to one of the pre-existing TC families or were considered to be potential transport proteins. These 21 proteins proved to either comprise novel subfamilies within the existing families or were assigned to novel TC families in subclass 9.B, putative transporters of ill-defined function. These proteins are listed in Table 6 according to their TC#s, together with their UniProt accession numbers and relevant information.

The first of these proteins belongs to the Major Facilitator Superfamily (MFS) [44] and was assigned TC# 2.A.1.77.1. This represents a new subfamily of the MFS. As expected for an MFS permease, it has 12 TMSs in the usual 6 plus 6 TMS arrangement [44]. Genome context analyses did not provide clues as to its function.

Four of the newly discovered proteins proved to belong to the Drug Metabolite Transporter (DMT) Superfamily [132, 133]. Two of these are distant members of the DME family, and were assigned TC#s 2.A.7.3.45 and 46. The gene encoding the former was adjacent to one encoding a cytoplasmic inosine/uridine-preferring nucleoside hydrolase, suggesting that this transporter could be a nucleoside uptake permease. The gene encoding the latter protein was adjacent to one encoding a secreted DUF1501 protein (Q7UPP6) that could be a sulfatase. This transporter might take up sulfate or an organic product of the esterase reaction. Genome context analyses did not provide clues as to the function of TC# 2.A.7.29.1, but the protein

assigned TC# 2.A.7.30.1 (Q7URM2) is encoded by a gene in a gene cluster with three secreted hydrolases, (1) an α/β hydrolase (Q7URM1), (2) a putative gluconolactonase (Q7URM0) and (3) a membrane-integrated metaloprotease (Q7URM3). α/β-hydrolase superfamily members include peptidases, lactonases and lipases. It seems likely that one or more product(s) of these hydrolase reactions is/are the substrate(s) of the transporter.

One of the newly discovered *R. baltica* proteins belongs to the 4-Toluene Sulfonate Uptake Permease (TSUP) family [134]. It was assigned TC# 2.A.102.2.3. Known transporters within this subfamily of the TSUP family are believed to export sulfite and sulfoacetate, and operon analyses have suggested that most members of this family transport sulfur-containing compounds [134]. It is probable, therefore, that the *R. baltica* protein transports one or more small sulfur-containing compounds. Interestingly, the gene encoding this transporter is adjacent to another putative transporter (Q7URC1) assigned to the TerC family with TC# 9.A.30.5.2 (see below).

Two proteins identified proved to belong to the ABC-2 topological type [54] of the ATP-binding Cassette (ABC) Superfamily. One *R. baltica* ABC transporter, assigned TC# 3.A.1.105.8, has six or seven TMSs and is a member of the Drug Exporter-1 (Drug E1) family, several members of which export various antibiotics [54]. Because the two genes encoding this system are adjacent to a squalene-hopenecyclase (Q7UE59), the natural substrate of the transporter could be a triterpene. The second of these ABC system, assigned TC# 3.A.1.132.7, is a member of the Gliding Motility (Gld) Family. Although the functions of members of this family have not been unequivocally assigned, one has been suggested to be an exopolysaccharide exporter (TC# 3.A.1.132.1; [135]), while another has been proposed to be a copper exporter (TC# 3.A.1.132.2; [136]). Genome context analyses revealed that the gene encoding the *R. baltica* homologue is flanked by two genes, one of which (Q7UXN6) codes for a lysine-2,3-amino mutase and the other (Q7UXN4) an epimerase. Possibly Q7UXN5 codes for an exporter specific for a lysine derivative. No ATPase was encoded next to this putative transporter.

The remaining proteins identified belong to TC class 9, putative transporters of unknown mechanism of action [49, 137]. The first two of these belong to family TC# 9.A.30, the Tellurite Resistance (TerC) family. Few members of this large family have been functionally characterized. Tellurite-resistant bacteria take up tellurite and convert it to black metallic crystals of tellurium, yielding black colonies on agar plates [138]. However, the available evidence suggests that members of the family may be diverse in function; thus, little can be said about the specific substrates of the two *R. baltica* proteins. They have been assigned TC#s 9.A.30.5.1 and 2.

Two proteins in *R. baltica* were identified as members of the DedA family (TC# 9.B.27). Members of this family have 5 or 6 putative TMSs. *E. coli* DedA proteins have been implicated in selenite transport [139], and a homologue in *Formitopsis palustris* appears to play a role in oxalate secretion [140]. It has been proposed that various DedA homologues play essential roles in membrane homeostasis in some bacteria [141]. These proteins are also related to SNARE-associated proteins in eukaryotes. The *R. baltica* homologues have been assigned TC#'s 9.B.27.1.3 and 9.B.27.1.4.

A member of the Rhomboid protease family was identified and assigned TC# 9.B.104.5.1. These 6 TMS enzymes (N- and C- termini inside) cleave integral membrane substrate proteins [142]. High resolution x-ray structures of these proteins are available, revealing an active site cavity that could allow release of products into either the cytoplasm or the external milieu [143]. It would be interesting to know in which membrane(s) the *R. baltica* protein is found and which orientation it assumes.

A member of the Lead Resistance Fusion Protein (PbrBC) Family was identified in *R. baltica* and assigned TC# 9.B.105.1.4. A role of homologues in *Ralstonia (Cupriavidus) metallidurans* as potential Pb^{2+} exporters has been suggested [131]. However, members of this family show limited sequence similarity to integral membrane phospholipid phosphatases and signal peptidases. Consequently, it is not possible to assign the function of the *R. baltica* protein. However, it is interesting to note that the gene encoding the *R. baltica* protein is adjacent to a putative RND lipid exporter (Q7UF33) assigned to TC# 2.A.6.7.6. Thus, it could be a phospholipid phosphatase.

Two proteins appear to be integral membrane glycosyl transferases, one representing a new subfamily of TC family 9.B.142 and assigned TC# 9.B.142.9.1, and the other representing a new family, the MurG Family (TC# 9.B.146), involved in peptidoglycan biosynthesis. This second *R. baltica* protein was assigned TC# 9.B.146.1.1. Several integral membrane glycosyl transferases have been shown to function in a group translocation process in which the transfer reaction is coupled to export of the substrate from the cytoplasm with release of the product to the external cell surface [53, 144]. The *murG* gene is adjacent to the *natAB* genes encoding one of the *R. baltica* Na^+ extrusion ABC pumps (TC# 3.A.1.115.2; see Table 1).

Remaining integral membrane proteins identified in *R. baltica* appear to represent founding members of novel TC families, all of unknown function. These have been assigned TC#'s 9.B.144.1.1 (the DUF 3367 Family), 9.B.145.1.1 (the DUF 389 Family), 9.B.147.1.1 and 9.B.147.2.1 (named the 10-IMP Family) and 9.B.148.2.1 (named the 4-DMT Family). The DUF 3367-encoding gene is adjacent to a cyclic diGMP hydrolase-encoding gene, suggesting a role in biofilm regulation. The DUF 389 protein gene is adjacent to a glucosamine 6-P deaminase gene, suggesting a role in amino-sugar metabolism; the 10-IMP family proteins are both adjacent to glycohydrolase genes, suggesting a role in carbohydrate metabolism, and one of them (QTUER8) is also adjacent to a putative sulfatase, again suggesting a role in extracellular complex carbohydrate metabolism.

The 4-DMT family entry (TC# 9.B.148.2.1) showed limited sequence similarities with members of the DMT Superfamily (TC# 2.A.7), but homology was not established. Interestingly, most members of this novel 4-DMT family are from Actinobacteria.

A few identified integral membrane proteins had homologues only in *Rhodopirellula* species. None of them contained recognizable conserved domains in the conserved domain database (CDD). These genus-specific proteins were not studied further.

4. Discussion

In this communication, we have provided the first comprehensive analysis of the complement of transport proteins found in a marine Planctomycetes. Not surprisingly, considering the unique cellular characteristics of members of this phylum, their unusual ecologically important associations with other organisms and their importance as agents of marine environmental regeneration, we find a set of transporters never observed for a previously studied organism. First, we find many systems that allow *R. baltica* to tolerate and flourish in a marine environment. The presence of DMSO and TMAO reductases allows *R. baltica* to generate reducing power from compounds prevalent in the oceans.

Second, *R. baltica* has numbers of sodium-dependent uptake transporters with a corresponding diminution in the numbers of proton-dependent systems as observed for other marine bacteria. Third, we find a wide variety of Na^+-extrusion porters that use primary sources of energy (ATP, organic carboxylic acids, electron donors) to generate an *smf*.

Fourth, *R. baltica* appears to have a full set of proton-extruding electron carrier complexes to generate a *pmf*. Fifth, it has Na^+:H^+ and K^+:H^+ antiporters potentially for the interconversion of the *pmf*, *smf*, and potassium electrochemical gradients. Sixth, this organism apparently has the cannibalizing SdpAB, SdpI and SdpC proteins, previously recognized only in a restricted group of biofilm-forming firmicutes [123, 126]. Finally, it has apparently duplicated transport systems such as Na^+-transporting F-type ATPases, Na^+-extruding ABC transporters and both Na^+:H^+ and K^+:H^+ antiporters. These characteristics are illustrated schematically in Figure 4, assuming that their significance reflects the *R. baltica* compartmentalized cell structure. Alternatively, the dual occurrence of these similar pairs of transporters could be used for transport during the two phases of their dimorphic life cycle, the motile free swimming stage and the sessile biofilm stage as form on the surfaces of macroalgae. It is likely that many genes are expressed in only one, but not both, of these two stages. The use of the *smf* as the primary chemiosmotic form of energy is consistent with the marine environment of *R. baltica*.

The speculative model presented in Fig. 4 proposes that the duplication of several types of sodium-transporting systems reflects the unusual compartmentalization of the *R. baltica* cell. This schematic diagram suggests that *R. baltica* can create a membrane potential across both the CM and the ICM using one each of the pair of enzymes identified for sodium pumping and electrochemical gradient interconversion. Thus, this scheme suggests that the two distinct F-type ATPases are present in two dissimilar membranes, as is also proposed for the two sodium pumping ABC transporters. The model can serve as a template for future experimentation, e.g. regarding membrane potential generation across the ICM as well as the CM, and locations of transport proteins using immunogold/electron microscopy or membrane fractionation combined with proteomics.

van Niftrik *et al*. [145] demonstrated that the F-type ATPase of the anammox Planctomycete *Kuenenia* is associated with both the ICM and the anammoxosome membrane. This fact is possibly relevant to *R. baltica*, even though the former anaerobic Planctomycete is rather specialized for ammonium metabolism. It does have the fundamental shared structure of other Planctomycetes with an ICM and a paryphoplasm as well as a cytoplasmic membrane. Since the ATPase in this organism is present in both the ICM and the anammoxosome membrane, it is reasonable to suggest that the two *R. baltica* enzymes function to energize both the CM and the ICM.

Several features of *R. baltica* are worthy of note. (1) Although our understanding of the utilization of DMSO and TMAO for bacterial growth in a marine environment is incomplete, it is understandable that these compounds would be used for energy given their prevalence in marine environments. This same environment explains the use of the Na^+ currency rather than (or in addition to) the H^+ currency as a primary type of chemiosmotic energy. (2) The ability of *R. baltica* to extrude Na^+ using three distinct primary sources of energy allows it to utilize its marine environment to maximal benefit while protecting itself from the potentially deleterious effects of high intracellular Na^+.

(3) Its proton-extruding electron carriers provide a *pmf*, allowing the use of some types of transporters, especially exporters, that cannot or do not use the *smf*. (4) The presence of Na^+:H^+ and K^+:H^+ antiporters (which may also be capable of functioning by Na^+:K^+ antiport) allows the regulated interconversion of three forms of chemiosmotic energy, conferring upon these organisms flexibility of energy utilization. (5) The presence of a complete Sdp “cannibalism/fratricide” system, like that characterized in *Bacillus subtilis*, may be expected to play an important role in relegating responsibilities to different members of the bacterial community in complex biofilms although such a suggestion must be considered tentative in the absence of direct evidence for *R. baltica*. (6) The apparent

duplication of the Na^+-translocating F-type ATPase, a Na^+-pumping ABC exporter, a Na^+:H^+ antiporter and a K^+:H^+ antiporter may either allow these organisms to independently control the ionic compositions of two different compartments in the cell, as suggested in Figure 4, or to control these compositions under different growth conditions such as the free swimming versus the sessile state.

All of these observations and postulates provide a wealth of information seeking documented explanations and fertile ground for further investigation. The unique features of *R. baltica* as the model system for understanding the life styles of marine Planctomycetes are just now coming to be understood. Nevertheless, it should be noted that the transport features of *R. baltica* may not be shared aspects of Planctomycetes in general, since the environments of these organisms vary from terrestrial soil, to freshwater, to termite guts, to marine and hypersaline solutions. Some of the properties described here, particularly the importance of Na^+-dependent transporters, may therefore be specific to marine bacteria.

The types of transporters identified and the closest homologues previously identified in other organisms proved to be typical of the bacterial domain with few exceptions. They therefore do not represent horizontally transferred genes from eukaryotes, and they do not imply a recent common origin or shared genomic composition with eukaryotes. This conclusion agrees with that of Fuchsman & Rocap [146], as well as of Jogler *et al*. [147]. However, although almost all of the top hits of *R. baltica* transporters in TCDB proved to be of bacterial rather than eukaryotic origin, this topic warrants further consideration. An evolutionary model for cell organization involving reductive evolution of Planctomycetes from a complex proto-eukaryote-like last universal common ancestor has been considered [148]. Moreover, alternative models for the origins of the unique Planctomycete cell plan have been presented. Overall, the structural and molecular evidence may not be consistent with convergent evolution of eukaryote-like features in a bacterium [148].

R. baltica is not believed to have a typical Gram-negative bacterial outer membrane, and it lacks the full complement of enzymes required for peptidoglycan cell wall biosynthesis. Still, it has the rudiments of these enzymes, presumably reflecting of its bacterial ancestry. Additionally, we found the constituents of several outer membrane transporters characteristic of two-membrane envelope Gram-negative bacteria. These proteins are lacking in Gram-positive bacteria possessing a single membrane. Thus, *R.baltica* has (1) outer membrane porins such as FadL, (2) several TolRQ systems that might function for energizing transport across an outer membrane and ensuring outer membrane stability [149] and (3) an OmpIP (BAM) outer membrane β-structural porin insertion apparatus [75]. Nothing is known of the trafficking of membrane transporters in Planctomycetes, but these organisms have several protein secretion systems as indicated in Table 1 under TC# 3.A. These include the general secretory pathway (Sec translocase), which normally exports proteins across bacterial cytoplasmic membranes, and the main terminal branch (type II secretion system), which normally transports proteins across the outer membranes of typical Gram-negative bacteria such as *Klebsiella pneumoniae* [150]. They also have ABC-type protein exporters that together with auxiliary proteins allow export of proteins across both membranes of the Gram-negative bacterial cell envelope in a single energy-coupled step. Other protein secretion systems appear to be incomplete and are probably non-functional. It should be noted that we do not know that the genes encoding putative outer membrane proteins and their insertion/secretion complexes are functional in *R. baltica*. In fact, very few outer membrane β-barrel proteins were found in *R. baltica* compared to well-characterized Gram-negative bacteria. However, one would expect that if these genes are non-functional, they would have been lost or converted to pseudogenes rather than exhibiting the characteristics of intact functional genes as appears to be the case [151]. The observation that outer membrane proteins equivalent to those of other Gram-negative

bacteria are present suggests to us that there must be a functional basis for retention of certain genes including those for outer membrane porins, the outer membrane insertion apparatus and type 2 outer membrane secretion systems (the main terminal branch). While it is possible that these represent the residue of an ancestral organism that did have a cell wall and outer membrane, it is also possible that the Planctomycetes have utilized these proteins for a function unique to organisms of this phylum.

Many questions remain concerning the compartmentalization and biogenesis of the different membranes identified in *R. baltica* and other *Planctomycetes*. It is known that *R. baltica* encodes within its genome more than 100 sulfatases that presumably function in the degradation of the extracellular sulfate-containing polysaccharides of macroalgae [152]. We have examined several of these sulfatases and have found that they do in fact have N-terminal signal sequences, and that P-SORT predicts many of them to be periplasmic proteins. They undoubtedly are exported from the cytoplasm via the general secretory pathway (Sec translocon). However, it is not possible at this point to predict in which of the membranes in *R. baltica* the Sec translocon resides, although several cell surface proteins have been identified [12] and the *R. baltica* proteome has been analyzed in some detail [10, 37, 40, 153]. Further, there is no information as to whether the transporters described in this report and the protein secretion/insertion apparati responsible for their biogenesis are enriched in one or more of the membrane compartments shown in Figure 4. Thus, we have little information about the subcellular localization of these systems.

Further experimentation will be necessary to provide clues as to the trafficking and insertion of membrane proteins that are the substrates of the *R. baltica* secretion/insertion systems discussed in Results section 3.5. Is it naive to assume that each membrane will prove to possess unique secretory systems with multiple main terminal branches? Perhaps some of the protein secretion systems identified in this report will prove to reflect protein translocation between compartments as well as to the extracellular environment. Alternatively, is there reason to believe that these systems will serve the same functions during the different lifecycle stages of this organism [6]? These open questions indicate that *R. baltica* provides fertile waters for further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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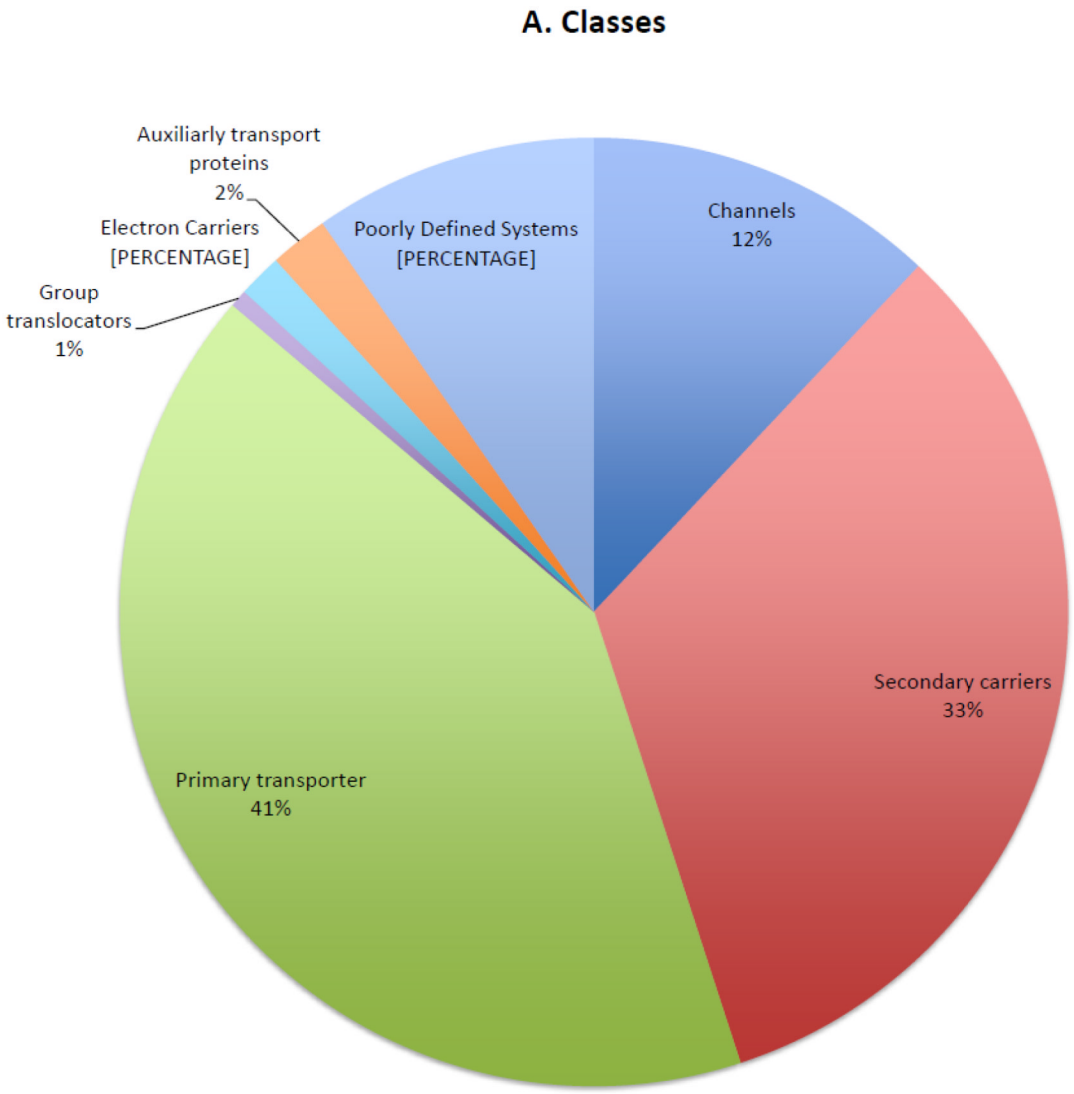
- *R. baltica* is an unusual prokaryote with intracellular membranes.
- *R. baltica* prominently uses the sodium currency (the sodium motive force, smf).
- F-type ATPases, ABC pumps, flagella, dehydrogenases and decarboxylases transport Na^+ rather than H^+.
- Genome analyses of transport proteins have revealed unprecedented duplications.
- Duplicated systems include Na^+-F-ATPases, Na^+-ABC pumps and Na^+:H^+ and K^+:H^+ antiporters.



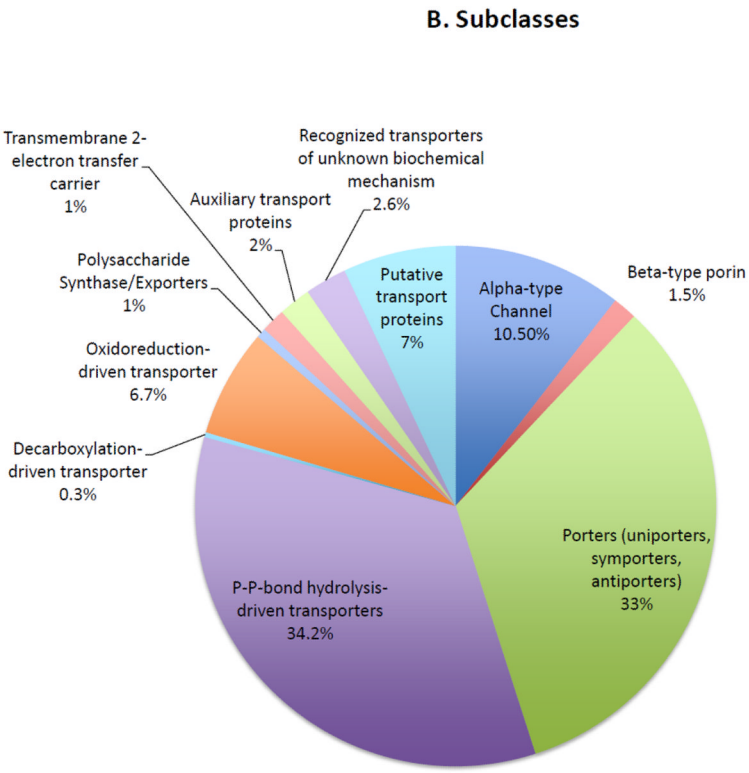


Figure 1.
Distribution of transporters based on TC (A) classes and (B) subclasses in *R. baltica*.

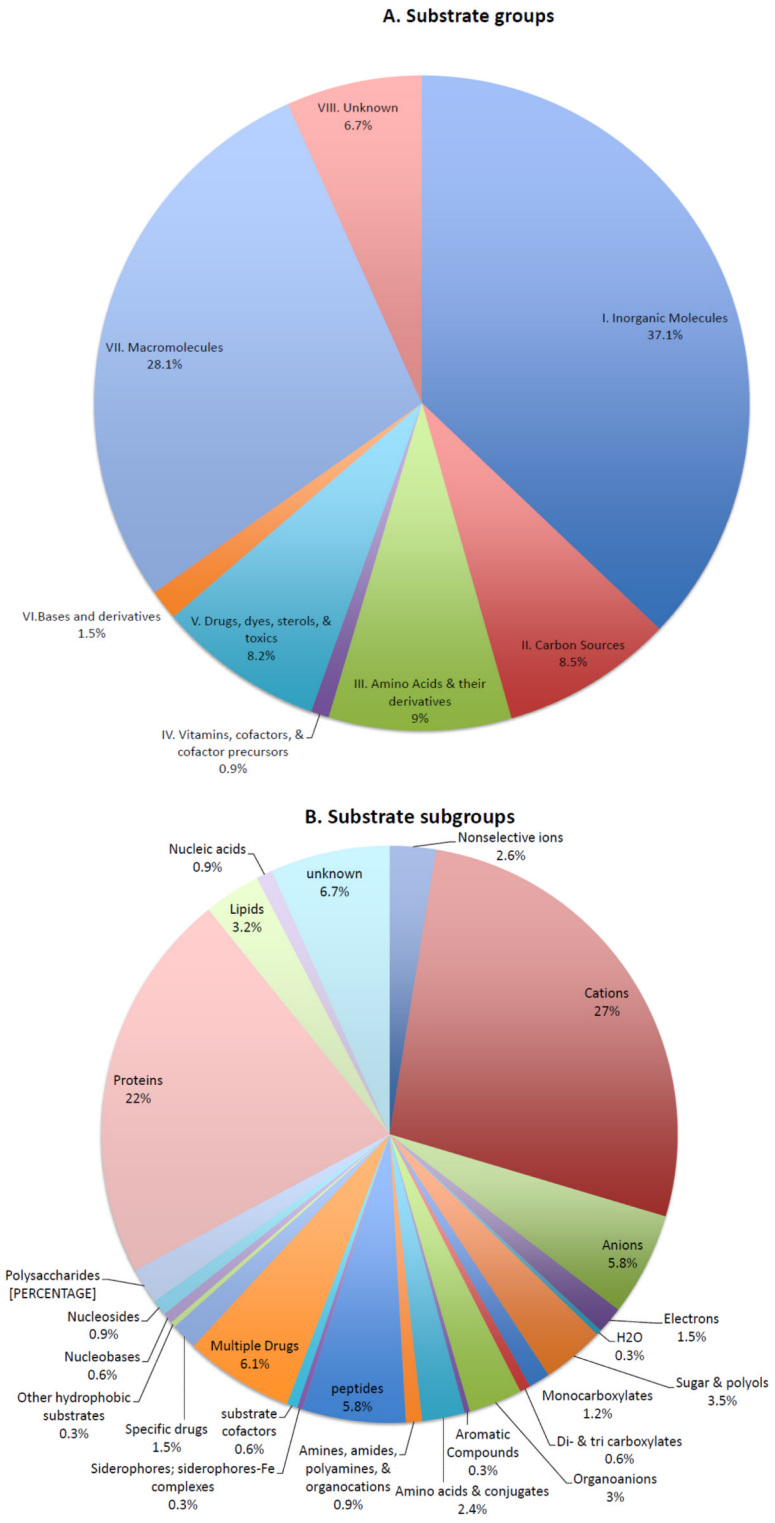
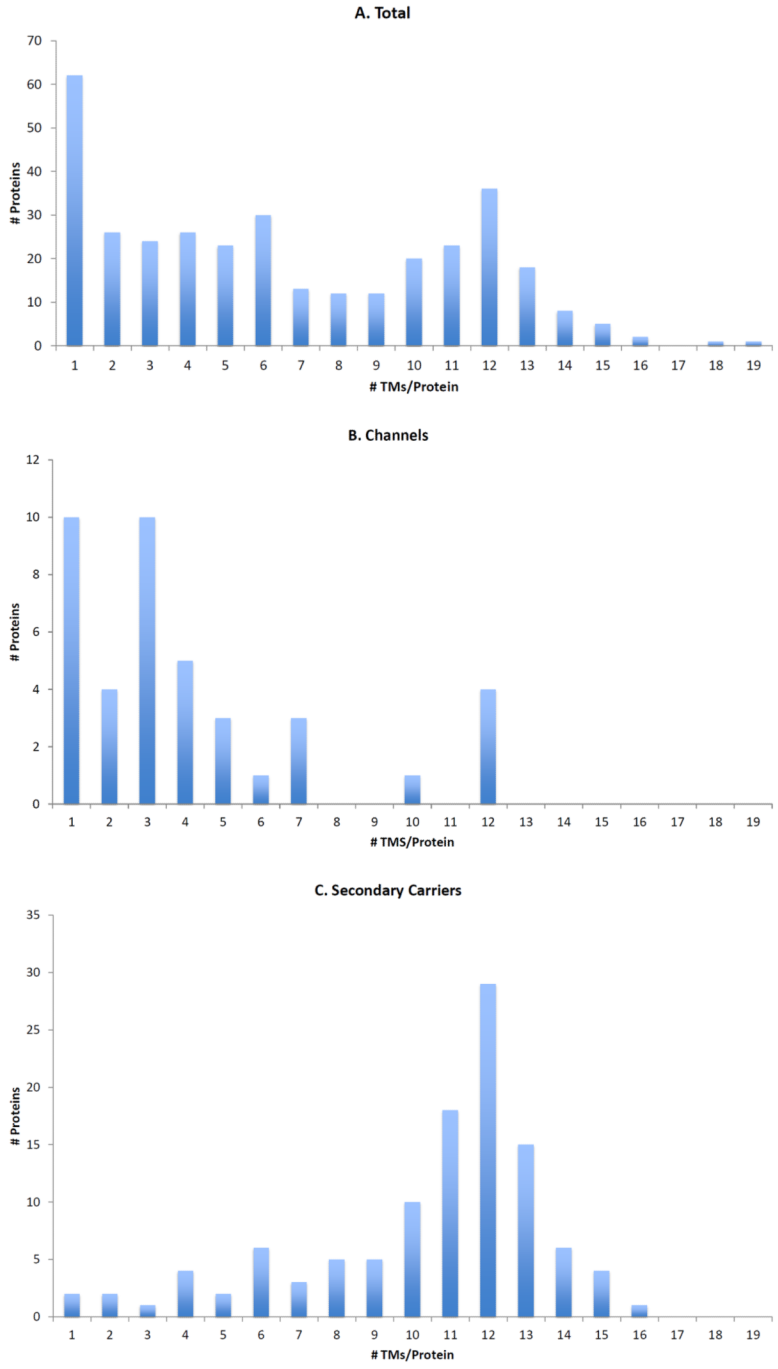


Figure 2.
Transporter distribution based on (A) substrate groups and (B) subgroups in *R. baltica*.



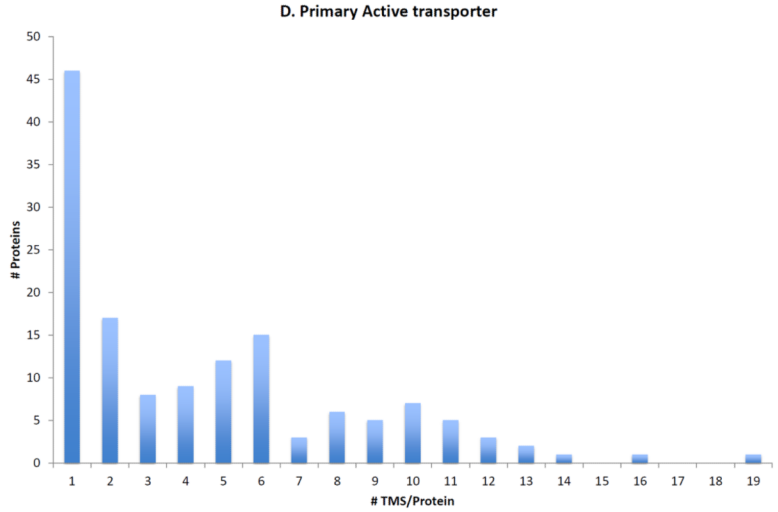


Figure 3.

Topological analysis of (A) all *R. baltica* integral membrane transport proteins included in this study, (B) channels, (C) secondary carriers and (D) primary active transporters. Proteins predicted to have 0 TMSs in either the query or the hit sequence were eliminated from this study as described under Methods (section 2.2).

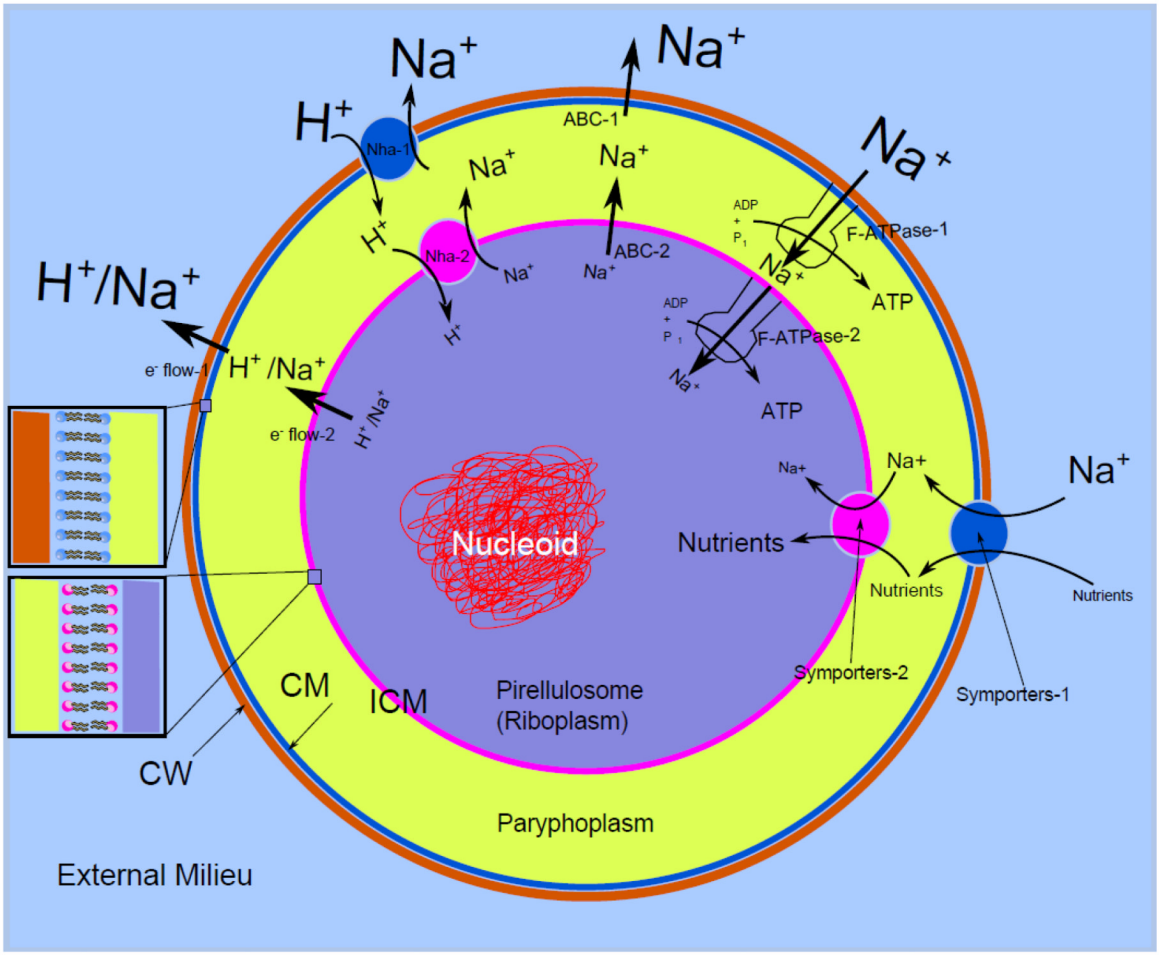


Figure 4.

Schematic interpretation of the unusual dual occurrence of several Na^+-transport systems in the compartmentalized *R. baltica* cell. The cell wall (CW) is color coded brown. F-type ATPases are proposed to be present in both the intracellular membrane (ICM; color coded green) and the cytoplasmic membrane (CM; dark blue) as has been documented for the two membranes in *Candidatus Kuenenia stuttgartiensis* [145]. This would imply that there is a proton motive force (*pmf*) and/or a sodium-motive force (*smf*) across both membranes which might also suggest that both membranes contain constituents of electron transfer chains although this has not been demonstrated for *R. baltica*. The yellow compartment is the paryphoplasm and the light blue compartment is the pirellulosome. The nucleoid is represented by a tangled red line. This color-coding is adopted from Fuerst and Sagulenko (2013) [23]. 'Parypho' is derived from the classical Greek word paryphe for border of a robe. The nucleoid of the *R. baltica* cell is only enclosed by a single membrane, that of the ICM, not to be confused with the structure of *Gemmata obscuriglobus*, which has a nuclear envelope within the pirellulosome surrounding the nucleoid. The whole compartment enclosed by the ICM is called the pirellulosome, but there is no additional nuclear body as in *Gemmata*, so the nucleoid is naked and bounded only by the ICM as are the other pirellulosome contents. No periplasm between the CW and CM is drawn because no evidence for its existence has been published. This arrangement could result in the generation of an *smf* as well as a *pmf* across both the CM and the ICM, and the former could be used for ATP synthesis via the two distinct F-type-ATPases, F-ATPase-1 and F-

ATPase-2 in the CM and ICM. The diagram illustrates the use of dual Na^+:H^+ antiporters, Nha-1 and Nha-2 as well as two ABC-type Na^+ pumps, ABC-1 and ABC-2.

Table 1

Family TC#	Family name	Hit TCID	Acc. in TCDB	Hit TMS #	Substrate Group	Specific Substrate	Uniprot Accession #	Query TMS #
1.A. Alpha-Type Channel -forming proteins and Peptides								
1.A.1	Voltage-gated Ion Channel (VIC) Superfamily	1.A.1.1.2	P06550	3	cations	K^+	Q7ULF5	7
		1.A.1.13.2	O27564	2	cations	K^+	Q7UFW6	3
		1.A.1.13.5	Q9xa52	2	nonselective	nonselective	Q7UEH3	3
		1.A.1.24.3	Q1d027	5	cations	K^+	Q7UIN7	5
1.A.8	Major Intrinsic Protein (MIP) Family	1.A.8.2.1	P18156	7	sugar	polyols, H_2O	Q7UMF0	6
		1.A.8.12.7	Q8LFP7	6	H2O	H_2O	Q7UH29	7
1.A.11	Ammonia Channel Transporter (Amt) Family	1.A.11.1.4	O67997	12	cations	ammonia	Q7UYZ3	12
		1.A.11.2.5	Q0IDE4	12	cations	ammonium/methyl ammonium	Q7UGU7	12
		1.A.11.2.7	O28528	12	cations	$NH_4^+/CH_3NH_3^+$	Q7UK31	10
1.A.13	Epithelial Chloride Channel (E-ClC) Family	1.A.13.3.1	A9WIT9	3	anion	Cl^-	Q7UIP4	3
		1.A.13.3.1	A9WIT9	3	anion	Cl^-	Q7UNM0	2
		1.A.13.4.1	F8CM01	1	anion	Cl^-	Q7ULH3	2
1.A.14	Testis-Enhanced Gene Transfer (TEGT) Family	1.A.14.3.6	Q9HC24	7	cations	Ca^{2+}	Q7UU92	7
1.A.22	Large Conductance Mechanosensitive Ion Channel (MscL) Family	1.A.22.1.1	P0A742	2	nonselective	nonselective (slightly cation selective)	Q7UW50	2
1.A.23	Small Conductance Mechanosensitive Ion Channel (MscS) Family	1.A.23.1.1	P77338	11	nonselective	nonselective	Q7URB6	12
		1.A.23.1.3	P39285	13	nonselective	nonselective	Q7UKC6	12
		1.A.23.2.1	P0C0S1	4	nonselective	nonselective	Q7UW85	4
		1.A.23.2.1	P0C0S1	4	nonselective	nonselective	Q7USJ8	4
		1.A.23.2.1	P0C0S1	4	nonselective	nonselective	Q7UF95	3
		1.A.23.2.1	P0C0S1	4	nonselective	nonselective	Q7USJ6	3
		1.A.23.2.1	P0C0S1	4	nonselective	nonselective	Q7UIX1	3
1.A.26	Mg2+ Transporter-E (MgtE) Family	1.A.26.1.2	Q5SMG8	5	cations	Mg^{2+}	Q7UXN8	5
		1.A.26.1.2	Q5SMG8	5	cations	Mg^{2+}	Q7UM21	5

Family TC#	Family name	Hit TCID	Acc. in TCDB	Hit TMS #	Substrate Group	Specific Substrate	Uniprot Accession #	Query TMS #
1.A.30	H^+- or Na+-translocating Bacterial Flagellar Motor/ExbBD Outer Membrane Transport Energizer (Mot-Exb) Superfamily	1.A.30.1.2	O06873	4	cations	H^+ or Na^+	Q7ULV0	4
		1.A.30.1.4	P39064	1	cations	H^+ or Na^+	Q7ULU9	1
		1.A.30.2.2	P0ABU9	3	cations	H^+ or Na^+	Q7UKD7	3
		1.A.30.2.3	Q1DFL7	3	cations	H^+ or Na^+	Q7UXR6	4
		1.A.30.2.4	Q1D3D7	1	cations	H^+ or Na^+	Q7UXR7	1
		1.A.30.2.5	Q1CYB8	3	cations	H^+ or Na^+	Q7UTE3	3
		1.A.30.2.6	Q1DE42	3	cations	H^+ or Na^+	Q7UJ89	4
		1.A.30.2.6	Q1DE42	3	cations	H^+ or Na^+	Q7UQK6	3
		1.A.30.2.7	Q1D0D2	1	cations	H^+ or Na^+	Q7TU06	1
		1.A.30.2.7	Q1D0D2	1	cations	H^+ or Na^+	Q7UTE2	1
		1.A.30.2.7	Q1D0D2	1	cations	H^+ or Na^+	Q7UKD5	1
1.A.35	CorA Metal Ion Transporter (MIT) Family	1.A.35.3.2	Q9WZ31	3	cations	Mg^{2+}	Q7UQV5	3
1.A.77	Mg2+/Ca2+ Uniporter (MCU) Family	1.A.77.7.1	Q8TN54	2	cations	H^+	Q7UH10	2
1.B. Outer Membrane Porins								
1.B.9	FadL Outer Membrane Protein (FadL) Family	1.B.9.2.1	O65943	1	aromatic compounds	toluene/m-xylene	Q7UUD0	1
1.B.33	Outer Membrane Protein Insertion Porin (Bam Complex) (OmpIP) Family	1.B.33.1.3	P77774	1	peptide	peptide	Q7UMR7	1
		1.B.33.1.3	P77774	1	peptide	peptide	Q7UZ93	1
		1.B.33.1.3	P77774	1	peptide	peptide	Q7UJ81	1
		1.B.33.1.4	Q9A7R7	1	peptide	peptide	Q7UKJ0	1
2.A Carrier-type Facilitators								
2.A.1	Major Facilitator Superfamily (MFS)	2.A.1.1.3	P0AGF4	12	sugar	xylose:H^+ symporter	Q7UF68	12
		2.A.1.2.27	Q7VW14	12	siderophores	alcaligin siderophore	Q7UEL1	11
		2.A.1.2.39	Q5JAK9	12	specific drugs	tetracycline	Q7UXZ3	12
		2.A.1.3.26	P36554	14	multiple drugs	novobiocin/deoxycholate	Q7UPX7	12
		2.A.1.3.45	E5Y3Y1	13	unknown	unknown	Q7UW47	8
		2.A.1.7.1	P11551	12	sugar	L-Fucose:H+ symporter	Q7UR88	12

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		2.A.1.10.4	P76417	12	nucleoside	putative nucleoside transporter	Q7UIP7	12
		2.A.1.14.1	P42237	12	dicarboxylate	glucarate	Q7UTN9	12
		2.A.1.15.9	O51798	12	mono-carboxyl	4-methylmuconolactone	Q7UE09	11
		2.A.1.30.1	Q9X4X4	12	other hydrophobic	abietane	Q7UXH6	12
		2.A.1.42.2	Q89SS6	14	lipids	lysophospholipid	Q7UKU3	12
		2.A.1.42.2	Q89SS6	14	lipids	lysophospholipid	Q7UQ42	5
		2.A.1.70.3	A4X2L1	12	unknown	unknown	Q7UUW0	12
2.A.3	Amino Acid-Polyamine-Organocation (APC) Superfamily	2.A.3.3.17	Q9F2U9	10	unknown	unknown	Q7UY13	12
		2.A.3.6.1	O28661	13	amino acids	cationic amino acid	Q7UFY5	12
		2.A.3.8.12	O34739	12	amino acids	Ser/Thr, aromatic amino acids	Q7ULF6	12
2.A.4	Cation Diffusion Facilitator (CDF) Family	2.A.4.1.3	O07084	5	cations	Cd^{2+} or Zn^{2+}:H^+ + K^+ antiporter	Q7UMH1	4
2.A.5	Zinc (Zn2+)-Iron (Fe2+) Permease (ZIP) Family	2.A.5.4.11	Q2KXZ6	7	cations	Zn^{2+} and Cd^{2+} uptake	Q7UJZ2	6
		2.A.5.5.2	Q8N1S5	8	cations	Zn^{2+}	Q7UV47	7
		2.A.5.7.1	Q9NUM3	8	cations	divalent heavy-metal cations	Q7UMH2	8
2.A.6	Resistance-Nodulation-Cell Division (RND) Superfamily	2.A.6.1.2	P13511	12	cations	Co^{2+}; Zn^{2+}; Cd^{2+}	Q7UEM8	11
		2.A.6.1.5	Q88RT6	12	cations	Zn^{2+}, Cd^{2+}, Pb^{2+}	Q7USF5	14
		2.A.6.2.6	P52002	12	multiple drugs	β-lactams, fluoroquinolones, tetracycline, macrolides, chloramphenicol, biocides, and a toxic indole compound	Q7UUN6	11
		2.A.6.2.15	Q9HVI9	12	multiple drugs	β-lactams, fluoroquinolones, tetracycline, macrolides, chloramphenicol, biocides, including levofloxacin, carbenicillin, aztreonam, ceftazidime, cefepime, cefoperazone, piperacillin, erythromycin, azithromyein, chloramphenicol,	Q7UVX2	14
		2.A.6.2.16	Q9I0Y8	12	multiple drugs	luoroquinolones,	Q7UJT6	14

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						chloramphenicol, biocides, xenobiotics and chloramphenicol		
		2.A.6.2.25	Q8ZRG9	12	cations	Au^{2+}	Q7ULF2	12
		2.A.6.2.27	Q9I6X4	12	specific drugs	triclosan	Q7UJY1	12
		2.A.6.2.27	Q9I6X4	12	specific drugs	triclosan	Q7UH35	9
		2.A.6.2.30	A6P7H3	11	multiple drugs	antimicrobials	Q7UY73	13
		2.A.6.2.37	A6P7H1	12	multiple drugs	multidrug	Q7UGN1	11
		2.A.6.2.39	Q9I0V5	1	multiple drugs	aztreonam, macrolides, novobiocin and tetracycline	Q7UY71	1
		2.A.6.3.1	P25197	12	polysaccharide	lipooligosaccharide	Q7URR8	13
		2.A.6.3.1	P25197	12	polysaccharide	lipooligosaccharide	Q7UZ48	12
		2.A.6.3.1	P25197	12	polysaccharide	lipooligosaccharide	Q7UJP2	11
		2.A.6.4.2	O32047	12	proteins	pre-proteins	Q7US98	15
		2.A.6.5.1	Q53902	9	specific drugs	actinorhodin	Q7UUN7	11
		2.A.6.5.1	Q53902	9	specific drugs	actinorhodin	Q7ULN1	11
		2.A.6.7.1	O29039	12	unknown	unknown	Q7UF33	14
2.A.7	Drug/Metabolite Transporter (DMT) Superfamily	2.A.7.1.4	P69937	4	multiple drugs	cetylpyridinium, cetyldimethyl ethylammonium, hexadecyltrimethyl ammonium	Q7UJY5	4
		2.A.7.2.1	P29939	5	unknown	unknown	Q7UHH1	5
		2.A.7.7.2	P27844	10	multiple drugs	antibiotic	Q7UQ61	10
		2.A.7.21.4	Q3SAW5	10	nucleobases	orotate	Q7UTT1	4
		2.A.7.23.1	P42243	10	amino acids	tryptophan	Q7UGZ9	10
2.A.8	Gluconate:H+ Symporter (GntP) Family	2.A.8.1.2	P39344	10	monocarboxyl	L-idonate/D-gluconate:H^+ symporter	Q7UNE5	13
		2.A.8.1.7	Q46892	14	monocarboxyl	gluconate:H^+ Symporter	Q7UET7	12
2.A.9	Cytochrome Oxidase Biogenesis (Oxal) Family	2.A.9.3.1	P25714	3	protein	proteins	Q7UFZ2	6
2.A.12	ATP:ADP Antiporter (AAA) Family	2.A.12.4.1	Q6MDZ0	12	nucleotides	NAD^+:ADP antiporter	Q7UQZ9	12
2.A.14	Lactate Permease (LctP) Family	2.A.14.2.1	Q57251	15	unknown	unknown	Q7UY15	16
2.A.17	Proton-dependent Oligopeptide Transporter (POT) Family	2.A.17.4.4	Q9ES07	11	peptide	peptide	Q7UIT9	12
2.A.19	Ca2+:Cation Antiporter (CaCA) Family	2.A.19.4.6	Q71RS6	11	cations	Na^+/Ca^{2+} antiporter Sodium/potassium/calcium	Q7UGA8	10

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		2.A.19.5.2	Q0ZAI3	10	cations	cation proton antiporter	Q7UX07	10
2.A.21	Solute:Sodium Symporter (SSS) Family	2.A.21.2.2	Q7A4Q7	13	amino acids	sodium/proline symporter	Q7UTC3	13
		2.A.21.3.7	Q5E733	13	sugar	sialic acid	Q7UMJ2	13
		2.A.21.3.7	Q5E733	13	sugar	sialic acid	Q7UNL7	13
		2.A.21.3.7	Q5E733	13	sugar	sialic acid	Q7UUE1	13
		2.A.21.3.8	A1S2A8	15	sugar	mannose	Q7USD7	15
		2.A.21.3.9	A8H019	13	sugar	galactose	Q7UQ37	14
		2.A.21.6.1	P33413	15	amines	urea, polyamines	Q7UHC8	13
		2.A.21.7.2	P32705	14	monocarboxyl	acetate/glyoxalate	Q7UJJ4	13
		2.A.21.8.1	Q9JMD7	13	amines	choline: Na+ symporter	Q7UFM6	13
2.A.23	Dicarboxylate/ Amino Acid:Cation (Na+ or H+) Symporter (DAACS) Family	2.A.23.2.10	Q10901	9	amino acids	amino acid	Q7UV95	11
2.A.25	Alanine or Glycine:Cation Symporter (AGCS) Family	2.A.25.1.3	Q6LX42	11	amino acids	alanine:Na+ symporter	Q7UKE2	12
2.A.27	Glutamate:Na+Symporter (ESS) Family	2.A.27.2.1	B1XKD9	11	amino acids	glutamate:sodium	Q7UGS6	12
2.A.33	NhaA Na+:H +Antiporter (NhaA) Family	2.A.33.1.2	Q56725	10	cations	Na^+, K^+:H^+ antiporter	Q7UTY3	11
2.A.35	NhaC Na+:H+ Antiporter (NhaC) Family	2.A.35.2.1	Q6LZF0	14	cations	Na^+/H^+ antiporter	Q7URI6	10
2.A.36	Monovalent Cation:Proton Antiporter-1 (CPA1) Family	2.A.36.2.1	O26854	13	cations	Na^+/H^+ exchanger	Q7UQM5	13
		2.A.36.4.4	Q9P937	13	cations	Na^+, K^+, Li^+ and Rb^+ (alkali metals)	Q7UY93	12
		2.A.36.6.3	Q87KV8	13	cations	K^+:H^+ antiporter	Q7UU07	12
		2.A.36.6.4	Q0ZAH6	13	cations	$K^+(NH4^+)$:H^+ antiporter	Q7UJT8	13
2.A.38	K+ Transporter (Trk) Family	2.A.38.1.3	Q6T3V7	10	cations	K^+	Q7UNB4	11
2.A.39	Nucleobase:Cation Symporter-1 (NCS1) Family	2.A.39.5.1	Q082R8	14	sugar	mannitol	Q7UYE6	12
		2.A.39.5.1	Q082R8	14	sugar	mannitol	Q7UVW0	13
2.A.41	Concentrative Nucleoside Transporter (CNT) Family	2.A.41.2.5	Q9UA35	13	nucleoside	nucleoside:Na+ cotransporter	Q7UKU0	9

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2.A.45	Arsenite-Antim onite (ArsB) Efflux Family	2.A.45.2.2	P0A607	11	anions	arsenite and/or antimonite	Q7UPU4	12
2.A.47	Divalent Anion:Na+ Symporter (DASS) Family	2.A.47.1.1	Q2FMC1	14	anions	anion	Q7UUK9	15
		2.A.47.4.5	Q9K7H7	14	anions	Na^+:SO_4 symporter	Q7UTC7	11
2.A.49	Chloride Carrier/ Channel (ClC) Family	2.A.49.6.1	P74477	11	anions	Cl^-	Q7UUY6	11
2.A.50	Glycerol Uptake (GUP) Family	2.A.50.2.1	P39580	12	substrate cofactors	activated alanine	Q7UTL2	11
2.A.52	Ni2+-Co2+ Transporter (NiCoT) Family	2.A.52.2.4	Q58492	6	cations	nickel/cobalt	Q7UHP3	6
2.A.53	Sulfate Permease (SulP) Family	2.A.53.3.1	O07488	13	anions	sulfate	Q7UFF6	10
		2.A.53.3.8	Q8F8H7	10	anions	bicarbonate uptake	Q7UF60	13
		2.A.53.3.8	Q8F8H7	10	anions	bicarbonate uptake	Q7UJU1	11
2.A.55	Metal Ion (Mn2+-iron) Transporter (Nramp) Family	2.A.55.3.1	P0A769	11	cations	Mn^{2+}, Fe^{2+}, Cd^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+}:H^+ symporter	Q7UFP5	11
		2.A.55.3.4	Q93JK1	11	cations	metal ion	Q7TU10	11
		2.A.55.3.4	Q93JK1	11	cations	metal ion	Q7UTF4	12
		2.A.55.3.4	Q93JK1	11	cations	metal ion	Q7UL02	10
2.A.58	Phosphate:Na+ Symporter (PNaS) Family	2.A.58.1.2	O87918	8	anions	phosphate	Q7UJU6	10
		2.A.58.2.1	P0AF43	9	anions	unknown (maybe phosphate)	Q7UMC5	9
2.A.59	Arsenical Resistance-3 (ACR3) Family	2.A.59.1.5	A6TP80	10	anions	arsenite	Q7UY98	9
2.A.63	Monovalent Cation (K+ or Na+):Proton Antiporter-3 (CPA3) Family	2.A.63.1.2	Q9KDA0	3	cations	Na^+:H^+ antiporter	Q7UXB7	3
		2.A.63.1.2	Q7AJV9	4	cations	Na^+:H^+ antiporter	Q7UXC0	4
		2.A.63.1.2	Q9KD98	14	cations	Na^+:H^+ antiporter	Q7UXC2	15
		2.A.63.1.4	O05227	3	cations	Na^+:H^+ antiporter	Q7UXB8	2
2.A.64	Monovalent Cation (K+ or Na+):Proton Antiporter-3 (CPA3) Family	2.A.64.2.1	Q9XH75	1	proteins	proteins	Q7UQP6	1
		2.A.64.3.1	O31467	1	proteins	proteins	Q7UQP7	2
		2.A.64.3.2	O05523	6	proteins	proteins	Q7UFI8	6

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2.A.66	Multidrug/ Oligosaccharidyl-lipid/ Polysaccharide (MOP) Flippase Superfamily	2.A.66.1.9	Q9F5N7	12	multiple drugs	norfloxacin, polymyxin B	Q7UWD3	12
		2.A.66.1.12	Q9I3Y3	12	multiple drugs	benzalkonium chloride, fluoroquinolone, ethidium bromide, acriflavin, tetraphenylphosphonium chloride	Q7USS5	13
		2.A.66.2.4	Q44575	11	polysaccharide	acetan	Q7UVP7	14
		2.A.66.5.3	A0YL48	12	polysaccharide	polysaccharide	Q7UF06	12
		2.A.66.7.1	Q4K6F5	12	lipids	lipids	Q7UKF5	12
2.A.69	Auxin Efflux Carrier (AEC) Family	2.A.69.4.5	Q48797	10	dicarbonate	malate	Q7USB9	10
2.A.79	Threonine/Serine Exporter (ThrE) Family	2.A.79.1.2	Q1DBY8	10	amino acids	threonine/Serine	Q7UJS3	10
2.A.81	Aspartate:Alanine Exchanger (AAEx) Family	2.A.81.1.2	Q5LCC7	11	cations	cobalt	Q7UH36	11
2.A.86	Autoinducer-2 Exporter (AI-2E) Family	2.A.86.1.1	P0AFI9	7	organoanions	autoinducer-2 furanosyl borate diester	Q7UIW9	6
		2.A.86.1.1	P0AFI9	7	organoanions	autoinducer-2	Q7USJ9	8
		2.A.86.1.2	P0AGM0	7	organoanions	autoinducer-2	Q7ULF1	6
		2.A.86.1.2	P0AGM0	7	organoanions	autoinducer-2	Q7USP8	7
		2.A.86.1.2	P0AGM0	7	organoanions	autoinducer-2	Q7UYF5	7
		2.A.86.2.3	Q3IS50	7	organoanions	autoinducer-2	Q7UPF9	8
2.A.93	Unknown BART Superfamily-1 (UBS1) Family	2.A.93.1.6	Q01E11	12	unknown	unknown	Q7ULP6	9
2.A.98	Putative Sulfate Exporter (PSE) Family	2.A.98.1.4	F3LB58	11	anions	sulfate	Q7ULI3	12
2.A.102	Putative 4-Toluene Sulfonate Uptake Permease (TSUP) Family	2.A.102.4.2	E7BBJ3	8	organoanions	organo-sulfur-containing compound	Q7UEN7	8
3.A P-P-Bond Hydrolysis-driven Transporters								
3.A.1	ATP-binding Cassette (ABC) Superfamily	3.A.1.2.13	A6VKS9	9	organoanions	autoinducer-2	Q7ULN8	8
		3.A.1.2.13	A6VKS9	9	organoanions	autoinducer-2	Q7UU56	8
		3.A.1.2.13	A6VKS9	9	organoanions	autoinducer-2	Q7UR04	8
		3.A.1.2.15	A8H4W6	9	sugar	xylitol	Q7UXV9	10
		3.A.1.5.22	Q5V9S0	6	peptide	peptide	Q7UHZ2	6

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		3.A.1.5.22	Q5V9S1	6	peptide	peptide	Q7UHZ1	6
		3.A.1.7.1	P07654	6	anions	Phosphate	Q7UP20	6
		3.A.1.7.1	P0AGH8	6	anions	Phosphate	Q7UP19	6
		3.A.1.10.1	P21408	1	cations	Fe^{3+}	Q7UYK9	1
		3.A.1.15.6	Q9RNI8	7	cations	Cu^{2+}, Zn^{2+}, Fe^{2+}	Q7UIM9	9
		3.A.1.15.8	P96116	1	cations	Mn^{2+}, Zn^{2+}, Fe^{2+}	Q7UIM5	1
		3.A.1.15.8	P96118	7	cations	Mn^{2+}, Zn^{2+}, Fe^{2+}	Q7UIM8	9
		3.A.1.16.2	Q7BW13	6	anions	bispecific cyanate/nitrite	Q7UYV0	6
		3.A.1.17.6	Q9CLG9	6	nucleobases	hydroxymethylpyrimidine, N-formyl-4-amino-5-(aminomethyl)-2-methylpyrimidine	Q7UNX6	6
		3.A.1.27.3	P64606	5	lipids	lipids	Q7UP62	6
		3.A.1.105.3	Q70J76	6	multiple drugs	dideacetyl-chromomycin, chromomycin and mithramycin	Q7UJF6	6
		3.A.1.106.2	Q2G2M9	5	multiple drugs	doxorubicin, verapamil, ethidium, tetraphenylphosphonium, vinblastine and the fluorescent dye	Q7ULL7	6
		3.A.1.106.2	Q2G2M9	5	multiple drugs	doxorubicin, verapamil, ethidium, tetraphenylphosphonium, vinblastine and the fluorescent dye	Q7UKL4	5
		3.A.1.106.8	O07550	6	multiple drugs	multiple drugs	Q7UXT8	6
		3.A.1.109.4	Q9I2M0	1	proteins	proteins	Q7UVG4	2
		3.A.1.113.1	P33951	7	peptide	peptide	Q7ULB4	5
		3.A.1.114.1	O50295	6	lipids	glycolipid	Q7UG93	5
		3.A.1.114.1	O50295	6	lipids	glycolipid	Q7UTR1	5
		3.A.1.115.1	P46904	6	cations	Na^{+}	Q7UQ82	12
		3.A.1.115.1	P46904	6	cations	Na^{+}	Q7UND7	11
		3.A.1.117.1	P97046	7	multiple drugs	multidrug	Q7UG09	6
		3.A.1.122.1	P75830	1	multiple drugs	14- and 15-membered lactone macrolides including erythromycin, heat-stable enterotoxin II, L-cysteine	Q7UL96	1
		3.A.1.122.1	P75831	4	multiple drugs	14- and 15-membered lactone macrolides including erythromycin, heat-stable enterotoxin II, L-cysteine	Q7ULB5	4
		3.A.1.122.1	P75831	4	multiple drugs	14- and 15-membered lactone macrolides	Q7UST5	4

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						including erythromycin, heat-stable enterotoxin II, L-cysteine		
		3.A.1.122.2	O31712	4	peptides	antimicrobial peptide	Q7UPF1	4
		3.A.1.122.7	A8TDX0	4	multiple drugs	macrolide	Q7UQ59	4
		3.A.1.122.8	Q73MJ4	4	multiple drugs	macrolide	Q7UH40	4
		3.A.1.125.1	P75957	1	proteins	lipoprotein	Q7UH39	2
		3.A.1.125.1	P75958	4	proteins	lipoprotein	Q7UPK4	4
		3.A.1.125.2	Q7D911	10	proteins	lipoprotein	Q7UJV6	10
		3.A.1.125.3	Q2J9P4	10	proteins	lipoprotein	Q7UPA2	10
		3.A.1.125.3	Q2J9P4	10	proteins	lipoprotein	Q7UJV3	11
		3.A.1.125.3	Q2J9P4	10	proteins	lipoprotein	Q7UST0	4
		3.A.1.132.1	Q93LN1	6	polysaccharide	exopolysaccharide	Q7ULI7	12
		3.A.1.132.4	Q2SDB0	8	unknown	unknown	Q7UXL8	10
		3.A.1.141.1	P74757	5	multiple drugs	ethyl viologen	Q7UQG6	6
		3.A.1.141.2	Q8R6Q5	6	unknown	unknown	Q7UQG5	7
3.A.2	H+- or Na+-translocating F-type, V-type and A-type ATPase (F-ATPase) Superfamily	3.A.2.1.2	P21904	1	cations	Na^+	Q7UH06	1
		3.A.2.1.2	P21904	1	cations	Na^+	Q7UFB9	2
		3.A.2.1.2	P21905	2	cations	Na^+	Q7UFC0	2
		3.A.2.1.2	P21905	2	cations	Na^+	Q7UH07	2
		3.A.2.1.2	P21903	6	cations	Na^+	Q7UFC2	6
		3.A.2.1.2	P21903	6	cations	Na^+	Q7UH08	5
3.A.3	P-type ATPase (P-ATPase) Superfamily	3.A.3.5.4	Q9ZHC7	9	cations	Ag^+	Q7UYX3	8
		3.A.3.25.2	Q92Z60	7	cations	cation	Q7UH21	7
		3.A.3.27.3	A6Q500	7	cations	cation, heavy-metal	Q7UQF3	9
3.A.5	The General Secretory Pathway (Sec) Family	3.A.5.2.2	P0A5Z2	10	proteins	proteins	Q7UN01	10
3.A.6	Type III (Virulence-related) Secretory Pathway (IIISP) Family	3.A.6.1.1	Q7BFA7	2	proteins	flagellar protein	Q7UXG4	2
		3.A.6.2.1	P15928	2	proteins	flagellar protein	Q7UNQ1	1
		3.A.6.2.1	P54700	5	proteins	flagellar protein	Q7UXG5	5
		3.A.6.2.1	P40727	4	proteins	flagellar protein	Q7UXG2	4
		3.A.6.2.1	P54702	6	proteins	flagellar protein	Q7UXG3	5
		3.A.6.2.1	P40729	7	proteins	flagellar protein	Q7UFV2	8

Family TC#	Family name	Hit TCID	Acc. in TCDB	Hit TMS #	Substrate Group	Specific Substrate	Uniprot Accession #	Query TMS #
3.A.7	Type IV (Conjugal DNA-Protein Transfer or VirB) Secretory Pathway (IVSP) Family	3.A.7.15.1	Q9XC05	5	proteins	fimbrium (pilus) subunit	Q7UHP6	5
		3.A.7.15.1	Q9XC05	5	proteins	fimbrium (pilus) subunit	Q7UR20	5
		3.A.7.15.1	Q9XC05	5	proteins	fimbrium (pilus) subunit	Q7UXS4	5
3.A.11	Bacterial Competence-related DNA Transformation Transporter (DNA-T) Family	3.A.11.1.1	P39695	12	nucleic acids	DNA	Q7UKZ4	11
		3.A.11.1.3	Q8VRL3	3	nucleic acids	DNA	Q7UE43	3
3.A.12	Septal DNA Translocator (S-DNA-T) Family	3.A.12.1.1	P21458	4	nucleic acids	DNA	Q7UJL4	4
3.A.15	Outer Membrane Protein Secreting Main Terminal Branch (MTB) Family	3.A.15.1.1	P15746	1	proteins	proteins	Q7UHC0	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UZ25	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UX69	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UHB2	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UPI1	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UPY8	2
		3.A.15.1.1	P15746	1	proteins	proteins	Q7US61	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7URZ8	2
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UJS1	2
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UPU1	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UZ90	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UG14	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UG16	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UMN3	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UHB6	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UY53	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7URP9	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UXC9	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UXW8	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7URU1	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UQ90	1

Family TC#	Family name	Hit TCID	Acc. in TCDB	Hit TMS #	Substrate Group	Specific Substrate	Uniprot Accession #	Query TMS #
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UUB4	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UGB3	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UWU9	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UPL9	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UG03	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UKK7	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UPI7	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UES8	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7USE1	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UGP9	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UUG9	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UJV0	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UKN2	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UYA1	1
		3.A.15.1.1	P15746	1	proteins	proteins	Q7UNZ5	1
		3.A.15.1.1	P15745	3	proteins	proteins	Q7UZ86	6
		3.A.15.1.1	P15754	5	proteins	proteins	Q7UFL4	9
		3.A.15.2.1	P22609	4	proteins	fimbrium (pilus) subunit	Q7UMN7	3
		3.A.15.2.1	P22609	4	proteins	fimbrium (pilus) subunit	Q7UJJ8	3
		3.A.15.2.1	P22609	4	proteins	fimbrium (pilus) subunit	Q7UMN8	3
		3.A.15.2.1	P22609	4	proteins	fimbrium (pilus) subunit	Q7UU65	3
		3.A.15.3.1	Q9XD71	1	proteins	fimbrium (pilus) subunit	Q7UI36	1
		3.A.15.3.1	Q9XD71	1	proteins	fimbrium (pilus) subunit	Q7UNA9	2
		3.A.15.3.1	Q9XD71	1	proteins	fimbrium (pilus) subunit	Q7ULT6	1
		3.A.15.3.1	Q9XD71	1	proteins	fimbrium (pilus) subunit	Q7UME3	2
		3.A.15.3.1	Q9XD71	1	proteins	fimbrium (pilus) subunit	Q7UJW2	2
		3.A.15.3.1	Q9XD71	1	proteins	fimbrium (pilus) subunit	Q7ULZ4	1
		3.A.15.3.1	Q9XD71	1	proteins	fimbrium (pilus) subunit	Q7UR34	1
		3.A.15.3.1	Q9XD71	1	proteins	fimbrium (pilus) subunit	Q7UJW1	1
		3.A.15.3.1	Q9XD71	1	proteins	fimbrium (pilus) subunit	Q7UU34	1
		3.A.15.3.1	O68433	6	proteins	fimbrium (pilus) subunit	Q7UT71	11

Family TC#	Family name	Hit TCID	Acc. in TCDB	Hit TMS #	Substrate Group	Specific Substrate	Uniprot Accession #	Query TMS #
3.A.23	Type VI Symbiosis/Virulence Secretory Pathway (VISP) Family	3.A.23.2.1	A8YQS0	1	proteins	protein	Q7UL75	1
3.B Decarboxylation-driven transporters								
3.B.1	Na+-transporting Carboxylic Acid Decarboxylase (NaT-DC) Family	3.B.1.1.5	Q9V0A4	3	cation	Na^+	Q7UHX0	2
3.D Oxidoreduction-driven Active Transporters								
3.D.1	H+ or Na+-translocating NADH Dehydrogenase (NDH) Family	3.D.1.4.1	Q8U101	3	cations	H^+	Q7UXC1	3
		3.D.1.4.1	Q8U107	3	cations	H^+	Q7UXB6	3
		3.D.1.4.1	Q8U100	15	cations	H^+	Q7UF67	14
		3.D.1.4.1	Q8U100	15	cations	H^+	Q7UXC5	16
		3.D.1.6.2	P05510	19	cations	H^+	Q7UF66	11
		3.D.1.8.1	P56752	17	cations	H^+	Q7UXT4	13
3.D.2	Proton-translocating Transhydrogenase (PTH) Family	3.D.2.2.1	P0C187	3	cations	H^+	Q7UIW2	3
		3.D.2.2.1	P0C188	10	cations	H^+	Q7UIW3	10
3.D.4	Proton-translocating Cytochrome Oxidase (COX) Superfamily	3.D.4.3.2	P0ABK2	9	cations	H^+	Q7UKW6	8
		3.D.4.3.2	P0ABJ9	9	cations	H^+	Q7UKW7	9
		3.D.4.3.3	D9IA45	2	cations	H^+	Q7UQF7	1
		3.D.4.3.3	H7F0T0	12	cations	H^+	Q7UQF9	13
		3.D.4.4.1	P24010	14	cations	H^+	Q7USM4	19
		3.D.4.4.1	P24010	14	cations	H^+	Q7UI91	12
		3.D.4.4.2	Q9AEL8	5	cations	H^+	Q7UI89	7
		3.D.4.4.3	Q5SLI2	2	cations	H^+	Q7USM3	2
		3.D.4.4.3	Q5SLI2	2	cations	H^+	Q7UI92	2
		3.D.4.6.2	Q3J5F6	7	cations	H^+	Q7US88	5
3.D.5	Na+-translocating NADH:Quinone Dehydrogenase (Na-NDH) Family	3.D.5.1.1	Q56587	13	cations	Na^+	Q7UWS4	10
		3.D.5.1.1	Q56582	2	cations	Na^+	Q7UWS3	2
		3.D.5.1.1	Q56584	2	cations	Na^+	Q7UWS0	2

Family TC#	Family name	Hit TCID	Acc. in TCDB	Hit TMS #	Substrate Group	Specific Substrate	Uniprot Accession #	Query TMS #
		3.D.5.1.1	Q56589	6	cations	Na^+	Q7UWS1	6
		3.D.5.1.1	Q57095	6	cations	Na^+	Q7UWS2	5
4.D Polysaccharide Synthase/Exporters								
4.D.1	Putative Vectorial Glycosyl Polymerization (VGP) Family	4.D.1.1.3	P75905	5	sugar	UDP-N-acetylglucosamine	Q7UJE8	4
4.D.2	COG0392; UPF0104 Putative Transporter (COG0392) Family	4.D.2.1.6	F2KQZ0	8	polysaccharide	polysaccharide	Q7UHG9	14
5.A Transmembrane 2-electron transfer carriers								
5.A.1	Disufide Bond Oxidoreductase D (DsbD) Family	5.A.1.5.1	O33918	7	electron	electron	Q7UJI4	9
		5.A.1.6.1	A3DGJ1	6	electron	electron	Q7UQF4	6
5.A.3	Prokaryotic Molybdopterin-containing Oxidoreductase (PMO) Family	5.A.3.3.3	Q9HR72	10	electron	electron	Q7UI98	10
		5.A.3.4.1	P33226	1	electron	electron	Q7UJN4	1
5.A.4	Prokaryotic Succinate Dehydrogenase (SDH) Family	5.A.4.1.1	Q65GF3	5	electron	electron	Q7UEU4	5
8.A. Auxiliary transport proteins								
8.A.1	Membrane Fusion Protein (MFP) Family	8.A.1.1.3	B1LPP9	1	unknown	unknown	Q7UST2	2
		8.A.1.1.3	B1LPP9	1	unknown	unknown	Q7ULG3	2
		8.A.1.6.2	P76397	1	unknown	unknown	Q7UJP1	1
8.A.3	Cytoplasmic Membrane-Periplasmic Auxiliary-1 (MPA1) Protein with Cytoplasmic (C) Domain (MPA1-C or MPA1 + C)	8.A.3.1.1	P33698	3	unknown	unknown	Q7UID4	1
		8.A.3.3.3	Q45409	3	unknown	unknown	Q7UVS9	3
8.A.21	Stomatin/Podocin/ Band 7/Nephrosis.2/SPFH (Stomatin) Family	8.A.21.2.1	O59180	3	unknown	unknown	Q7UP83	2
		8.A.21.2.1	O59179	7	unknown	unknown	Q7UNB5	5
9.A Transporters of Unknown Classification								
9.A.8	Ferrous Iron Uptake	9.A.8.1.6	Q7MV19	9	cations	ion, Fe^{2+}	Q7UP65	8

Family TC#	Family name	Hit TCID	Acc. in TCDB	Hit TMS #	Substrate Group	Specific Substrate	Uniprot Accession #	Query TMS #
	(FeoB) Family							
9.A.24	The Mitochondrial Outer Membrane Insertion Pathway (MOM-IP) Family	9.A.24.1.1	Q6ICF9	4	lipids	cholesterol	Q7UEK4	4
		9.A.24.1.1	Q6ICF9	4	lipids	cholesterol	Q7UED9	4
9.A.31	Putative SdpC Peptide Antibiotic-like Killing Factor Exporter, SdpAB (SdpAB) Family	9.A.31.1.1	O34616	6	peptides	peptide (toxic)	Q7UWK8	7
9.A.40	The HlyC/CorC (HCC) Family of Putative Transporters	9.A.40.2.1	P54428	4	cations	Fe^{2+}	Q7UQW5	3
		9.A.40.3.4	Q6P4Q7	5	cations	metal ion	Q7UQM0	3
9.A.47	Tight Adherence (Pilus) Biogenesis Apparatus (TABA) Family	9.A.47.1.1	Q9S4A8	5	proteins	proteins (pili, fimbrae)	Q7UXS3	4
		9.A.47.1.1	Q9S4A8	5	proteins	proteins (pili, fimbrae)	Q7UR19	4
9.B putative Uncharacterized Transporters								
9.B14	Putative Heme Handling Protein (HHP) Family	9.B.14.3.1	Q7VHG9	14	cofactors	heme	Q7UMT1	18
9.B.18	SecDF-associated Single Transmembrane Protein, YajC (YajC) Family	9.B.18.1.1	P0ADZ7	1	proteins	proteins	Q7US99	1
9.B.20	Putative Mg2+ Transporter-C (MgtC) Family	9.B.20.2.2	B9TCJ7	5	cations	Mg^{2+}	Q7USM2	4
9.B.27	DedA or YdjX-Z (DedA) Family	9.B.27.1.1	P76219	6	anions	anions	Q7UEP9	6
		9.B.27.1.2	P76221	5	anions	anions	Q7UEL8	5
		9.B.27.2.5	D6GX19	5	anions	anions	Q7USM1	3
9.B.28	Putative Permease Duf318 (Duf318) Family	9.B.28.1.1	B5LWZ8	8	amines	Ethanolamine	Q7UHR4	9
9.B.30	Hly III (Hly III) Family	9.B.30.1.1	P54176	7	unknown	unknown	Q7UGF9	7
9.B32	SdpC (Peptide-Antibiotic Killer Factor) Immunity Protein, SdpI (SdpI) Family	9.B.32.1.2	P0ADK5	2	unknown	unknown	Q7ULG4	3

Family TC#	Family name	Hit TCID	Acc. in TCDB	Hit TMS #	Substrate Group	Specific Substrate	Uniprot Accession #	Query TMS #
9.B.45	Arg/Asp/Asp (RDD) Family	9.B.45.1.2	Q465I3	3	unknown	unknown	Q7UXA5	4
		9.B.45.1.2	Q465I3	3	unknown	unknown	Q7UL03	4
9.B.59	Putative Peptide Transporter Carbon Starvation CstA (CstA) Family	9.B.59.1.3	Q5JIF7	15	peptides	peptides	Q7UP15	15
9.B.93	Spanin (Spanin) Family	9.B.93.1.2	E1IMB6	6	unknown	unknown	Q7UR84	6
9.B.97	Acyltransferase-3/ Putative Acetyl-CoA Transporter (ATAT) Family	9.B.97.4.1	D4I357	9	unknown	unknown	Q7UMB9	10
9.B.98	DUF95 (DUF95) Family	9.B.98.1.3	Q2JF02	6	unknown	unknown	Q7UT68	5
9.B.104	YedE/YeeE (YedE/YeeE) Family	9.B.104.1.1	P09391	6	peptides	peptide	Q7UVD2	6
		9.B.104.1.2	Q46G03	6	peptides	peptide	Q7UQD4	6
		9.B.104.1.2	Q46G03	6	peptides	peptide	Q7UIH5	6
		9.B.104.1.2	Q46G03	6	peptides	peptide	Q7UH51	5
		9.B.104.1.3	F0Z2G1	6	peptides	peptide	Q7UVY7	7
		9.B.104.1.3	F0Z2G1	6	peptides	peptide	Q7UIQ0	7
9.B.105	Lead Resistance Fusion Protein (PbrBC) Family	9.B.105.1.1	Q58AJ7	10	cations	Pb^{2+}	Q7UIY3	6
9.B.126	Putative Lipid Exporter (YhjD) Family	9.B.126.2.1	P0A8K8	6	lipids	lipids	Q7USG3	6
		9.B.126.2.1	P0A8K8	6	lipids	lipids	Q7UQD0	5
9.B.128	O-antigen Polymerase, WzyE (WzyE) Family	9.B.128.2.1	D7DZ35	12	lipids	lipids	Q7UVS2	13

Table 2

Integral membrane MFS superfamily proteins in *R. baltica* arranged by family.

TC Number	Family Name	Known Substrate Range	# in Rba
2.A.1.1	The Sugar Porter (SP) Family	sugar and sugar derivative (uniport; symport); urate (antiport)	1
2.A.1.2	The Drug:H+ Antiporter-1 (12 Spanner) (DHA1) Family	drug, polyamine, neurotransmitter, sugar, nucleobase/side, siderophore, lipid (antiport); vitamin (symport)	2
2.A.1.3	The Drug:H+ Antiporter-2 (14 Spanner) (DHA2) Family	drug, boron, bile acid, parquot, fatty acid, siderophore, amino acid (antiport); pyrimidine (symport)	2
2.A.1.7	The Fucose: H^+ Symporter (FHS) Family	sugar and sugar derivative (uniport; symport)	1
2.A.1.10	The Nucleoside: H^+ Symporter (NHS) Family	nucleosides (symport)	1
2.A.1.14	The Anion:Cation Symporter (ACS) Family	organic and inorganic anion, peptide, vitamin, amino acid, nucleotide (uniport; symport)	1
2.A.1.15	The Aromatic Acid:H+ Symporter (AAHS) Family	aromatic acid, vitamin (symport)	1
2.A.1.30	The Putative Abietane Diterpenoid Transporter (ADT) Family	diterpenoid (symport)	1
2.A.1.42	The Lysophospholipid Transporter (LplT) Family	lysophospholipid	2
2.A.1.70	Unidentified Major Facilitaor-18 (UMF18) Family	unknown	1
2.A.12	The ATP:ADP Antiporter (AAA) Family	ATP:ADP antiport	1
2.A.17	The Proton-dependent Oligopeptide Transporter (POT) Family	peptide, histidine, nitrate (symport; occasionally antiport)	1

Table 3

Integral membrane APC superfamily proteins in *R. baltica* arranged by family.

TC Number	Family	Known Substrate Range	# in Rba
2.A.3	The Amino Acid-Polyamine-Organocation (APC) Superfamily	amino acid-polyamine-organocation (symport;antiport)	3
2.A.21	The Solute:Sodium Symporter (SSS) Family	sugars, amino acids and organocations (Na^+ symport)	9
2.A.25	The Alanine or Glycine:Cation Symporter (AGCS) Family	alanine and/or glycine (symport with Na^+ and or H^+)	1
2.A.39	The Nucleobase:Cation Symporter-1 (NCS1) Family	nucleobases (cation symport)	2
2.A.53	The Sulfate Permease (SulP) Family	inorganic anion, or anion:anion exchange	3

Table 4

Integral membrane ABC import proteins in *R. baltica* arranged by family.

TC Number	Family	Known Substrate Range	# in Rba
3.A.1.2	The Carbohydrate Uptake Transporter-2 (CUT2) Family	carbohydrate	4
3.A.1.5	The Peptide/Opine/Nickel Uptake Transporter (PepT) Family	peptide, opine, nickel	2
3.A.1.7	The Phosphate Uptake Transporter (PhoT) Family	phosphate	2
3.A.1.10	The Ferric Iron Uptake Transporter (FeT) Family	ferric iron, Fe-hydroxamate	1
3.A.1.15	The Manganese/Zinc/Iron Chelate Uptake Transporter (MZT) Family	manganese, zinc ,iron chelates	3
3.A.1.16	The Nitrate/Nitrite/Cyanate Uptake Transporter (NitT) Family	nitrate, citrite, cyanate, bicarbonate	1
3.A.1.17	The Taurine Uptake Transporter (TauT) Family	taurine, aromatic sulfur, phthalate, hydroxymethylpyrimidine	1
3.A.1.27	The γ-Hexachlorocyclohexane (HCH) Family	cholesterol, chloroplast lipid, γ-hexachlorocyclohexane	1

Table 5

Integral membrane ABC export proteins in *R. baltica* arranged by family.

TC Number	Family Description	Known Substrate Range	# in Rba
3.A.1.105	The Drug Exporter-1 (DrugE1) Family	multidrug	1
3.A.1.106	The Lipid Exporter (LipidE) Family	lipid export	3
3.A.1.109	The Protein-1 Exporter (Prot1E) Family	proteins, multidrug	1
3.A.1.113	The Peptide-3 Exporter (Pep3E) Family	proteins, multidrug	1
3.A.1.114	The Probable Glycolipid Exporter (DevE) Family	glycolipid	2
3.A.1.115	The Na+ Exporter (NatE) Family	Na^+ efflux	2
3.A.1.117	The Drug Exporter-2 (DrugE2) Family	multidrug	1
3.A.1.122	The Macrolide Exporter (MacB) Family	multidrug	6
3.A.1.125	The Lipoprotein Translocase (LPT) Family	lipoprotein	6
3.A.1.132	The Gliding Motility ABC Transporter (Gld) Family	exopolysaccharide	2
3.A.1.141	The Ethyl Viologen Exporter (EVE) Family (DUF990 Family)	ethyl viologen	2

Table 6

Integral membrane (putative) transport proteins of *R. baltica* with scores of >0.001 to previous TC entries in G-BLAST searches.

TC #	UniProt acc#	Size (#aa's)	Query TMS #	Family Assignment
2.A.1.77.1	Q7UTR7	535	12	MFS
2.A.7.29.1	Q7UVJ3	362	10	DMT
2.A.7.3.45	Q7UYS1	363	10	DMT
2.A.7.3.46	Q7UPP7	350	10	DMT
2.A.7.30.1	Q7URM2	299	9	DMT
2.A.102.2.3	Q7URC0	258	8	TSUP
3.A.1.105.8	Q7UE57	442	7	ABC
3.A.1.132.7	Q7UXN5	627	13	ABC
9.A.30.5.1	Q7UHX7	286	7	TerC
9.A.30.5.2	Q7URC1	300	7	TerC
9.B.27.1.3	Q7UPC8	247	6	DedA
9.B.27.1.4	Q7USX5	195	5	DedA
9.B.104.5.1	Q7UL82	227	6	Rhomboid
9.B.105.1.4	Q7UF32	223	4	PbrBC
9.B.142.9.1	Q7UKY8	463	7	GT39
9.B.144.1.1	Q7UKM0	834	14	DUF3367
9.B.145.1.1	Q7UVM4	502	7	DUF389
9.B.146.1.1	Q7UQ81	532	11	MurG
9.B.147.1.1	Q7UER8	379	10	10-IMP
9.B.147.2.1	Q7UNA5	419	10	10-IMP
9.B.148.2.1	Q7UY28	131	4	4-DMT