Published in final edited form as:
Genomics. 2007 October ; 90(4): 424–446. doi:10.1016/j.ygeno.2007.06.002.

Comprehensive Analysis of Transport Proteins Encoded Within the Genome of *Bdellovibrio bacteriovorus*

Ravi D. Barabote, **Snjezana Rendulic**, **Stephan C. Schuster**, and **Milton H. Saier Jr.***
Division of Biological Sciences, University of California at San Diego, La Jolla, CA 92093-0116

Abstract

Bdellovibrio bacteriovorus is a bacterial parasite with an unusual lifestyle. It grows and reproduces in the periplasm of a host prey bacterium. The complete genome sequence of *B. bacteriovorus* has recently been reported. We have reanalyzed the transport proteins encoded within the *B. bacteriovorus* genome according to the current content of the transporter classification database (TCDB). A comprehensive analysis is given on the types and numbers of transport systems that *B. bacteriovorus* has. In this regard, the potential protein secretory capabilities of at least 4 types of inner membrane secretion systems and 5 types for outer membrane secretion are described. Surprisingly, *B. bacteriovorus* has a disproportionate percentage of cytoplasmic membrane channels and outer membrane porins. It has far more TonB/ExbBD-type systems and MotAB-type systems for energizing outer membrane transport and motility than does *E. coli*. Analysis of probable substrate specificities of its transporters provides clues to its metabolic preferences. Interesting examples of gene fusions and of potentially overlapping genes were also noted. Our analyses provide a comprehensive, detailed appreciation of the transport capabilities of *B. bacteriovorus*. They should serve as a guide for functional experimental analyses.

Keywords

Bacterial parasitism; transport; genome analyses; vectorial metabolism; protein secretion

Bdellovibrio bacteriovorus is a Gram-negative δ-proteobacterium that preys on other Gram-negative bacteria [1]. *B. bacteriovorus* penetrates the outer membrane of its prey and grows intraperiplasmically [2]. There it differentiates from the attack phase cell into the growth phase cell [1,3]. It loses its flagellum and initiates growth. At this point, *B. bacteriovorus* modifies the host cell peptidoglycan [4,5] and converts the host cell into a spherical structure called a bdelloplast in a process dependent on glycanase [2,6]. Not until 45 minutes after initiating the growth phase does DNA replication begin. During the following 2-3 hours, *B. bacteriovorus* causes extensive host cell damage and grows into a long coiled filament [7]. Late steps in the differentiation cycle can be completed outside of the host cell [8]. Although wild-type *B. bacteriovorus* is an obligate parasite, it can be mutated to grow in culture [9].

The *B. bacteriovorus* developmental cycle has been divided into eight phases according to morphological and physiological observations [7,10,11]: (1) The attack phase: *B.*

*Corresponding author: Telephone: (858) 534-4084 Fax: (858) 534-7108 msaier@ucsd.edu.

bacteriovorus swims rapidly and collides with its prey, remaining reversibly attached for a short "recognition" period [12]. (2) Irreversible attachment: Active adhesion, possibly involving multiple fimbriae, occurs at the pole opposite the flagellum. (3) Invasion: *B. bacteriovorus* forms a "penetration pore" in the host cell outer membrane and cell wall [13]. Invasion may involve retractive fimbriae pulling the prey through the pore [11]. Pore formation is believed to occur when *B. bacteriovorus* locally secretes hydrolytic enzymes to degrade outer envelope constituents. Before entry into the periplasm, *B. bacteriovorus* frequently sheds its flagellum [10]. The pore is ultimately resealed by the host cell. (4) Macromolecular synthesis: *B. bacteriovorus* initiates macromolecular (RNA, protein, lipid, polysaccharide) synthesis. The first round of DNA synthesis occurs [2,14]. Since *B. bacteriovorus* can synthesize only 11 amino acids, protein synthesis depends on the uptake of host degradation products [11]. (5) Bdelloplast formation: The rod-shaped host cell rounds up into the spherical bdelloplast [2,15], and cell growth continues. (6) Septation: After formation of a single long snake-like cell, *B. bacteriovorus* synchronously undergoes septation, generating multiple progeny cells. (7) Flagellation: The progeny cells synthesize flagella while in the exhausted host bdelloplast. (8) Exit phase: *B. bacteriovorus* secretes novel hydrolytic enzymes that cause bdelloplast lysis. Release of the progeny attack cells is achieved [16]. This progression of developmental events may be initiated and regulated by a set of sensor kinase/response regulator systems and orchestrated by a sigma factor cascade similar in principle to that established for *Bacillus* sporulation [11,17,18].

Throughout most of the growth phase, prey cytoplasmic and integral membrane proteins are degraded, as are other host cell macromolecules [19,20]. There is evidence that *B. bacteriovorus* secretes proteins, possibly porins [21] but definitely many degradative enzymes [20]. Many of these appear in the host cell cytoplasm although the mechanisms by which they get there are unknown. Upon completion of growth, the single filamentous cell septates, giving rise to multiple motile cells, their numbers depending on the size of the prey cell [22]. Extensive signaling between the predator and prey bacteria seems to be operative [23,24]. *B. bacteriovorus* has the potential of being a therapeutic agent for treatment of Gram-negative bacterial infections [11,25,26,27].

In 2004, the genome sequence of *B. bacteriovorus* was published [11], allowing prediction of its physiology from its gene content. The single circular chromosome contains 3.8 Mbp and includes an estimated 3,600 coding regions. Only 55% of the deducted proteins were assigned a putative function based on homology searches. The rest were of unknown function. The transport systems predicted by annotation of the genome sequence were shown to fall in the classes of ABC-type transporters and MFS permeases or secondary carriers belonging to other transporter families. In this analysis, we are updating the list of transport systems according to the content of the January 2006 version of the transporter classification database (TCDB; http://www.tcdb.org/). We propose possible substrates and functions for some of these transporters.

B. bacteriovorus exhibits a number of properties that suggest the need for a most unusual complement of transport proteins. Several of its metabolic pathways may be incomplete, based on the available gene function annotations [11]. Neither oxidation nor fermentation of carbohydrates, organic acids or alcohols has been demonstrated [1,28]. Biochemically, this organism lacks the phosphoenolpyruvate-dependent sugar transporting phosphotransferase system (PTS) [19]. It seems to depend primarily on non-carbohydrate macromolecular metabolism for carbon and energy [1,29,30]. It secretes many macromolecular degradative enzymes including carbohydrases, proteases, nucleases and lipases [1,19,20]. Thus, it must have a tremendous capacity for protein secretion across both of its membranes [20]. It appears to grow largely at the expense of host cell proteins, nucleic acids, and membrane and cell wall constituents [1,31]. For example, up to 80% of degraded host nucleic acids is

incorporated into *B. bacteriovorus* DNA [32]. However, this is just a fraction of the nucleotides required for growth, so *B. bacteriovorus* must be capable of making all of the nucleotides required for DNA synthesis. In fact, complete pathways for purine and pyrimidine biosynthesis are encoded within the genome [11].

Only a few molecular transport activities in *B. bacteriovorus* have been characterized, and even fewer transport proteins have been associated with these activities. The energy-dependent uptake of intact nucleotides (UMP and ATP) has been demonstrated [33,34]. Uptake of nucleotides appears to be a rare trait for a bacterium, but at least two such systems appear to exist in *B. bacteriovorus*. Neither has been characterized in molecular terms.

We have reanalyzed the complement of recognizable transporters encoded within the *B. bacteriovorus* genome 2 years after its original annotation, as a plethora of new sequences have been made publicly available during this time. The *B. bacteriovorus* genome analyses reported by Rendulic et al. [11] identified transporters as either permeases or ABC-type transporters. Here we present a systematic classification of the *B. bacteriovorus* transporters based on the TC system [35], which facilitates a more detailed understanding of transport function and evolution [36]. The methodology used has been described previously [37].

The genome of *B. bacteriovorus* reveals the presence of potential efflux pumps for hydrophobic and amphipathic drugs and organic solvents as well as potential uptake systems for amino acids, peptides and inorganic anions. The general secretory (Sec) system, the twin arginine targeting protein translocation system, and ABC-type protein secretion systems are also discussed. *B. bacteriovorus* encodes complete flagellar and fimbrial protein export systems and probably a type II main terminal branch (MTB; TC #3.A.15) for secretion of proteins across the outer membrane [38]. Several other protein export systems are described as well. These systems must account for the unusual parasitic lifestyle of this bacterium.

Results

Overview of Transporter Types

Table 1 presents an overall summary of the classes of transporters found in *B. bacteriovorus*. The 406 transport proteins make up 172 transport systems. 11.3% of the genes encode recognizable transport proteins, corresponding to established entries in TCDB. An additional 161 (4.5%) of its genes encode potential transporters that, however, do not give good hits in TCDB (see below). The total potential percent of transport protein encoding genes is therefore 15.8%. Since in most free-living bacteria, 10-15% of the genes encode transport proteins [39], *B. bacteriovorus* may contain an unusually large number of transporters especially considering the fact that the genomes of most intracellular parasites encode lower proportions of transport proteins [39]. The *B. bacteriovorus* genome encodes higher numbers of ABC-type transporters than most other bacterial genomes analyzed [11].

In most bacteria, approximately 3-8% of all the transport proteins encoded in the genome are channel-type transporters [39], *B. bacteriovorus* has a surprisingly large number of recognized channel proteins: 15 inner membrane channel proteins (3.7% of the total number of transport proteins) comprising 12 channels (7% of the transport systems), and 29 outer membrane porin-type channel-forming proteins (7.1% of the recognized transport proteins), corresponding to 16 systems, (9.3% of the transport systems). These numbers presumably reflect a need for rapid, low specificity uptake and export of ions and nutrients, consistent with the unusual lifestyle of *B. bacteriovorus*. Some of these channels may be expressed only at certain phases of the growth cycle (e.g., phase 4, see Introduction) or in response to specific stress conditions.

B. bacteriovorus has substantially more secondary carriers (70 systems, or 41% of the transport systems identified) than primary active transporters (55 systems, or 32%). The genome sequence gives no indication for group translocators of the phosphoenolpyruvate:sugar phosphotransferase system (PTS), confirming biochemical results of Romo et al. [19]. It has just two recognized transmembrane electron flow carrier, but 16 of its putative transporters fall into the TC class 9 category of poorly defined systems. Many (161) potential transport proteins (see below) have no counterpart in TCDB [35]. This is also an unusually large number for a bacterium with a genome of 3.8 Mbps. This observation may reflect a need for permeases of diverse function.

Transport Substrates

Figure 1 presents a breakdown of the transport systems according to substrate type. Forty-four (26%) of the recognized transporters are specific for inorganic molecules. Of these, the large majority transport cations (20%) while far fewer transport anions (3.5%). Nearly 2.5% are low specificity outer membrane porins.

Small organic molecule transporters show a strong bias for drugs and toxic compounds, there being three times as many of these transporters as there are sugar or vitamin transporters. Systems specific for organic acids [40] are only half as plentiful as those for amino acids [14]. A few systems probably transport aromatic compounds and nucleosides. However, 22 transporters (13%) are drug/toxic compound efflux systems. This last percentage is comparable to, but on the high side relative to that found in many other bacteria.

Half of the macromolecular transporters are probably protein export systems, but lipid and polysaccharide exporters are also present. Nearly 30% of the identified transporters fall into the “miscellaneous” or “unknown” category. We suggest the probable substrates and transport mechanisms of several of these transporters. These results will be discussed in more detail below and on our website (http://www.biology.ucsd.edu/~msaier/supmat/Bba).

Distribution of Topological Types

The TMHMM transmembrane helix prediction program [41,42] was used to predict the number of putative transmembrane segments (TMSs) in all the proteins encoded in the *B. bacteriovorus* genome. Of the 3587 recognized protein-encoding genes in *B. bacteriovorus*, 2736 (76%) are predicted to have 0 TMSs, and 849 (24%) are predicted to have 1 or more TMSs. Of the latter, 396 (11%) have only 1 TMS. Many of these will be secreted proteins such as periplasmic binding proteins, which require an N-terminal leader sequence to exit the cytoplasm via the general secretory (Sec) pathway. Remaining proteins with 2 or more TMSs (Fig. 2) include 453 proteins (13%), which have the greatest potential of being transporters. However, it should be noted some of these proteins are not involved in transport as discussed below. Further, only about half of the *B. bacteriovorus* proteins with 2 or more TMSs were classified into transporter families while a majority of the remainder were assigned as putative transporters with no functional information (see below). Of these, 152 (9.2%) have just 2 or 3 TMSs. Many of these are recognizable sensor histidine kinases, but those that function as transporters are likely to be oligomeric pore formers. No proteins with 3 or less TMSs per polypeptide chain have yet been identified that function as carriers [35,43]. Sixty-three proteins (1.8%) have 4 TMSs. These could be either carriers or channels. If carriers, they probably function as dimeric or tetrameric structures [44,45]. Proteins with 5 or more TMSs are likely to be secondary carriers or primary active transporters although some channel proteins are known to have 5 or more TMSs [46,47]. There are nearly equal numbers of predicted 5 and 6 TMS proteins (50 (1.4%) and 47 (1.3%), respectively) encoded within the *B. bacteriovorus* genome. Carriers of 5 or 6 TMSs

generally function as dimers. Transmembrane proteins of 5 TMSs are mostly ABC transporters while those with 6 are primarily secondary carriers (see below). A total of 4.6% of the proteins in *B. bacteriovorus* have 4-9 TMSs; 7, 8 and 9 TMS carriers are also known [47]. Just 2% of the proteins have 10 or more TMSs. Of these, most are predicted to have 10 or 12 TMSs. As shown in Figure 2, large (≥10 TMSs) proteins with even numbers of TMSs predominate over those predicted to have odd numbers (77% even; 23% odd). We believe this has to do with the pathways taken for their evolutionary appearance [43]. The distribution of topological types is not strikingly different than from those of other bacteria [48,49]. The actual proportion of proteins with even numbers of TMSs may be even greater due to errors in topological prediction.

The five largest transmembrane proteins have 16 (4 proteins) and 24 (1 protein) TMSs. The 24 TMS protein (GI:42523740) is a member of the monovalent cation:proton antiporter-3 (CPA3) family (TC #2.A.63) and is a fusion of two previously recognized subunits of this multi-protein transporter complex [50,51]. Transporters of the CPA3 family include subunits that are homologous to subunits of the NADH dehydrogenase complex (TC #3.D.5). Of the four 16 TMS proteins, two (GI:42523556, GI:42523724) are of the CPA2 family (TC #2.A.37). Typical $Na:H^+$ and $K^+:H^+$ antiporters of the CPA2 family have up to 14 TMSs (e.g., GmrA of *Bacillus megaterium*) [52]. The two *B. bacteriovorus* homologues proved to be fusion proteins where the integral membrane transporters are fused to soluble TrkA-like domains [53], and the two extra TMSs precede the TrkA domain, linking the transporter to this soluble regulatory domain.

The third 16 TMS protein (GI:42523211) is a member of the H^+-translocating pyrophosphatase family (TC #3.A.10), most members of which have 16 TMSs [54]. Finally, the fourth 16 TMS protein (GI:42525209) is a homologue of subunit L of the proton-translocating NADH dehydrogenase complex which in other organisms has 16 TMSs.

Predicted Subcellular Localizations of B. bacteriovorus Proteins

We used the PSORTb program [55,56] to predict the subcellular localizations of the proteins in *B. bacteriovorus*. Of the assigned proteins, 860 proteins (23%) were predicted to be cytoplasmic, while 522 (14.1%) were predicted to be in the cytoplasmic membrane. Of the remaining, 56 (1.4%) may be in the periplasm, 100 (2.5%) may be in the outer membrane, and 31 (0.8%) may be extracellular. However, 2018 proteins (58%) could not be assigned a subcellular location using this program. It should be noted that the PSORTb program predicts slightly more proteins (14.1%) to be cytoplasmic membrane proteins than based merely on the number of proteins with 2 or more predicted transmembrane helices (13%) as noted above. The PSORTb program uses the HMMTOP algorithm to predict the number of transmembrane segments in a protein. Different prediction programs used to predict the topology and subcellular localization of proteins, often yield varying results [57].

Channels (TC #1.A)

As noted above, *B. bacteriovorus* has a large number of channel types. Three are homologous to known chloride channels of the ClC family (TC #1.A.11; Table 2). A fourth protein (GI:42524203) shows statistically significant sequence similarity to a central portion of an epithelial chloride channel (E-ClC, TC #1.A.13), but the rest of the protein does not resemble members of the E-ClC family. There is therefore no clear evidence that this protein functions in anion transport.

B. bacteriovorus possesses five mechanosensitive channels, one of the MscL-type (TC #1.A.22) and four of the MscS-type (TC #1.A.23). Both types of channels are known to function in hypoosmotic stress adaptation [58]. Only one of these five channel proteins had been

discussed previously [11] although the NCBI Genbank records do include annotations noting that these proteins are putative mechanosensitive ion channels.

As reported previously [11], *B. bacteriovorus* encodes 3 MotA and 3 MotB homologues (TC #1.A.30.1). These occur within three operons that each contains a single *motA* and a single *motB* gene. This observation is surprising since *B. bacteriovorus* encodes only a single flagellum. Possibly the three flagellar "torque generators" act on a single flagellum under different conditions. For example, for swimming vs. swarming motility as is the case of *Bacillus subtilis* [59,60]. However, these Mot proteins are distantly related to gliding motility genes in *Myxococcus xanthus* [61], and gliding motility may be a characteristic of *B. bacteriovorus* [11]. One or more of these MotAB pairs may therefore function to energize gliding motility rather than flagellar rotation.

Finally, *B. bacteriovorus* has a single divalent metal ion channel of the MIT or CorA family (TC #1.A.35). CorA family members can be specific for a single divalent cation or can allow entry of several [62]. This homologue may provide a primary mechanism for divalent cation (Mg^{2+}, Co^{2+}, etc.) uptake in this organism.

Outer Membrane Porins (TC #1.B)

B. bacteriovorus has a fair complement of outer membrane β-structured porins. These include a single member of the 16 TMS sugar porin family (TC #1.B.3), 4 paralogues of the 8 TMS OmpA-type porin family (TC #1.B.6) and two members of the 12 TMS Tsx nucleoside-specific porin family (TC #1.B.10). Four outer membrane receptors (TC #1.B. 14) probably function in the energy-dependent uptake of Fe-siderophore complexes (3 systems) and vitamin B_{12} (1 system). There are also seven outer membrane factors (TC #1.B.17) that presumably function in conjunction with inner membrane efflux pumps. One of these resembles PtrF of *E. coli* and probably acts with an ABC-type protease exporter; a second most closely resembles NodT2 and may therefore catalyze oligosaccharide export; several others probably act with RND-type drug efflux pumps. TolC of *E. coli* can function with multiple transporters from different families, and three of the OMF family members in *B. bacteriovorus* proved most similar to TolC. Consequently, these proteins may be multifunctional. Surprisingly, we could identify only four membrane fusion proteins (MFP, TC #8.A.1) [63]. These proteins probably function with ABC- (3) and RND-type (1) drug exporters. All MFPs characterized to date function with a single efflux transporter, so at least 7 might be expected to be present. Since these proteins are sequence divergent, some MFPs encoded in the *B. bacteriovorus* genome may not have been identified by our current search and annotation techniques.

Two outer membrane secretins (TC #1.B.22) were found. One, a PilQ homologue, may function as a "porthole" in the export of type IV pilus subunits [64,65]. The other, an XcpQ homologue, is likely to serve as the porthole for a type II protein secretion system of the main terminal branch [66,67].

B. bacteriovorus has four homologues of *E. coli* YaeT (TC #1.B.33) and one homologue of *E. coli* Imp (OstA). It also has one homologue of YfiO and two of YfgL (none of NlpB) [68]. These *E. coli* proteins are known to function as a complex for the assembly and insertion of outer membrane macromolecules, proteins, lipids and/or lipopolysaccharides [68]. Other subunits of the *E. coli* complex may exist but have not yet been identified. One such candidate is encoded by a gene in an operon that also encodes several homologues of other constituents of the *E. coli* outer membrane biogenesis complex. It seems clear that *B. bacteriovorus* assembles its outer membrane as does *E. coli*. However, the presence of four YaeT homologues and two YfgL homologues suggests a level of complexity greater than observed for *E. coli*. There may be four distinct systems corresponding to the four YaeT

homologues, and these may share the other components of these systems. Alternatively, more than one YaeT homologue may participate in the formation of a single complex. Interestingly, *B. bacteriovorus* has a LolA-like outer membrane protein that may function in lipoprotein export [11].

Finally *B. bacteriovorus* encodes a single holin of the LrgA family (TC #1.E.14), not discussed previously [11], although the Genbank record does contain annotation suggesting homology to the LrgA family. This protein is encoded by a gene that is downstream of and within the same operon as a putative autolysin, a murine hydrolase. It would seem that *B. bacteriovorus* encodes a chromosomal holin/autolysin system that may function in programmed cell death [69]. A penicillin-binding protein, possibly also involved in cell wall metabolism, is encoded within the same operon.

Secondary Carriers (TC #2.A)

B. bacteriovorus has substantial representation of transporters of the Major Facilitator Superfamily (MFS, TC #2.A.1). These include 6 drug exporters of the DHA1 family (TC #2.A.1.2) and two sequence divergent members of the OPA family (TC #2.A.1.4) that may function in sugar-phosphate:inorganic phosphate antiport. One member of the MFS (TC #2.A.1.6) shows greatest sequence similarity to the KgtP α-ketoglutarate uptake transporter of *E. coli* (see TCDB). Two more show greatest similarity with a putative acriflavine uptake transporter (TC # 2.A.1.36). Such systems probably have some other aromatic compounds as their natural substrates. One putative MFS transporter closely resembles the AmpG transporter of *E. coli* (TC #2.A.1.25) which takes up cell wall degradation products [70,71]. Three MFS members in *B. bacteriovorus* could not be assigned a substrate type due to their low BLAST scores with functionally characterized transporters. They may be members of uncharacterized families not yet in TCDB.

Some other families in TCDB have been shown to be distantly related to the MFS [72,73,74]. Of these, *B. bacteriovorus* has one member in the GPH family (TC #2.A.2) of glycoside permeases which might be a melibiose uptake system, and one member of the POT family (TC #2.A.17) of peptide uptake systems. The presence of these two transporters expands the limited repertoire of transporters *B. bacteriovorus* has for taking up sugars and amino acid derivatives. A single member of the APC superfamily (TC #2.A.3) of transporters for amino acids and their derivatives is present in *B. bacteriovorus*, fewer than in most bacteria with a genome of comparable size. This transporter is a member of the ABT family (TC #2.A.3.6) for which no functionally characterized members are available.

Two members of the Cation Diffusion Facilitator (CDF) family (TC #2.A.4) are also present. CDF carriers function in prokaryotes as heavy metal (Co^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+} and Hg^{2+}) efflux pumps probably using a Me^{2+}/H^{+} antiport mechanism [75,76]. These pumps can exhibit broad or narrow specificities, so the two CDF carriers in *B. bacteriovorus* may be a broad and a narrow specificity system like the two proteins in TCDB (YiiP and CzcD, respectively) that they most closely resemble.

A single member of the ZIP family (TC #2.A.5) of heavy metal uptake carriers occurs in *B. bacteriovorus*. These carriers can be Zn^{2+}-specific or broad specificity (Fe^{2+}, Co^{2+}, Mn^{2+}, etc.) uptake systems. The *B. bacteriovorus* homologue most resembles ZupT, a broad specificity system of *E. coli* [77].

Three families within the RND superfamily (TC #2.A.6) are represented in *B. bacteriovorus*. The first family is the heavy metal efflux (HME) family (TC #2.A.6.1). The single *B. bacteriovorus* homologue in this family is most similar to the CzcA protein of *Ralstonia eutropha*, a Co^{2+}, Zn^{2+}, Cd^{2+} efflux system. The second family is the Hydrophobe/

Amphiphile Exporter (HAE1) family of drug efflux pumps (TC #2.A.6.2). Six of these paralogues are present in *B. bacteriovorus*. All of them most closely resemble MdtC (YegO) of *E. coli*, a broad specificity drug/detergent/organic solvent/lipid exporter [78]. These systems might protect the parasite against defense mechanisms of the host bacterium. The last *B. bacteriovorus* RND homologue is a member of the mostly archaeal HAE3 family (TC #2.A.6.7) which is still poorly characterized.

Within the Drug/Metabolite Transporter (DMT) superfamily (TC #2.A.7) are two members of the SMR family (TC #2.A.7.1) of small multidrug resistance systems. One most closely resembles the *E. coli* EmrE broad specificity cationic drug exporter, while the other resembles the *E. coli* SugE narrow specificity cationic drug exporter [44,79]. Three members of the DME family (TC #2.A.7.3) are probably metabolite efflux pumps. Two of these most closely resemble the RhtA protein of *E. coli* which is a threonine/homoserine exporter that may also be able to accommodate other semipolar amino acids [80]. Two more distantly related putative 10 TMS members of the DMT superfamily could not be assigned membership to an established family within the DMT superfamily. They may be members of new families.

B. bacteriovorus encodes a single Ca^{2+}:cation antiporter of the CaCA family (TC #2.A.19). All characterized members of this family function in Ca^{2+} extrusion from the cytoplasm using a monovalent cation antiport mechanism. Therefore, this is likely to be its function in *B. bacteriovorus*. Prokaryotic members of the NSS family (TC #2.A.22) are amino acid uptake systems, and the one from *B. bacteriovorus* most resembles the tryptophan uptake system of *Symbiobacterium thermophilum*, TnaT [81]. There is also a single member of the glutamate:sodium symporter (ESS) family (TC #2.A.27) represented in *B. bacteriovorus*, a protein resembling GltS of *E. coli* which transports both D- and L-glutamate as well as various glutamate derivatives.

B. bacteriovorus encodes within its genome many putative cation:proton antiporters, one of the NhaC family (TC #2.A.35). NhaC-type systems can function as Na^+:H^+ antiporters or malate • $2H^+$:lactate • Na^+ antiporters [82,83]. Thus, members of this family may merely act as cation exchangers, but they may also be capable of electroneutral transport of organic anions.

Five members of the CPA2 family (TC #2.A.37) of monovalent cation transporters are encoded within the *B. bacteriovorus* genome, and they most closely match four different transporters in TCDB. Two resemble *Bacillus* GrmA, a spore germination protein of unknown transport specificity, which, however, closely resembles the GerN Na^+/H^+-K^+ antiporter of *Bacillus cereus*; the second resembles the KefC glutathione-regulated K^+ efflux protein of *E. coli*; the third looks like the MagA iron-regulated transporter of a magnetotactic bacterium; and the fourth is like NhaS3 of *Synechocystis*, a Na^+:H^+ antiporter. It seems likely that each of these *B. bacteriovorus* homologues will prove to catalyze a different reaction, but always acting on monovalent cations.

Two additional monovalent cation transporters are found within the *B. bacteriovorus* genome. The first is a multicomponent Na^+ or K^+:H^+ antiporter of the CPA3 family (TC #2.A.63). All seven characteristic constituents of these systems were identified in *B. bacteriovorus* although only 6 had been annotated. One of them (GI:42523740) is a fusion protein of two previously recognized subunits of these systems. The system in *B. bacteriovorus* most closely resembles the Na^+:H^+ antiporter of the Gram-positive bacterium, *Staphylococcus aureus*, rather than the K^+:H^+ antiporter of the Gram-negative bacterium, *Rhizobium meliloti* [50,84]. It is therefore likely that the *B. bacteriovorus* homologue is a Na^+:H^+ antiporter. These complex Na^+:H^+ antiporters, with subunits homologous to

subunits in NADH dehydrogenase, are possibly dependent on $NADH_2$. Such a Na^+:H^+ antiporter has not been characterized in a Gram-negative bacterium before.

The K^+ uptake (KUP) permease (TC #2.A.72) of *E. coli* may use a K^+:H^+ symport mechanism, allowing a 10^6-fold accumulation of K^+ over the external medium [85,86]. This protein has an N-terminal 12 TMS topology (residues 1-450) followed by a hydrophilic domain of unknown function (residues 450-622). The same structure is observed for the *B. bacteriovorus* protein that it closely resembles. This suggests that the *B. bacteriovorus* homologue may function, and may be regulated, like the *E. coli* homologue.

Four members of the MOP superfamily (TC #2.A.66), from three different constituent families, were identified in *B. bacteriovorus*. One is probably an MDR efflux pump of the MATE family (TC #2.A.66.1) of drug:Na^+ antiporters; the second is likely to be a polysaccharide exporter of the PST family (TC #2.A.66.2); and the third and fourth are members of the “Mouse Virulence family” (TC #2.A.66.4) with no functionally characterized member.

B. bacteriovorus encodes one or two members of each of several small solute carrier families. One resembles the BenE benzoate:H^+ symporter of *Acinetobacter calcoaceticus* (TC #2.A.46) [87]; a second and third are members of the DASS family (TC #2.A.47) of divalent anion:Na^+ symporters within the IT superfamily [88]; the fourth is a full-sized chromate/sulfate transporter of the CHR family (TC #2.A.51) [89,90,91,92]; the fifth is a putative phosphate:Na^+ uptake symporter of the PNaS family (TC #2.A.58); the sixth is a member of the ArAE family (TC #2.A.85) which may export one or more aromatic acids [93] and functions with a Membrane Fusion Protein (TC #8.A.1) [94]; and the seventh and eighth are putative AbgT family (TC #2.A.68) homologues which might be peptide, p-aminobenzoyl-glutamate and/or drug uptake porters. One of these proteins (GI:42521870) had been annotated as a short chain fatty acid transporter like AtoE of *E. coli* (TC #2.A. 73.1.1).

Two remaining pmf-dependent systems listed in Table 2 under the 2.A category of TCDB are involved in protein trafficking. One resembles the YidC protein of the Oxa1 family (TC #2.A.9). This protein probably catalyzes protein insertion into the cytoplasmic membrane [95]. The other is the Twin Arginine Targeting and Translocation (TAT) protein secretion system (TC #2.A.69) [96]. All bacteria with a Tat system have at least 1 TatC constituent and at least 1 TatA constituent. *E. coli* and *B. bacteriovorus* have three TatA homologues, TatA, TatB and TatE [96]. *B. bacteriovorus* has a single TatC as does *E. coli*, and it is encoded within a bicistronic operon that also encodes a TatB homologue. Unlike the gene arrangement in *E. coli*, TatA and TatE are both encoded elsewhere on the chromosome.

Outer Membrane Receptor (OMR) Energizers for Active Transport Across the Outer Membrane

Category 2.C.1 in TCDB includes a single family of multicomponent, pmf-dependent transporter energizers. Two such systems are present in *E. coli*, and at least three constituents show sequence similarity between these two systems. The TonB/ExbB/ExbD system shows sequences and functions similar to the TolA/TolQ/TolR system. The latter system has additional auxiliary proteins called Pal (a lipoprotein), TolB and YbgF. *B. bacteriovorus* encodes at least one complete TolA-type system with minimally one copy of each of the recognized *E. coli* auxiliary constituents (6 non-homologous proteins). However, encoded in this *B. bacteriovorus* genome are 6 TonB/TolA homologues, six ExbB/TolQ-like constituents, nine ExbD/TolR-like proteins, three YbgF homologues, two TolB homologues and one Pal lipoprotein. Operon analyses revealed that one set of TolA, TolB, TolQ, and TolR are encoded together in a single operon. Five separate TolQ/TolR pairs are encoded in

five other distinct operons, and three of these also encode an extra ExbD/TolR-like homologue. Pal, TolB and YbgF homologues, present in 1, 2 and 3 copies, respectively (Table 2), are encoded at sites distant from each other and the other Tol genes, with the single exception noted above where a TolB homologue is encoded within an operon with TolA, TolQ and TolR homologues.

The operon structures were examined in the proximity of these genes. In brief, there are four OMR receptors in Bba; three are probably specific for complex iron; and one is specific for vitamin B_{12}. Six TolQ/TolR homologues are present, but there are fewer TolB, Pal and YbgF homologues. It is probable that these last mentioned proteins either can function with multiple co-transcribed pairs of TolQR proteins or are not required. This might suggest that each OMR interacts with its own TolQ/R pair, any of which can use the same TolB/Pal/YbgF complex. It is interesting to recall that TolQ/R homologues (MotA/B homologues) may function in adventurous gliding motility in *Myxococcus xanthus* [61](see acc. #AAO22857). The possibility that *B. bacteriovorus* is capable of gliding motility using type four pili has not been demonstrated [11].

Primary Active Transporters – ABC Superfamily (TC #3.A.1)

The ABC superfamily of ATP-driven transporters is the largest transporter superfamily represented in the *B. bacteriovorus* genome. Fourteen potential uptake systems and 20 potential efflux systems were identified, and all of these systems appear to be complete, having all of the expected constituents. One maltose-type system of the CUT1 family (TC #3.A.1.1) and one ribose-type system of the CUT2 family (TC #3.A.1.2) were identified. In the former system, the MalE binding protein and the MalF protein constituent are fused in a single polypeptide chain derived from a single fused gene, an unusual arrangement. In the latter system, one cytoplasmic (C) ATP-hydrolyzing protein (RbsA), one periplasmic receptor (R) and two membrane (M) constituents were found. This arrangement resembles that of a minority of CUT-2 transporters. Others have a single membrane constituent and thus have only three constituents, one C, one M and one R. The *E. coli* ribose system has the equivalent four gene products, RsbABCD, where A is the cytoplasmic ATPase, B is the periplasmic receptor, and C and D are the channel-forming membrane proteins.

One system for uptake of polar amino acids (PAAT family, TC #3.A.1.3) and one system for uptake of hydrophobic amino acids (HAAT family, TC #3.A.1.4) were found. Both systems appear to be complete with three constituents in the PAAT family system (1R, 1C and 1M) and five in the HAAT family system (1R, 2Cs and 2Ms).

There is a complete oligopeptide uptake system (TC #3.A.1.5) like that in *E. coli* with two Rs, two Cs and two Ms. However, a strange situation is observed for the dipeptide (Dpp) system where there is a single receptor (R) and two pairs of membrane proteins (M) but no ATPase (C). Possibly these two systems use the sequence similar OppD and OppF to energize transport. Complete ABC uptake transporters specific for (1) phosphate (resembling PstABC/PstS of *E. coli*; 4 constituents; TC #3.A.1.7), (2) phosphonates (most like PhnCDE of *E. coli*; 3 constituents; TC #3.A.1.9), (3) polyamines (PotABCD of *E. coli*; 4 constituents; TC #3.A.1.11), (4) zinc (ZnuABC of *E. coli*; 3 constituents; TC #3.A.1.15), (5) inorganic anions (nitrate, nitrite, cyanide and bicarbonate; 3 constituents; TC #3.A.1.16), taurine (3 constituents; TC #3.A.1.17), and (6) thiamin (3 constituents; TC #3.A.1.19) were found.

ABC efflux systems include (1) a single system specific for lipooligosaccharides (2 constituents; TC #3.A.1.102), (2) three systems specific for lipids and/or drugs (one constituent each; TC #3.A.1.106), (3) either one 4-component or two 2-component Na^+ exporter(s) (2 constituents each; TC #3.A.1.115), (4) a macrolide exporter (1 constituent; TC

#3.A.1.122), (5) two probable lipoprotein exporters (2 or 3 constituents each; TC #3.A. 1.125), and (6) two eukaryotic-like MDR pumps (TC #3.A.1.208), one resembling the plant AtMRP2 system and the other resembling the human MRP3 system. There are also about a dozen functionally unassigned ABC systems or orphan proteins. We have made functional predictions for some of these proteins when warranted (see Table 2). The Genbank records also include annotations of recognizable domains for many of these proteins.

Primary Active Transporters – Other Cation-transporting ATPases

B. bacteriovorus has one complete H^+-translocating F-type ATPase (TC #3.A.2) and one H^+-translocating pyrophosphatase (TC #3.A.10). Both enzymes can reversibly synthesize pyrophosphate bonds using the proton electrochemical gradient (the proton motive force, pmf) as the driving force. *B. bacteriovorus* also has five P-type ATPases (TC #3.A.3), one likely to be specific for Ca^{2+} (efflux), one for K^+ (uptake), and three heavy metal systems that could be either uptake or efflux systems. No other cation-translocating ATPases appear to be encoded within the *B. bacteriovorus* genome.

Primary Active Transporters – ATP-dependent Protein Secretion Systems

As reported previously [11], *B. bacteriovorus* has a complete (11 component) general secretory (Sec) system (TC #3.A.5) including SecYEG, SecA, SecDF, FtsY and Ffh, YjaC, the 4.5 S RNA and FtsE. A very sequence-divergent FtsX was also identified (see below). *B. bacteriovorus* has a complete (11 component) flagellar protein export system (TC #3.A.6) [11] and possesses a single member of the septal DNA translocator (S-DNA-T) family (TC #3.A.12), essential for DNA translocation after septum formation in many bacteria. This protein may be required to complete DNA translocation after synchronous cell division of *B. bacteriovorus* snakes (phase 6 in the developmental cycle) (see Introduction).

B. bacteriovorus has protein constituents resembling those of two related types of outer membrane protein secretion systems (TC #3.A.15). One of these is the type II protein secretion system or main terminal branch (MTB), like the PulC-O,S system found in *Klebsiella pneumoniae* (14 constituents) [67], and the other is the pilin secretion/fimbrial assembly system like the PilA-EQTU FimTU system of *Pseudomonas aeruginosa* (10 constituents) [97,98]. The system(s) found in *B. bacteriovorus* has(have) at least 13 constituents, 7 most closely resembling the Pul system, and 6 most closely resembling the Pil system. However, there are many other *pil* (pilus) and *fim* (fimbrium) genes present on the *B. bacteriovorus* chromosome. Rendulic et al. [11] suggested that these proteins comprise a single system, a Pil-type rather than an MTB-type system. If, as proposed, *B. bacteriovorus* has a type 4 pilus-driven system for passage through the host cell envelope, then the suggestion that it is a Pil-type system is valid. Indeed, Schwudke et al. [99] have shown that a pilus gene, *flp1*, shows increased expression in the attack phase of Bba, compared to the intracellular replication phase. However, it is known that *B. bacteriovorus* secretes many proteins across its two-membrane envelope, and consequently, a Pul-type system would be expected to be useful. The nine *pul* genes in *B. bacteriovorus* occur within two operons, and these are distant in sequence from any of the *pil* genes recognized using TC-BLAST [36]. Other *pil* annotated genes not listed in Table 2 are present in the *B. bacteriovorus* genome, but these genes are not homologous to genes encoding the proteins of the *P. aeruginosa pil* system in TCDB (TC #3.A.15.2.1). They also localized to regions of the chromosome distant from the two *pil* operons in *B. bacteriovorus* that encode five of the *pil* genes listed in Table 3. On a purely bioinformatic basis, it is difficult to distinguish the Pul-from the Pil-type systems with certainty. However, based on their degrees of sequence similarity, we propose that *B. bacteriovorus* has both a complete Pil biogenesis system and a functional Pul-type (type II) protein secretion system.

Many prokaryotes have Na^+-transporting organic acid decarboxylases (TC #3.B.1) which include α-, β- and γ-subunits [100,101]. *B. bacteriovorus* has two copies of both the α- and γ-subunits, but we could not identify a β-subunit. The β-subunit is the actual transporter, while α is the decarboxylase (often present without β), and γ, a Zn^{2+}-binding protein of catalytic importance [102,103] is thought to be the linker connecting α and β. The absence of a recognizable β leaves the question open as to whether *B. bacteriovorus* can couple decarboxylation to Na^+ expulsion.

Primary Active Transporters – Cation-translocating Electron Transfer Complexes

Many bacteria possess Na^+-translocating NADH dehydrogenase complexes of 14 dissimilar subunits [104,105,106]. In *B. bacteriovorus,* 13 proteins comprise the NADH dehydrogenase (TC #3.D.1), encoded within two operons. One of the 13 proteins is a fusion protein (Nqo5-Nqo4). Thus, the system is complete and is presumed to be functional. *B. bacteriovorus* also has a complete proton pumping cytochrome oxidase complex (TC #3.D.4) with all five expected proteins (Cox1-4 and X) encoded within a single operon. Surprisingly, it also has several homologues of cytochrome oxidase subunits that map elsewhere on the chromosome. A Cox1-like homologue and a Cox2-like homologue are encoded within a single operon and show low sequence similarity to the Cox subunits. They have high sequence identity with the two subunits of nitric oxide reductases [107,108], and therefore presumably serve this function. These enzyme complexes may be capable of coupling proton export to electron flow [107].

Transmembrane Electron Flow Carriers (TC #5.A)

B. bacteriovorus possesses two transmembrane electron flow systems that can influence cellular energetics. One is disulfide bond oxidoreductase D (DsbD; TC #5.A.1) in which electrons from an electron donor such as NADH in the cytoplasm are transferred sequentially via thioredoxin reductase, thioredoxin and DsbD to a periplasmic disulfide-containing protein electron acceptor. Several such periplasmic proteins (DsbC, DsbE and DsbG) can be reduced via the DsbD pathway, and some of them can further reduce other disulfide-containing periplasmic proteins in Gram-negative bacteria [109]. The DsbD pathway in *B. bacteriovorus* undoubtedly facilitates proper folding of and disulfide bond formation in periplasmic proteins as is the case for *E. coli* [110,111].

The second transmembrane electron flow carrier is a single member of the Prokaryotic Molybdopterin-containing Oxidoreductase (PMO) family (TC #5.A.3), probably a dimethylsulfoxide (DMSO) oxidoreductase. One large protein (1033 aas) is a DmsAB fusion protein including the equivalent of the α- and β-subunits while the other is a DmsC subunit (the γ-subunit) [112,113]. Both proteins show greatest sequence similarity with an archaeal enzyme in TCDB (Table 2).

(Putative) Transporters of Unknown Function or Mechanism

In the 9A category of incompletely characterized transporters, we find one FeoAB iron uptake system (TC #9.A.8), one MgtE magnesium uptake porter (TC #9.A.19), and one putative iron transporter of the Ferroportin family (TC #9.A.23), as reported previously [11]. Our preliminary analyses suggest that this last *B. bacteriovorus* protein may be distantly related to members of the Major Facilitator Superfamily in agreement with the Genbank annotation.

In the 9B series of putative permeases, we find two homologues of Bacterial Murein Precursor Exporters of the MPE family (TC #9.B.30) which are found in many, if not all, bacteria. These porters probably serve the function of exporting precursors essential for bacterial cell wall synthesis [114,115]. Members of the Putative Fatty Acid Transporter

family (TC #9.B.17) are acyl CoA synthetases that may in some cases function in fatty acid uptake coupled to esterification with cytoplasmic coenzyme A [116,117,118,119].

B. bacteriovorus encodes four potential hemolysins that could function in host cell lysis or pore formation in the cytoplasmic membrane. The two members of the HlyIII family (TC #9.B.30) are homologous to the *B. cereus* hemolysin III [120,121]. The two members of the HlyC/CorC family (TC #9.B.37) include one protein believed to be a hemolysin [122] and one protein believed to be a component (CorB) of the Ca^{2+}/Mg^{2+} uptake transporter [123]. All proteins that show homology with members of the 9B class are homologous to TC entries of unestablished functions (Table 2).

Updated annotations and distant homologues of established transporters

Table S1 on our website (http://www.biology.ucsd.edu/~msaier/supmat/Bba) lists the proteins originally annotated as ABC-type proteins (8) or permeases (10) for which we now suggest new annotations. In addition to identifying the proteins and providing the original annotations, this table presents the protein sizes and the predicted numbers of TMSs. In some cases where we suspected erroneous annotations, we noted that several homologues in the NCBI database also appeared to be annotated in the same way, thus providing an explanation for discrepancies noted in this analysis. This allowed us to identify erroneous annotations in the current databases as well as surprising examples of gene overlap in the *B. bacteriovorus* genome. These findings are described in our website (http://www.biology.ucsd.edu/~msaier/supmat/Bba).

Putative Transporters Lacking Functional Data or Close Homologues in TCDB

One hundred and nine putative transporters were identified on the basis of their predicted transmembrane α-helical topologies, PSORTb localizations, and operon associations (Table S2). Like some of the proteins mentioned in the previous section and on our website, some of the proteins included in Table S2 had been annotated as permease-like constituents, and these were also investigated. The results of these studies are presented on our website.

Discussion and overview

B. bacteriovorus is an organism with an unusual lifestyle. It grows only in the intraperiplasmic space of another host Gram-negative bacterium (see Introduction). It derives its nutrients by degrading host macromolecules and hence secretes degradative enzymes from its own cytoplasm to that of its host cell [20]. Such mechanisms are not yet understood.

We have analyzed the transporters in this organism to determine what systems might confer upon *B. bacteriovorus* its unique physiological characteristics. We identified several additional putative transport proteins than reported in the original genome annotation effort [11]. Our most interesting and provocative findings will be summarized here, and the potential physiological importance of some of our observations will be considered.

Monovalent Ion Transport

B. bacteriovorus has an unusually large complement of transporters for monovalent cations (H^+, K^+ and Na^+). Some of these are primary active transporters that promote the generation of electrochemical gradients (pmf and smf) that in part comprise the membrane potential. *B. bacteriovorus* probably uses respiratory function primarily for this purpose, NADH dehydrogenase to generate Na^+ gradients, and both cytochrome oxidase and nitric oxide reductase to create the proton electrochemical gradient. These are then used to make high-energy phosphodiester bonds in ATP and pyrophosphate via the F-type ATPase and the H^+-

translocating pyrophosphatase, respectively. The DsbD oxidoreductase and the dimethylsulfoxide (DMSO) reductase both catalyze transmembrane electron flow from the cytoplasm to the periplasm and thus tend to dissipate the pmf. The Kdp-like P-type K^+ uptake system uses ATP to bring K^+ into the cell, so if this is an electrogenic system, it should dissipate the pmf. This system is not likely to influence the membrane potential appreciably except under highly selective conditions [124]. The ABC-type Na^+ exporter(s) (NatE) also use ATP to pump Na^+ out of the cell, so this process is expected to enhance the pmf. If ATP or pyrophosphate is available, then the F-type ATPase or the H^+-translocating pyrophosphatase, respectively, can generate a pmf using these high-energy compounds.

All other recognized monovalent ion transporters are cation uniporters, antiporters and symporters, but these derive from many independent families as well as different subfamilies within the primary families. One NhaC cation/proton (or malate•$2H^+$/lactate Na^+) antiporter, and five CPA2 cation/proton antiporters, are encoded in the *B. bacteriovorus* genome. However, four of the 5 proteins from the latter family most closely resemble phylogenetically divergent TC entries within the NhaC family, and may therefore serve dissimilar functions. These homologous systems may, of course, be developmentally regulated, being activated only during specific stages in the *B. bacteriovorus* life cycle. Single members from (1) the multicomponent CPA3 family, which may use NADH to drive Na^+ efflux and (2) a KUP-type K^+/H^+ symporter for K^+ uptake are also present in *B. bacteriovorus*.

Divalent Cation Transporters

B. bacteriovorus appears to have three or four types of divalent cation uptake systems. One is an ABC-type system similar to the *E. coli* ZnuABC zinc transport system; a second is represented by three P-type copper ion/heavy metal uptake and/or efflux ATPases [125]; a third is a CorA-type divalent ion-specific channel-type system used primarily for cation uptake; and the fourth is a Zip family carrier-type system responsible for Zn^{2+}, Fe^{2+} and/or Mn^{2+} uptake. While the former two types of systems (1 and 2) are driven by ATP-hydrolysis, both of these last two mentioned systems (3 and 4) are probably driven by the membrane potential. These four types of systems could exhibit overlapping but distinct specificities and affinities, and be regulated in response to different stimuli.

Other systems are probably efflux systems. These include (1) a P-type Ca^{2+}-ATPase, (2) the three heavy metal P-type ATPase efflux systems mentioned above, (3) a Ca^{2+}/H^+ antiporter (for Ca^{2+} extrusion), (4) an RND-type heavy metal efflux system, and (5) two CDF-type heavy metal efflux systems, one possibly of broad specificity and the other of narrow specificity (see Results and TCDB). While (1 and 2) are ATP hydrolysis-driven, (3-5) are pmf-driven. These systems probably interact functionally to maintain essential concentrations while preventing the accumulation of toxic levels of these substances.

Anion Transporters

Many systems in *B. bacteriovorus* transport organic and inorganic solutes with inwardly directed polarity, and in some cases we cannot be sure if the natural substrates are one or the other or both. Of the potential organic anion transporters, there is one system for aromatic acid uptake and one for aromatic acid efflux belonging to the Major Facilitator (TC #2.A. 1.15) and ArAE (TC #2.A.85) families, respectively. There is also a member of a family of ABC systems for the uptake of organic phosphonates. Members of the DASS family in the IT superfamily may take up either organic and/or inorganic anions.

At least one member of each of 5 different families is likely to transport inorganic anions. There are two transporters that are probably specific for sulfate (the CHR and SulP

families), two likely to be specific for phosphate (the PNaS family of phosphate:Na^+ symporters and the PP family of ATP-dependent phosphate uptake permeases in the ABC superfamily), and one specific for various anions (NO_3^-, NO_2^-, CN^- and HCO_3^-) in the NNP family within the ABC superfamily.

Nutrient Transport

B. bacteriovorus appears to have numerous uptake systems for amino acids and peptides but relatively few for sugars. The three probable sugar uptake permeases are (1) a member of the GPH family, probably specific for glycosides such as melibiose, (2) an ABC Cut1 family system specific for maltose and malto-oligosaccharides, and (3) a Cut2 family system specific for ribose. In contrast, numerous systems are present for the uptake (and efflux) of amino acids and their derivatives. Five potential peptide uptake permeases derive from four TC families: (1) a potential peptide transporter of the AbgT family in the IT superfamily, (2) an AmpG-like carrier, probably specific for cell wall degradation products in the MF superfamily, (3) a peptide transporting permease within another family of the MFS, the POT family [72], and (4) an oligopeptide (OPP) system and either one or two dipeptide (DPP) system(s), all within the ABC superfamily. In this last case, only a single pair of ATPase subunits was identified, although receptors and membrane proteins were found for two or three systems. Consequently, we postulate that the former ATPase subunits may be shared by both or all three systems. Although we know of no precedence for such a postulate in the ABC superfamily, the sharing of energy-coupling proteins by multiple transporters has been amply documented in the bacterial phosphotransferase system (PTS) superfamily [126].

Uptake systems for amino acids include: (1) a member of the BAT family within the APC superfamily (of unknown specificity, but almost certainly specific for amino acids and their derivatives as are all members of this superfamily), (2) a member of the NSS family, possibly a tryptophan uptake system, (3) a member of the ESS family, likely to transport acidic amino acids such as D- and L-glutamate and their derivatives, and (4) two ABC-type systems, one specific for polar amino acids (the PAAT family) and one specific for hydrophobic amino acids (the HAAT family).

Candidates for the functionally characterized ATP/ADP antiporter(s) in *B. bacteriovorus* [33,34] include two sequence-divergent members of the organophosphate/phosphate antiporter (OPA) family in the MF superfamily and a sequence divergent member of the phosphate transporter family (PNaS; TC #2.A.58; see our website). If these do in fact prove to be the “missing” antiporters, they will represent new specificities within these families. Other members of the MFS, and members of other families, distantly related to any of the functionally characterized members, potentially could also fulfill this role.

Drugs and Hydrophobic Substances

B. bacteriovorus encodes drug/amphiphile exporters from all superfamilies that are known to include MDR pumps. Thus, there are (1) six MFS (H^+ antiport) systems, (2) six RND (H^+ antiport) systems, (3) five ABC (ATP-dependent) systems from three different ABC families, one from a predominantly eukaryotic family, (4) two cationic drug exporters of the SMR family within the DMT H^+-antiporter superfamily, one probably of narrow specificity and one of broad specificity, and (5) one Na^+ antiporter from the MATE family within the MOP superfamily. Altogether there are 20 putative drug/amphiphile exporters, more than 10% of the total number of transporters identified. These pumps may protect *B. bacteriovorus* against efforts of the host cell to defend itself against bacterial predation using toxic chemicals.

Macromolecular Exporters

Protein secretion and membrane insertion must play a major role in the predatory lifestyle of *B. bacteriovorus*. It has been suggested that this parasite somehow exports hydrolytic enzymes directly from its own cytoplasm to that of its prey across three membranes (see ref. [20] for a review). The only systems known to achieve this feat in a single step are the type III [127] and type IV [64] secretion systems of Gram-negative bacteria, but these systems are lacking in *B. bacteriovorus*. An alternative mechanism must therefore be proposed. We suggest that *B. bacteriovorus* secretes these enzymes into the host periplasm, and that the host cell then takes them up by retrotranslocation [128]. Although such a process has not been documented in prokaryotes, it is well documented in eukaryotes where other mechanisms may also be operative [129].

All living organisms studied to date have a general secretory pathway for exporting unfolded polypeptide chains across the cytoplasmic membrane. However, not all constituents are common to all organisms. Only the SecYEG, Ffh and FtsY proteins are found ubiquitously. *B. bacteriovorus* has these proteins plus all of the auxiliary constituents found in *E. coli* [130] that are thought to increase the efficiency of protein export. It also possesses a 4 component Tat system for secreting folded proteins across this membrane. Many organisms have simplified 2 or 3 component Tat systems [96]. Thus, the *B. bacteriovorus* Sec and Tat systems are just as complicated as those in *E. coli*.

Potential hemolysins that could destabilize the host cell membrane if exported to the host periplasm and a holin that probably exports an autolysin across the *B. bacteriovorus* cytoplasmic membrane were also detected. But how does *B. bacteriovorus* export proteins across its own outer membrane? At least four types of such systems were positively identified. (1) Type I ABC systems export proteins across both membranes in a single energy-coupled step. These systems are usually substrate protein specific and are therefore limited in scope. (2) The flagellar export machinery has been shown to be capable of exporting pathogenicity-related proteins in addition to flagellar proteins, but again, this process appears to be of limited physiological significance [131,132]. (3) The same arguments apply to pilus (Pil) and fimbrial (Fim) protein secretion systems: they appear to be specific for the subunits of the pilus or fimbrium they assemble. (4) *B. bacteriovorus* also has several “autotransporters” that have C-terminal domains that insert into the outer membrane and translocate their N-terminal domains across this structure. However, each autotransporter is usually specific for its own N-terminal protein domain [133].

What system, then, is responsible for secreting the majority of the many exported proteins that *B. bacteriovorus* encodes in its genome across the outer membrane? We identified many and perhaps all of the essential components of the Main Terminal Branch (MTB) (Type II) pathway (TC #3.A.15) [67,134]. In many Gram-negative bacteria, such systems provide the principal pathway for protein secretion across the outer membrane. Although these systems are distantly related to Pil-type systems, the sequence similarity of the putative *B. bacteriovorus* “Pul” proteins to authentic Pul proteins of *Klebsiella* was much greater than that of the characterized Pil homologues. Also, the putative *pul* genes of *B. bacteriovorus* were found in operons that did not include and mapped distantly from any of the *pil* genes. These observations lead us to suggest that the MTB provides the primary route for the export of secreted protein across the outer membrane of *B. bacteriovorus*. It is noteworthy that *E. coli* lacks such a system.

The genome also encodes other potential macromolecular (polysaccharide, lipid and lipopolysaccharide) exporters including several of the ABC, RND and MOP superfamilies [135,136,137]. Many other observations suggested an unusual degree of complexity in *B. bacteriovorus* that is lacking in most well studied bacteria such as *E. coli* and *B. subtilis*. For

example, we noted unusual numbers of homologues of the flagellar motor (3 each of MotA and MotB), of the TolAQR energizers of outer membrane OMR-type importers and of the outer membrane assembly complexes (see Table 3). In each case, the numbers of homologues suggest that although the molecular mechanisms are similar in the two organisms, the degree of functional complexity is much greater in *B. bacteriovorus* than in *E. coli*. These pmf- or smf-dependent energizers may also provide novel functions such as serving as the motor for gliding motility [61].

An interesting observation relates to the relatively large proportion of fusion proteins in *B. bacteriovorus* that in most bacteria are encoded by two distinct genes. Why *B. bacteriovorus* has a disproportionate number of such fusion proteins is not clear. Fusion often facilitates complex formation and increases both stability and efficiency of the assembled complex [138,139,140], but why *B. bacteriovorus* as compared to other bacteria would prefer to use this mechanism remains to be determined. Possibly *B. bacteriovorus* is subject to a wider range of stress conditions by virtue of its parasitic lifestyle. This possibility might also explain the large proportion of stress-relieving channel proteins and multidrug efflux pumps.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH grants GM077402, GM55434 and GM64368. We thank Mary Beth Hiller for her assistance in the preparation of this manuscript.

References

1. Stolp H. The bdellovibrios: bacterial parasites of bacteria. Ann. Rev. Phytopathol. 1973; 11:53–76.
2. Tudor JJ, McCann MP, Acrich IA. A new model for the penetration of prey cells by bdellovibrios. J. Bacteriol. 1990; 172:2421–2426. [PubMed: 2185219]
3. Starr MP, Huang JC. Physiology of the bdellovibrios. Adv. Microb. Physiol. 1972; 8:215–261. [PubMed: 4579073]
4. Ruby, EG. Cell-envelope modifications accompanying intracellular growth of *Bdellovibrio bacteriovorus*. In: Moulder, JW., editor. Intracellular Parasitism. CRC Press; Boca Raton, FL: 1989. p. 17-34.Ch. 2
5. Ruby EG, Rittenberg SC. Attachment of diaminopimelic acid to bdelloplast peptidoglycan during intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J. J. Bacteriol. 1984; 158:597–602. [PubMed: 6202674]
6. Araki Y, Ruby EG. A soluble enzyme activity that attaches free diaminopimelic acid to bdelloplast peptidoglycan. Biochemistry. 1988; 27:2624–2629. [PubMed: 3289615]
7. Rittenberg SC, Shilo M. Early host damage in the infection cycle of *Bdellovibrio bacteriovorus*. J. Bacteriol. 1970; 102:149–160. [PubMed: 4908670]
8. Ruby EG, Rittenberg SC. Differentiation after premature release of intraperiplasmically growing *Bdellovibrio bacteriovorous*. J. Bacteriol. 1983; 154:32–40. [PubMed: 6403505]
9. Diedrich DL, Denny CF, Hashimoto T, Conti SF. Facultatively parasitic strain of *Bdellovibrio bacteriovorus*. J. Bacteriol. 1970; 101:989–996. [PubMed: 4908792]
10. Lambert C, Evans KJ, Till R, Hobley L, Rendulic S, Schuster SC, Aizawa SI, Sockett RE. Characterising the flagellar filament and the role of motility in bacterial prey-penetration by *Bdellovibrio bacteriovorus*. Mol. Microbiol. 2006; 60:274–286. [PubMed: 16573680]
11. Rendulic S, Jagtap P, Rosinus A, Eppinger M, Baar C, Lanz C, Keller H, Lambert C, Evans KJ, Goesmann A, Meyer F, Sockett RE, Schuster SC. A predator unmasked: life cycle of *Bdellovibrio bacteriovorus* from a genomic perspective. Science. 2004; 303:689–692. [PubMed: 14752164]

12. Thomashow LS, Rittenberg SC. Waveform analysis and structure of flagella and basal complexes from *Bdellovibrio bacteriovorus* 109J. J. Bacteriol. 1985; 163:1038–1046. [PubMed: 4030690]
13. Hasegawa H, Naganuma K, Nakagawa Y, Matsuyama T. Membrane filter (pore size, 0.22-0.45 μm; thickness, 150 μm) passing-through activity of *Pseudomonas aeruginosa* and other bacterial species with indigenous infiltration ability. FEMS Microbiol. Lett. 2003; 223:41–46. [PubMed: 12798998]
14. Rittenberg, SC. Bdellovibrios–intraperiplasmic growth. In: Burns, RG.; Slater, JH., editors. Experimental Microbial Ecology. Blackwell Scientific; Oxford, UK: 1982. p. 379-391.
15. Thomashow MF, Rittenberg SC. Intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J: solubilization of *Escherichia coli* peptidoglycan. J. Bacteriol. 1978; 135:998–1007. [PubMed: 357428]
16. Fackrell HB, Robinson J. Purification and characterization of a lytic peptidase produced by *Bdellovibrio bacteriovorus* 6-5-S. Can. J. Microbiol. 1973; 19:659–666. [PubMed: 4709167]
17. Dworkin J, Losick R. Developmental commitment in a bacterium. Cell. 2005; 121:401–409. [PubMed: 15882622]
18. Stragier P, Losick R. Cascades of sigma factors revisited. Mol. Microbiol. 1990; 4:1801–1806. [PubMed: 2127951]
19. Romo AJ, Ruby EG, Saier MH Jr. Effect of *Bdellovibrio bacteriovorus* infection on the phosphoenolpyruvate:sugar phosphotransferase system in *Escherichia coli*: evidence for activation of cytoplasmic proteolysis. Res. Microbiol. 1992; 143:5–14. [PubMed: 1322553]
20. Saier MH Jr. Protein uptake into *E. coli* during *Bdellovibrio* infection: A process of reverse secretion? FEBS Lett. 1994; 337:14–17. [PubMed: 8276104]
21. Tudor JJ, Karp MA. Translocation of an outer membrane protein into prey cytoplasmic membranes by bdellovibrios. J. Bacteriol. 1994; 176:948–952. [PubMed: 8106336]
22. Eksztejn M, Varon M. Elongation and cell division in *Bdellovibrio bacteriovorus*. Arch. Microbiol. 1977; 114:175–181. [PubMed: 410384]
23. Gray KM, Ruby EG. Prey-derived signals regulating duration of the developmental growth phase of *Bdellovibrio bacteriovorus*. J. Bacteriol. 1990; 172:4002–4007. [PubMed: 2193927]
24. Thomashow MF, Cotter TW. *Bdellovibrio* host dependence: the search for signal molecules and genes that regulate the intraperiplasmic growth cycle. J. Bacteriol. 1992; 174:5767–5771. [PubMed: 1522057]
25. Hampton T. Researchers eye “predatory” bacterium for novel antimicrobial strategies. JAMA. 2004; 291:1188–1189. [PubMed: 15010430]
26. Moore A. Finding my enemy's enemies. EMBO Rep. 2004; 5:754–757. [PubMed: 15289822]
27. Sockett RE, Lambert C. Bdellovibrio as therapeutic agents: a predatory renaissance? Nat. Rev. Microbiol. 2004; 2:669–675. [PubMed: 15263901]
28. Seidler RJ, Starr MP. Isolation and characterization of host-independent Bdellovibrios. J. Bacteriol. 1969; 100:769–785. [PubMed: 4901359]
29. Ruby EG, McCabe JB. Metabolism of periplasmic membrane-derived oligosaccharides by the predatory bacterium *Bdellovibrio bacteriovorus* 109J. J. Bacteriol. 1988; 170:646–652. [PubMed: 3276663]
30. Starr MP, Seidler RJ. The Bdellovibros. Annu. Rev. Microbiol. 1971; 25:649–678. [PubMed: 4949041]
31. Barel G, Sirota A, Volpin H, Jurkevitch E. Fate of predator and prey proteins during growth of *Bdellovibrio bacteriovorus* on *Escherichia coli* and *Pseudomonas syringae prey*. J. Bacteriol. 2005; 187:329–335. [PubMed: 15601717]
32. Matin A, Rittenberg SC. Kinetics of deoxyribonucleic acid destruction and synthesis during growth of *Bdellovibrio bacteriovorus* strain 109D on *Pseudomonas putida* and *Escherichia coli*. J. Bacteriol. 1972; 111:664–673. [PubMed: 4559819]
33. Ruby EG, McCabe JB. An ATP transport system in the intracellular bacterium, *Bdellovibrio bacteriovorus* 109J. J. Bacteriol. 1986; 167:1066–1070. [PubMed: 3745115]
34. Ruby EG, McCabe JB, Barke JI. Uptake of intact nucleoside monophosphates by *Bdellovibrio bacteriovorus* 109J. J. Bacteriol. 1985; 163:1087–1094. [PubMed: 4030692]

35. Busch W, Saier MH Jr. The Transporter Classification (TC) System. CRC Crit. Rev. Biochem. Mol. Biol. 2002; 37:287–337. 2002. [PubMed: 12449427]
36. Saier MH Jr. Tran CV, Barabote RD. TCDB: the Transporter Classification Database for membrane transport protein analyses and information. Nucl. Acids Res. 2006; 34:D181–D186. (Database issue). [PubMed: 16381841]
37. Lorca GL, Barabote RD, Zlotopolski V, Tran C, Winnen B, Hvorup RN, Stonestrom AJ, Nguyen E, Huang LW, Kim DS, Saier MH Jr. Transport capabilities of eleven gram-positive bacteria: Comparative genomic analyses. Biochim. Biophys. Acta. 2007; 1768:1342–1366. [PubMed: 17490609]
38. Saier MH Jr. Protein secretion systems in Gram-negative bacteria. Microbe. 2006; 1:414–419.
39. Ren Q, Paulsen IT. Comparative analyses of fundamental differences in membrane transport capabilities in prokaryotes and eukaryotes. PLoS Comput. Biol. 2005; 3:e27. [PubMed: 16118665]
40. Barel G, Jurkevitch E. Analysis of phenotypic diversity among host-independent mutants of *Bdellovibrio bacteriovorus* 109J. Arch. Microbiol. 2001; 176:211–216. [PubMed: 11511869]
41. Sonnhammer EL, von Heijne G, Krogh A. A hidden Markov model for predicting transmembrane helices in protein sequences. Proc. Int. Conf. Intell. Syst. Mol. Biol. 1998; 6:175–182. [PubMed: 9783223]
42. Krogh A, Larsson B, von Heijne G, Sonnhammer EL. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J. Mol. Biol. 2001; 305:567–580. [PubMed: 11152613]
43. Saier MH Jr. Tracing pathways of transport protein evolution. Mol. Microbiol. 2003; 48:1145–1156. [PubMed: 12787345]
44. Chung Y-J, Saier MH Jr. SMR-type multidrug resistance pumps. Curr. Opin. Drug Discov. Dev. 2001; 4:237–245.
45. Pornillos O, Chang G. Inverted repeat domains in membrane proteins. FEBS Lett. 2006; 580:358–362. [PubMed: 16406365]
46. Saier MH Jr. Families of proteins forming transmembrane channels. J. Membr. Biol. 2000a; 175:165–180. [PubMed: 10833527]
47. Saier MH Jr. A functional-phylogenetic classification system for transmembrane solute transporters. Microbiol. Mol. Biol. Rev. 2000b; 64:354–411. [PubMed: 10839820]
48. Paulsen IT, Chauvaux S, Choi P, Saier MH Jr. Characterization of glucose-specific catabolite repression-resistant mutants of *Bacillus subtilis*: identification of a novel Hexose:H^+ symporter. J. Bacteriol. 1998; 180:498–504. [PubMed: 9457850]
49. Paulsen IT, Nguyen L, Sliwinski MK, Rabus R, Saier MH Jr. Microbial genome analyses: comparative transport capabilities in eighteen prokaryotes. J. Mol. Biol. 2000; 301:75–100. [PubMed: 10926494]
50. Hiramatsu T, Kodama K, Kuroda T, Mizushima T, Tsuchiya T. A putative multisubunit Na^+/H^+ antiporter from *Staphylococcus aureus*. J. Bacteriol. 1998; 180:6642–6648. [PubMed: 9852009]
51. Ito M, Guffanti AA, Wang W, Krulwich TA. Results of non-polar mutations in each of the seven *Bacillus subtilis mrp* genes suggest complex interactions among the gene products in support of Na^+- and Alkali- but not cholate-resistance. J. Bacteriol. 2000; 182:5663–5670. [PubMed: 11004162]
52. Tani K, Watanabe T, Matsuda H, Nasu M, Kondo M. Cloning and sequencing of the spore germination gene of *Bacillus megaterium* ATCC 12872: similarities to the NaH-antiporter gene of *Enterococcus hirae*. Microbiol. Immunol. 1996; 40:99–105. [PubMed: 8867604]
53. Roosild TP, Miller S, Booth IR, Choe S. A mechanism of regulating transmembrane potassium flux through a ligand-mediated conformational switch. Cell. 2002; 109:781–791. [PubMed: 12086676]
54. Au KM, Barabote RD, Hu KY, Saier MH Jr. Evolutionary appearance of H^+-translocating pyrophosphatases. Microbiology. 2006; 152:1243–1247. [PubMed: 16622041]
55. Gardy JL, Laird MR, Chen F, Rey S, Walsh CJ, Ester M, Brinkman FS. PSORTb v.2.0: expanded prediction of bacterial protein subcellular localization and insights gained from comparative proteome analysis. Bioinformatics. 2005; 21:617–623. [PubMed: 15501914]

56. Rey S, Acab M, Gardy JL, Laird MR, deFays K, Lambert C, Brinkman FS. PSORTdb: a protein subcellular localization database for bacteria. Nucleic Acids Res. 2005; 33:D164–168. (Database issue). [PubMed: 15608169]
57. Kall L, Sonnhammer EL. Reliability of transmembrane predictions in whole-genome data. FEBS Lett. 2002; 532:415–418. [PubMed: 12482603]
58. Pivetti CD, Yen M-R, Miller S, Busch W, Tseng Y-H, Booth IR, Saier MH Jr. Two families of prokaryotic mechanosensitive channel proteins. Microbiol. Mol. Biol. Rev. 2003; 67:66–85. [PubMed: 12626684]
59. Calvio C, Celandroni F, Ghelardi E, Amati G, Salvetti S, Ceciliani F, Galizzi A, Senesi S. Swarming differentiation and swimming motility in *Bacillus subtilis* are controlled by *swrA*, a newly identified dicistronic operon. J. Bacteriol. 2005; 187:5356–5366. [PubMed: 16030230]
60. Ito M, Hicks DB, Henkin TM, Guffanti AA, Powers BD, Zvi L, Uematsu K, Krulwich TA. MotPS is the stator-force generator for motility of alkaliphilic Bacillus, and its homologue is a second functional Mot in *Bacillus subtilis*. Mol. Microbiol. 2004; 53:1035–1049. [PubMed: 15306009]
61. Youderian P, Burke N, White DJ, Hartzell PL. Identification of genes required for adventurous gliding motility in *Myxococcus xanthus* with the transposable element mariner. Mol. Microbiol. 2003; 49:555–570. [PubMed: 12828649]
62. Kehres DG, Lawyer CH, Maguire ME. The CorA magnesium transporter gene family. Microb. Comp. Genomics. 1998; 3:151–169. [PubMed: 9775386]
63. Dinh T, Paulsen IT, Saier MH Jr. A family of extracytoplasmic proteins that allow transport of large molecules across the outer membranes of Gram-negative bacteria. J. Bacteriol. 1994; 176:3825–3831. [PubMed: 8021163]
64. Cao TB, Saier MH Jr. Conjugal Type IV macromolecular transfer systems of Gram-negative bacteria: Organismal distribution, structural constraints and evolutionary conclusions. Microbiology. 2001; 147:3201–3214. [PubMed: 11739753]
65. Collins RF, Ford RC, Kitmitto A, Olsen RO, Tønjum T, Derrick JP. Three-dimensional structure of the *Neisseria meningitidis* secretin PilQ determined from negative-stain transmission electron microscopy. J. Bacteriol. 2003; 185:2611–2617. [PubMed: 12670986]
66. Chami M, Guilvout I, Gregorini M, Remigy HW, Muller SA, Valerio M, Engel A, Pugsley AP, Bayan N. Structural insights into the secretin PulD and its trypsin-resistant core. J. Biol. Chem. 2005; 280:37732–33741. [PubMed: 16129681]
67. Sandkvist M. Biology of type II secretion. Mol. Microbiol. 2001; 40:271–283. [PubMed: 11309111]
68. Wu T, Malinverni J, Ruiz N, Kim S, Silhavy TJ, Kahne D. Identification of a multicomponent complex required for outer membrane biogenesis in *Escherichia coli*. Cell. 2005; 121:235–245. [PubMed: 15851030]
69. Bayles KW. Are the molecular strategies that control apoptosis conserved in bacteria? Trends Microbiol. 2003; 11:306–311. [PubMed: 12875813]
70. Chahboune A, Decaffmeyer M, Brasseur R, Joris B. Membrane topology of the *Escherichia coli* AmpG permease required for recycling of cell wall anhydromuropeptides and AmpC beta-lactamase induction. Antimicrob. Agents Chemother. 2005; 49:1145–1149. [PubMed: 15728916]
71. Cheng Q, Park JT. Substrate specificity of the AmpG permease required for recycling of cell wall anhydro-muropeptides. J. Bacteriol. 2002; 184:6434–6436. [PubMed: 12426329]
72. Chang AB, Lin R, Studley WK, Tran CV, Saier MH Jr. Phylogeny as a guide to structure and function of membrane transport proteins. Mol. Membrane Biol. 2004; 21:171–181.
73. Pao SS, Paulsen IT, Saier MH Jr. The major facilitator superfamily. Microbiol. Mol. Biol. Rev. 1998; 62:1–32. [PubMed: 9529885]
74. Saier MH Jr. Beatty JT, Goffeau A, Harley KT, Heijne WHM, Huang S-C, Jack DL, Jahn PS, Lew K, Liu J, Pao SS, Paulsen IT, Tseng T-T, Virk PS. The major facilitator superfamily. J. Mol. Microbiol. Biotechnol. 1999; 1:257–279. [PubMed: 10943556]
75. Chao Y, Fu D. Kinetic study of the antiport mechanism of an *Escherichia coli* zinc transporter, ZitB. J. Biol. Chem. 2004; 279:12043–12050. [PubMed: 14715669]

76. Guffanti AA, Wei Y, Rood SV, Krulwich TA. An antiport mechanism for a member of the cation diffusion facilitator family: divalent cations efflux in exchange for K^+ and H^+ Mol. Microbiol. 2002; 45:145–153. [PubMed: 12100555]
77. Grass G, Franke S, Taudte N, Nies DH, Kucharski LM, Maguire ME, Rensing C. The metal permease ZupT from *Escherichia coli* is a transporter with a broad substrate spectrum. J. Bacteriol. 2005; 187:1604–1611. [PubMed: 15716430]
78. Yu EW, Aires JR, Nikaido H. AcrB multidrug efflux pump of *Escherichia coli*: composite substrate-binding cavity of exceptional flexibility generates its extremely wide substrate specificity. J. Bacteriol. 2003; 185:5657–5664. [PubMed: 13129936]
79. Chung YJ, Saier MH Jr. Overexpression of the *Escherichia coli* sugE gene confers resistance to a narrow range of quaternary ammonium compounds. J. Bacteriol. 2002; 184:2543–2545. [PubMed: 11948170]
80. Livshits VA, Zakataeva NP, Aleshin BB, Vitushkina MV. Identification and characterization of the new gene *rhtA* involved in threonine and homoserine efflux in *Escherichia coli*. Res. Microbiol. 2003; 154:123–135. [PubMed: 12648727]
81. Androutsellis-Theotokis A, Goldberg NR, Ueda K, Beppu T, Beckman ML, Das S, Javitch JA, Rudnick G. Characterization of a functional bacterial homologue of sodium-dependent neurotransmitter transporters. J. Biol. Chem. 2003; 278:12703–12709. [PubMed: 12569103]
82. Ito M, Guffanti AA, Zemsky J, Ivey DM, Krulwich TA. Role of the *nhaC*-encoded Na^+/H^+ antiporter of alkaliphilic *Bacillus firmus* OF4. J. Bacteriol. 1997; 179:3851–3857. [PubMed: 9190799]
83. Wei Y, Guffanti AA, Ito M, Krulwich TA. *Bacillus subtilis* YqkI is a novel malic/Na^+-lactate antiporter that enhances growth on malate at low protonmotive force. J. Biol. Chem. 2000; 275:30287–30292. [PubMed: 10903309]
84. Putnoky P, Kereszt A, Nakamura T, Endre G, Grosskopf E, Kiss P, Kondorosi A. The *pha* gene cluster of *Rhizobium meliloti* involved in pH adaptation and symbiosis encodes a novel type of K^+ efflux system. Mol. Microbiol. 1998; 28:1091–1101. [PubMed: 9680201]
85. Trchounian A, Kobayashi H. Kup is the major K^+ uptake system in *Escherichia coli* upon hyper-osmotic stress at a low pH. FEBS Lett. 1999; 447:144–148. [PubMed: 10214935]
86. Zakharyan E, Trchounian A. K^+ influx by Kup in *Escherichia coli* is accompanied by a decrease in H^+ efflux. FEMS Microbiol. Lett. 2001; 204:61–64. [PubMed: 11682179]
87. Neidle EL, Hartnett C, Ornston LN, Bairoch A, Rekik M, Harayama S. Nucleotide sequences of the *Acinetobacter calcoaceticus benABC* genes for benzoate 1,2-dioxygenase reveal evolutionary relationships among multicomponent oxygenases. J. Bacteriol. 1991; 173:5385–5395. [PubMed: 1885518]
88. Prakash S, Cooper G, Singhi S, Saier MH Jr. The ion transporter superfamily. Biochim. Biophys. Acta. 2003; 1618:79–92. [PubMed: 14643936]
89. Alvarez AH, Moreno-Sánchez R, Cervantes C. Chromate efflux by means of the ChrA chromate resistance protein from *Pseudomonas aeruginosa*. J. Bacteriol. 1999; 181:7398–7400. [PubMed: 10572148]
90. Nicholson ML, Laudenbach DE. Genes encoded on a cyanobacterial plasmid are transcriptionally regulated by sulfur availability and CysR. J. Bacteriol. 1995; 177:2143–2150. [PubMed: 7536734]
91. Nies D, Koch S, Wachi S, Peitzsch N, Saier MH Jr. CHR, a novel family of prokaryotic proton motive force-driven transporters probably containing chromate/sulfate antiporters. J. Bacteriol. 1998; 180:5799–5802. [PubMed: 9791139]
92. Pimentel BE, Moreno-Sanchez R, Cervantes C. Efflux of chromate by *Pseudomonas aeruginosa* cells expressing the ChrA protein. FEMS Microbiol. Lett. 2002; 212:249–254. [PubMed: 12113942]
93. Van Dyk TK, Templeton LJ, Cantera KA, Sharpe PL, Sariaslani FS. Characterization of the *Escherichia coli* AaeAB efflux pump: a metabolic relief valve? J. Bacteriol. 2004; 186:7196–7204. [PubMed: 15489430]
94. Harley KT, Saier MH Jr. A novel ubiquitous family of putative efflux transporters. J. Mol. Microbiol. Biotechnol. 2000; 2:195–198. [PubMed: 10939244]

95. Yen M-R, Harley KT, Tseng Y-H, Saier MH Jr. Phylogenetic and structural analyses of the Oxa1 family of putative protein translocation constituents. FEMS Microbiol. Lett. 2001; 204:223–231. [PubMed: 11731127]
96. Yen M-R, Tseng Y-H, Nguyen EH, Wu L-F, Saier MH Jr. Sequence and phylogenetic analyses of the twin arginine targeting (Tat) protein export system. Arch. Microbiol. 2002; 177:441–450. [PubMed: 12029389]
97. Darzins A, Russell MA. Molecular genetic analysis of type-4 pilus biogenesis and twitching motility using *Pseudomonas aeruginosa* as a model system–a review. Gene. 1997; 192:109–115. [PubMed: 9224880]
98. Hobbs M, Mattick JS. Common components in the assembly of type 4 fimbriae, DNA transfer systems, filamentous phage and protein-secretion apparatus: a general system for the formation of surface-associated protein complexes. Mol. Microbiol. 1993; 10:233–243. [PubMed: 7934814]
99. Schwudke D, Bernhardt A, Beck S, Madela K, Linscheid MW, Appel B, Strauch E. Transcriptional activity of the host-interaction locus and a putative pilin gene of *Bdellovibrio bacteriovorus* in the predatory life cycle. Curr. Microbiol. 2005; 51:310–316. [PubMed: 16195835]
100. Buckel W. Sodium ion-translocating decarboxylases. Biochim. Biophys. Acta. 2001; 1505:15–27. [PubMed: 11248185]
101. Dimroth P, Jockel P, Schmid M. Coupling mechanism of the oxaloacetate decarboxylase Na^+ pump. Biochim. Biophys. Acta. 2001; 1505:1–14. [PubMed: 11248184]
102. Schmid M, Wild MR, Dahinden P, Dimroth P. Subunit gamma of the oxaloacetate decarboxylase Na^+ pump: interaction with other subunits/domains of the complex and binding site for the Zn^{2+} metal ion. Biochemistry. 2002; 41:1285–1292. [PubMed: 11802728]
103. Dahinden P, Pos KM, Dimroth P. Identification of a domain in the α-subunit of the oxaloacetate decarboxylase Na^+ pump that accomplishes complex formation with the γ-subunit. FEBS J. 2005; 272:846–855. [PubMed: 15670164]
104. Hirst J. The dichotomy of complex I: a sodium ion pump or a proton pump. Proc. Natl. Acad. Sci. USA. 2003; 100:773–775. [PubMed: 12552138]
105. Sapra R, Bagramyan K, Adams MWW. A simple energy-conserving system: proton reduction coupled to proton translocation. Proc. Natl. Acad. Sci. USA. 2003; 100:7545–7550. [PubMed: 12792025]
106. Steuber J, Schmid C, Rufibach M, Dimroth P. Na^+ translocation by complex I (NADH: quinone oxidoreductase) of *Escherichia coli*. Mol. Microbiol. 2000; 35:428–434. [PubMed: 10652103]
107. Flock U, Reimann J, Adelroth P. Proton transfer in bacterial nitric oxide reductase. Biochem. Soc. Trans. 2006; 34(Pt 1):188–190. [PubMed: 16417518]
108. Meakin GE, Jepson BJ, Richardson DJ, Bedmar EJ, Delgado MJ. The role of *Bradyrhizobium japonicum* nitric oxide reductase in nitric oxide detoxification in soya bean root nodules. Biochem. Soc. Trans. 2006; 34(Pt 1):195–196. [PubMed: 16417520]
109. Kimball RA, Martin L, Saier MH Jr. Reversing transmembrane electron flow: The DsbD and DsbB protein families. J. Mol. Microbiol. Biotechnol. 2003; 5:133–149. [PubMed: 12766342]
110. Katzen F, Beckwith J. Transmembrane electron transfer by the membrane protein DsbD occurs via a disulfide bond cascade. Cell. 2000; 103:769–779. [PubMed: 11114333]
111. Krupp R, Chan C, Missiakas D. DsbD-catalyzed transport of electrons across the membrane of *Escherichia coli*. J. Biol. Chem. 2001; 276:3696–3701. [PubMed: 11085993]
112. Hille R. Molybdenum and tungsten in biology. Trends Biochem. Sci. 2002; 27:360–367. [PubMed: 12114025]
113. McEwan AG, Hanson GR, Bailey S. Dimethylsulphoxide reductase from purple phototrophic bacteria: structures and mechanism(s). Biochem. Soc. Trans. 1998; 26:390–396. [PubMed: 9765885]
114. Boyle DS, Khattar MM, Addinall SG, Lutkenhaus J, Donachie WD. *ftsW* is an essential cell-division gene in *Escherichia coli*. Mol. Microbiol. 1997; 24:1263–1273. [PubMed: 9218774]
115. Gérard P, Vernet T, Zapun A. Membrane topology of the *Streptococcus pneumoniae* FtsW division protein. J. Bacteriol. 2002; 184:1925–1931. [PubMed: 11889099]

116. Faergeman NJ, Black PN, Zhao XD, Knudsen J, DiRusso CC. The acyl-CoA synthetases encoded within FAA1 and FAA4 in *Saccharomyces cerevisiae* function as components of the fatty acid transport system linking import, activation, and intracellular utilization. J. Biol. Chem. 2001; 276:37051–37059. [PubMed: 11477098]

117. Hall AM, Wiczer BM, Herrmann T, Stremmel W, Bernlohr DA. Enzymatic properties of purified murine fatty acid transport protein 4 and analysis of acyl-CoA synthetase activities in tissues from FATP4 null mice. J. Biol. Chem. 2005; 280:11948–11954. [PubMed: 15653672]

118. Hirsch D, Stahl A, Lodish HF. A family of fatty acid transporters conserved from mycobacterium to man. Proc. Natl. Acad. Sci. USA. 1998; 95:8625–8629. [PubMed: 9671728]

119. Zou Z, DiRusso CC, Ctrnacta V, Black PN. Fatty acid transport in *Saccharomyces cerevisiae*. Directed mutagenesis of FAT1 distinguishes the biochemical activities associated with Fat1p. J. Biol. Chem. 2002; 277:31062–31071. [PubMed: 12052836]

120. Baida G, Budarina ZI, Kuzmin NP, Solonin AS. Complete nucleotide sequence and molecular characterization of hemolysin II gene from *Bacillus cereus*. FEMS Microbiol. Lett. 1999; 180:7–14. [PubMed: 10547438]

121. Baida GE, Kuzmin NP. Cloning and primary structure of a new hemolysin gene from *Bacillus cereus*. Biochim. Biophys. Acta. 1995; 1264:151–154. [PubMed: 7495855]

122. ter Huurne AA, Muir S, van Houten M, van der Zeijst BA, Gaastra W, Kusters JG. Characterization of three putative *Serpulina hyodysenteriae* hemolysins. Microb. Pathog. 1994; 16:269–282. [PubMed: 7968456]

123. Gibson MM, Bagga DA, Miller CG, Maguire ME. Magnesium transport in *Salmonella typhimurium*: the influence of new mutations conferring Co^{2+} resistance on the CorA Mg^{2+} transport system. Mol. Microbiol. 1991; 5:2753–2762. [PubMed: 1779764]

124. Epstein W. The roles and regulation of potassium in bacteria. Prog. Nucleic Acid Res. Mol. Biol. 2003; 75:293–320. [PubMed: 14604015]

125. Odermatt A, Suter H, Krapf R, Solioz M. Primary structure of two P-type ATPases involved in copper homeostasis in *Enterococcus hirae*. J. Biol. Chem. 1993; 268:12775–12779. [PubMed: 8048974]

126. Tchieu JH, Norris V, Edwards JS, Saier MH Jr. The complete phosphotransferase system in *Escherichia coli*. J. Mol. Microbiol. Biotechnol. 2001; 3:329–346. [PubMed: 11361063]

127. Nguyen L, Paulsen IT, Tchieu J, Hueck CJ, Saier MH Jr. Phylogenetic analyses of the constituents of type III protein secretion systems. J. Mol. Microbiol. Biotechnol. 2000; 2:125–144. [PubMed: 10939240]

128. Ahner A, Brodsky JL. Checkpoints in ER-associated degradation: excuse me, which way to the proteasome? Trends Cell Biol. 2004; 14:474–478. [PubMed: 15350974]

129. Schmitz A, Herzog V. Endoplasmic reticulum-associated degradation: exceptions to the rule. Eur. J. Cell Biol. 2004; 83:501–509. [PubMed: 15679096]

130. Cao TB, Saier MH Jr. The general protein secretory pathway: Phylogenetic analyses leading to evolutionary conclusions. Biochim. Biophys. Acta. 2003; 1609:115–125. [PubMed: 12507766]

131. Saier MH Jr. Evolution of bacterial type III protein secretion systems. Trends Microbiol. 2004; 12:113–115. [PubMed: 15001186]

132. Song YC, Jin S, Louie H, Ng D, Lau R, Zhang Y, Weerasekera R, Al Rashid S, Ward LA, Der SD, Chan VL. FlaC, a protein of *Campylobacter jejuni* TGH9011 (ATCC43431) secreted through the flagellar apparatus, binds epithelial cells and influences cell invasion. Mol. Microbiol. 2004; 53:541–553. [PubMed: 15228533]

133. Loveless BJ, Saier MH Jr. A novel family of channel-forming, autotransporting, bacterial virulence factors. Mol. Membr. Biol. 1997; 14:113–123. [PubMed: 9394291]

134. Ma Q, Zhai Y, Schneider CJ, Ramseier TM, Saier MH Jr. Protein secretion systems of *Pseudomonas aeruginosa* and *P. fluorescens*. Biochim. Biophys. Acta. 2003; 1611:223–233. [PubMed: 12659964]

135. Hvorup RN, Winnen B, Chang AB, Jiang Y, Zhou XF, Saier MH. The multidrug/oligosaccharidyl-lipid/polysaccharide (MOP) exporter superfamily. Eur. J. Biochem. 2003; 270:799–813. [PubMed: 12603313]

136. Reizer J, Reizer A, Saier MH Jr. A new subfamily of bacterial ABC-type transport systems catalyzing export of drugs and carbohydrates. Prot. Sci. 1992; 1:1326–1332.

137. Tseng T-T, Gratwick KS, Kollman J, Park D, Nies DH, Goffeau A, Saier MH Jr. The RND permease superfamily: an ancient, ubiquitous and diverse family that includes human disease and development proteins. J. Mol. Microbiol. Biotechnol. 1999; 1:107–125. [PubMed: 10941792]

138. Amar P, Ballet P, Barlovatz-Meimon G, Benecke A, Bernot G, Bouligand Y, Bourguine P, Delaplace F, Delosme JM, Demarty M, Fishov I, Fourmentin-Guilbert J, Fralick J, Giavitto JL, Gleyse B, Godin C, Incitti R, Kepes F, Lange C, Le Sceller L, Loutellier C, Michel O, Molina F, Monnier C, Natowicz R, Norris V, Orange N, Pollard H, Raine D, Ripoll C, Rouviere-Yaniv J, Saier M Jr. Soler P, Tambourin P, Thellier M, Tracqui P, Ussery D, Vincent JC, Vannier JP, Wiggins P, Zemirline A. Hyperstructures, genome analysis and I-cells. Acta Biotheor. 2002; 50:357–373. [PubMed: 12675536]

139. Liu N, Caderas G, Deillon C, Hoffmann S, Klauser S, Cui T, Gutte B. Fusion proteins from artificial and natural structural modules. Curr. Protein Pept. Sci. 2001; 2:107–121. [PubMed: 12370019]

140. Norris V, Gascuel P, Guespin-Michel J, Ripoll C, Saier MH Jr. Metabolite-induced metabolons: the activation of transporter-enzyme complexes by substrate binding. Mol. Microbiol. 1999; 31:1592–1595. [PubMed: 10200976]

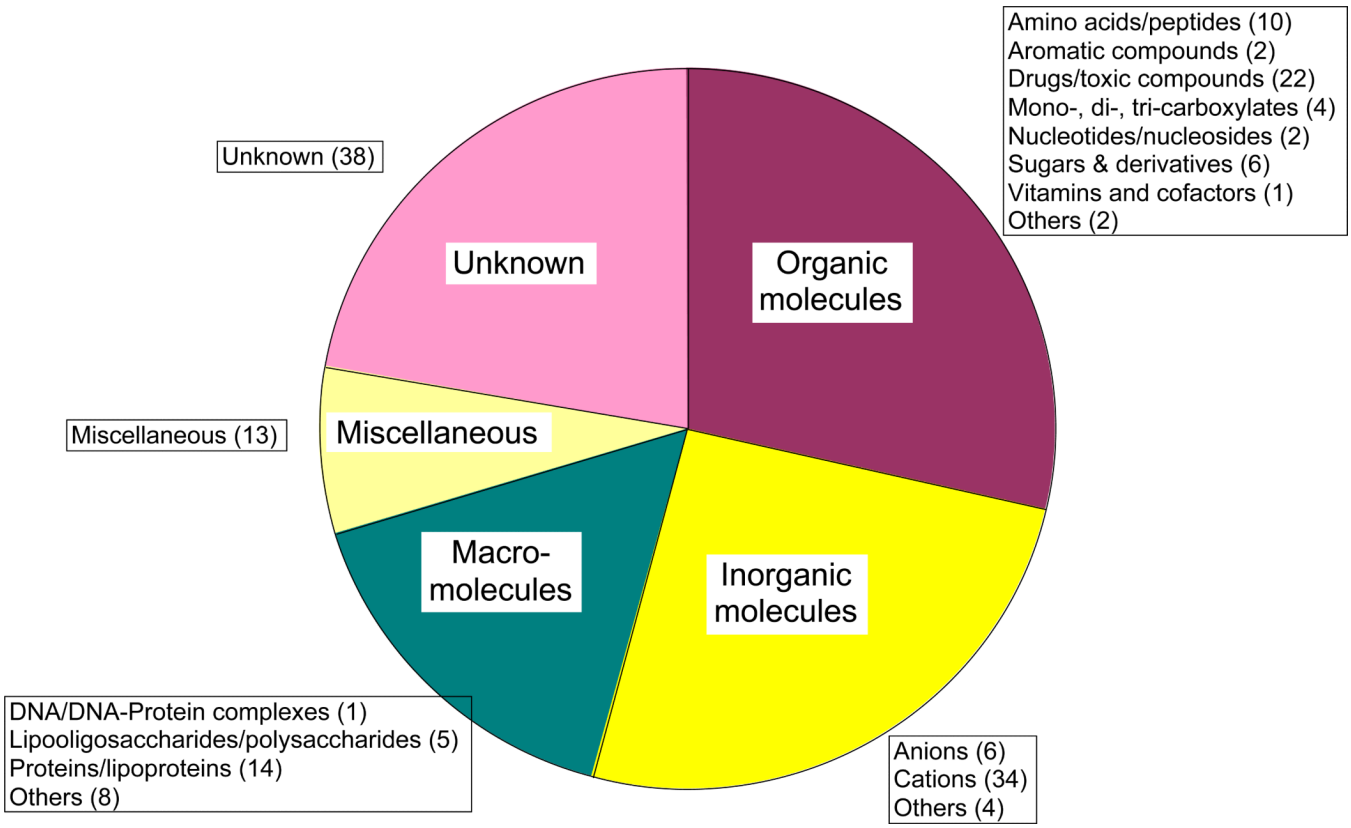

Figure 1.

Pie chart of transporters in *B. bacteriovorus* according to predicted substrate specificity. Five different categories are shown. More detailed analyses, including numbers of systems found in each subcategory, specific for each substrate type are provided in the boxes adjacent to the five pieces of the pie. The chart is based on the data presented in Table 2 as discussed in detail in the text.

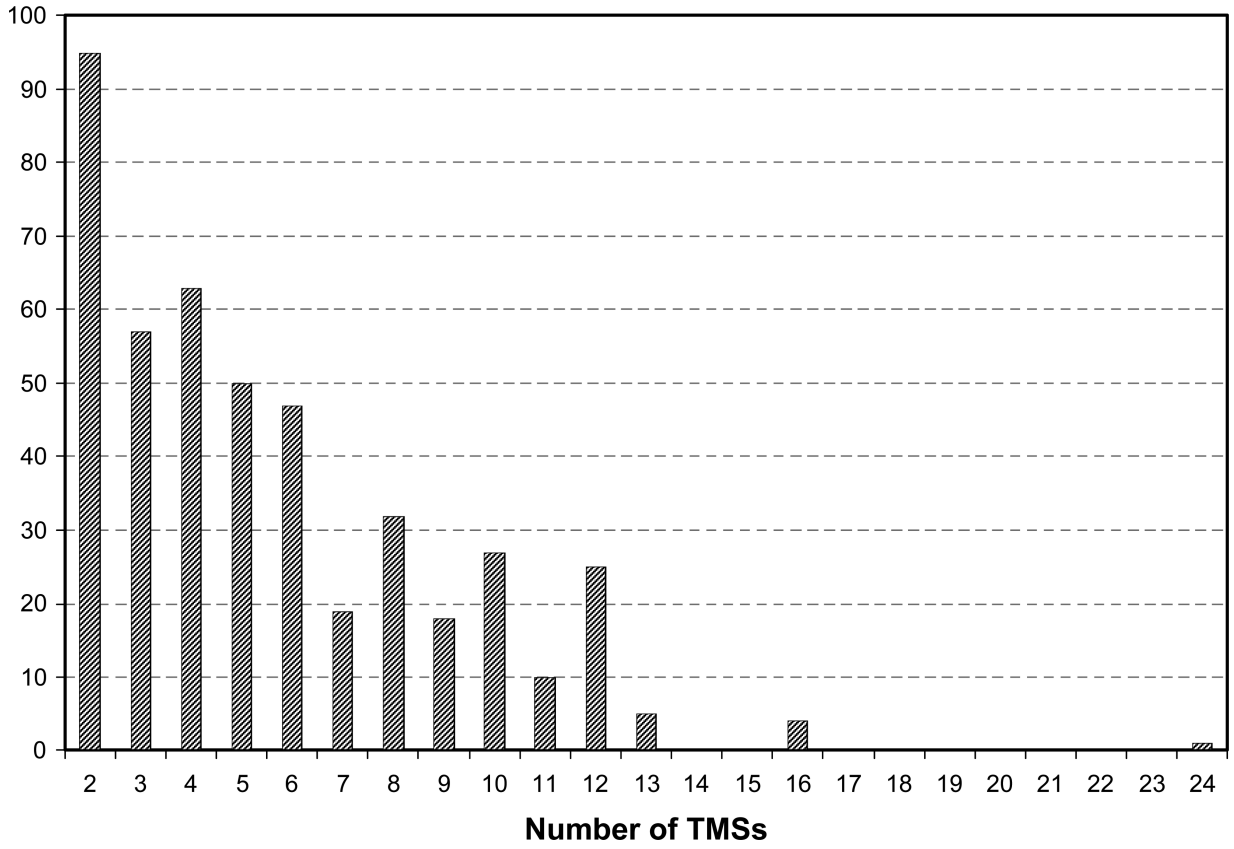

Figure 2.

Distribution of topological types of putative membrane proteins with 2-24 predicted TMSs. Number of proteins of a particular predicted topological structure is plotted on the X-axis versus the number of TMSs in that protein, plotted on the Y-axis. The plot illustrates the greater prevalence of proteins with even numbers of TMSs than odd numbers of TMSs with the sole exception of the putative 5 TMS protein. These 5 TMS proteins are common in secondary carriers and ATP-hydrolysis-driven uptake transporters of the ABC superfamily (see text). We believe this distribution reflects the evolutionary pathway taken for their appearance [43].

Table 1

Overview of the *Bdellovibrio bacteriovorus* transporter analyses.

TC Class[a]	Class Description	No. of Transport Proteins[b]	TC Subclass	Subclass Description	No. of Transport Proteins[b]
1.	Channels	45 (29)	1.A	α-type channels	15 (12)
			1.B	β-barrel porins	29 (16)
			1.C	Pore-forming toxins (proteins and peptides)	–
			1.D	Nonribosomally synthesized channels	–
			1.E	Holins	1 (1)
2.	Secondary carriers	100 (70)	2.A	Porters (uniporters, symporters, antiporters)	73 (64)
			2.B	Nonribosomally synthesized porters	–
			2.C	Ion-gradient-driven energizers	27 (6)
3.	Primary active transporters	227 (55)	3.A	P-P-bond-hydrolysis-driven transporters	202 (51)
			3.B	Decarboxylation-driven transporters	4 (-)
			3.C	Methyltransfer-driven transporters	–
			3.D	Oxidoreduction-driven transporters	21 (4)
			3.E	Light absorption-driven transporters	–
4.	Group translocators	0	4.A	Phosphotransfer-driven group translocators	-
			4.B	Nicotinamide ribonucleoside (NR) uptake permeases	-
5.	Transmembrane electron carriers	4 (2)	5.A	Transmembrane 2-electron transfer carriers	4 (2)
			5.B	Transmembrane 1-electron transfer carriers	–
8.	Auxiliary transport proteins	7 (-)	8.A	Auxiliary transport proteins	7 (-)
9.	Poorly defined system	23 (16)	9.A	Recognized transporters of unknown biochemical mechanism	4 (3)
			9.B	Putative uncharacterized transport proteins	19 (13)
			9.C	Functionally characterized transporters lacking identified sequences	–
	Total	406 (172)			406 (172)

[a]Transporter classes 6 & 7 have not been assigned in the TC system yet and therefore are absent from this table.

[b]Numbers in parentheses represent the number of transport systems.

Table 2

TC classification and functional predictions of putative transport proteins from *Bdellovibrio bacteriovorus.*

TC Family	Family Name	Number of transport systems[a]	Bba Protein ID	Size	# of TMSs[b]	Best blast-hit in TCDB and/or comments
1.A.11	Chloride Channel (ClC) Family	3	42523302	442	8	EriC [*E. coli*]
			42523636	406	10	MJ0305 [*M. jannaschii*]
			42523824	442	10	EriC [*E. coli*]
1.A.22	Large Conductance Mechanosensitive Ion Channel (MscL) Family	1	42521798	144	2	MscL [*E. coli*]
1.A.23	Small Conductance Mechanosensitive Ion Channel (MscS) Family	4	42521793	396	5	KefA [*E. coli*]
			42523536	351	4	MscMJLR-like [*M. jannaschii*]
			42524113	380	4	MscMJLR [*M. jannaschii*]
			42524226	316	2	KefA [*E. coli*]
1.A.30.1	H^+- or Na^+-translocating Bacterial Flagellar Motor (Mot) Family	3	42521781	262	4	MotA [*B. subtilis*]
			42521782	249	1	MotS [*B. subtilis*]
			42524415	319	1	MotS [*B. subtilis*]
			42524416	293	4	PomA [*V. alginolyticus*]
			42524629	318	0	MotB [*E. coli*]
			42524630	265	3	MotA [*E. coli*]
1.A.35	CorA Metal Ion Transporter (MIT) Family	1	42522589	308	2	CorA [*M. jannaschii*]
1.B.3	Sugar Porin (SP) Family	1	42522760	447	0	LamB [*E. coli*]
1.B.6	OmpA-OmpF Porin (OOP) Family	4	42521994	171	0	OmpA [*E. coli*]
			42522454	368	0	OmpATb-like [*M. tuberculosis*]
			42524036	440	0	OmpATb-like [*M. tuberculosis*]
			42524180	214	0	OmpATb [*M. tuberculosis*]
1.B.10	Nucleoside-specific Channel-forming Outer Membrane Porin (Tsx) Family	2	42522254	269	0	OmpK [*V. parahaemolyticus*]
			42524633	253	1	OmpK-like
1.B.14	Outer Membrane Receptor (OMR) Family	4	42522910	698	0	FhuA [*E. coli*]
			42523077	709	0	ViuA [*V. cholerae*]
			42524068	687	0	FecA [*E. coli*]
			42524920	610	0	BtuB [*E. coli*]
1.B.17	Outer Membrane Factor (OMF) Family		42522293	426	1	NodT2 [*R. leguminosarum*] [with 3.A.1.115]

TC Family	Family Name	Number of transport systems[a]	Bba Protein ID	Size	# of TMSs[b]	Best blast-hit in TCDB and/or comments
			42522458	434	0	TolC [*E. coli*] [with 2.A.6.2]
			42522925	462	1	ToxI-like [2.A.6.2] [*B. glumae*]
			42523285	485	0	TolC-like
			42523393	437	0	PrtF [*E. coli*]
			42523686	404	0	TolC [*E. coli*]
			42524451	509	0	VceC-like [*V. cholerae*]
1.B.22	Outer Bacterial Membrane Secretin (Secretin) Family		42522439	726	0	PilQ [*P. aeruginosa*]
			42524863	222	0	XcpQ [*P. aeruginosa*]
1.B.33	Outer Membrane Protein Insertion Porin (OmpIP) Family	4	42521763	245	0	YfiO [*E. coli*] ComL
			42523001	799	0	YaeT [*E. coli*]
			42523064	573	0	D15-like [*H. influenzae*]
			42523505	392	0	YfgL [*E. coli*]
			42523581	803	1	Imp [*E. coli*]
			42523924	929	0	YaeT-like [*E. coli*]
			42524276	423	0	YfgL-like [*E. coli*]
			42524950	392	0	Omp85-like [*N. meningitidis*]
1.B.x	Unclassified outer membrane protein	1	42525165	216	1	LolA-like OMP
1.E.14	LrgA Holin (LrgA Holin) Family	1	42524812	129	4	LrgA [*S. aureus*]
2.A.1	MFS Superfamily					
2.A.1.2	Drug:H^+ Antiporter-1 (12 Spanner) (DHA1) Family	6	42522009	396	12	Bcr [*E. coli*]
			42522069	405	10	TetA [*E. coli*]
			42522244	367	11	TetA [*E. coli*]
			42522883	393	12	YdeA [*E. coli*]
			42523627	412	12	PbuE/YdhL [*B. subtilis*]
			42525027	397	12	NepI [*E. coli*]
2.A.1.4	Organophosphate:Pi Antiporter (OPA) Family	2	42523618	513	13	UhpC [*E. coli*]
			42524319	497	12	Hpt [*C. pneumoniae*]
2.A.1.6	Metabolite:H^+ Symporter (MHS) Family	1	42523790	454	12	KgtP [*E. coli*]
2.A.1.24	Unknown Major Facilitator-1 (UMF1) Family	1	42522938	392	11	YCL038c [*S. cerevisiae*]

TC Family	Family Name	Number of transport systems[a]	Bba Protein ID	Size	# of TMSs[b]	Best blast-hit in TCDB and/or comments
2.A.1.25	Peptide-Acetyl-Coenzyme A Transporter (PAT) Family	1	42521955	447	12	AmpG [*E. coli*]
2.A.1.30	Putative Abietane Diterpenoid Transporter (ADT) Family	1	42523759	398	12	DitE [*P. abietaniphila*]
2.A.1.36	Acriflavin-sensitivity (YnfM) Family	2	42522279	394	9	YnfM [*E. coli*]
			42524997	408	12	YnfM [*E. coli]*
2.A.1.x	Member of the MFS Superfamily	3	42522316	405	10	Putative MFS
			42523216	418	12	Putative MFS
			42523082	395	10	Putative MFS
2.A.2	Glycoside-Pentoside-Hexuronide (GPH): Cation Symporter Family	1	42524653	422	10	MelB [*E. coli*]
2.A.3	Amino Acid/Polyamine/Organocation (APC) Superfamily					
2.A.3.6	Archaeal/Bacterial Transporter (ABT) Family	1	42523390	412	12	Cat-1 [*A. fulgidus*]
2.A.4	Cation Diffusion Facilitator (CDF) Family	2	42521983	345	6	YiiP [*E. coli*]
			42523674	310	5	CzcD [*A. eutrophus*]
2.A.5	Zinc (Zn^{2+})-Iron (Fe^{2+}) Permease (ZIP) Family	1	42524041	251	7	ZupT [*E. coli*]
2.A.6	RND Superfamily					
2.A.6.1	Heavy Metal Efflux (HME) Family	1	42523684	1010	9	CzcA [*A. eutrophus*]
2.A.6.2	(Largely Gram-negative Bacterial) Hydrophobe/Amphiphile Efflux-1 (HAE1) Family	6	42522459	1032	12	MdtC/YegO [*E. coli*]
			42522562	1050	10	MdtC/YegO [*E. coli*]
			42522687	1033	10	MdtC/YegO [*E. coli*]
			42523244	1039	12	MdtC/YegO [*E. coli*]
			42523286	1033	11	MdtC/YegO [*E. coli*]
			42524450	1070	12	MdtC/YegO [*E. coli*]
2.A.6.7	(Largely Archaeal Putative) Hydrophobe/Amphiphile Efflux-3 (HAE3) Family	1	42523469	1013	13	MJ1562 [*M. jannaschii*]
2.A.7	DMT Superfamily					
2.A.7.1	4 TMS Small Multidrug Resistance (SMR) Family	2	42523246	145	4	EmrE [*E. coli*]
			42523933	131	4	SugE [*E. coli*]
2.A.7.3	10 TMS Drug/Metabolite Exporter (DME) Family	3	42521888	295	10	PecM [E. *chrysanthemi*]
			42522043	285	10	RhtA [*E. coli*]
			42524639	298	10	RhtA [*E. coli*]
2.A.7.x	Member of the DMT Superfamily	2	42523696	309	9	Putative DMT

TC Family	Family Name	Number of transport systems[a]	Bba Protein ID	Size	# of TMSs[b]	Best blast-hit in TCDB and/or comments
			42523878	311	10	Putative DME [2.A.7.3]
2.A.9	Cytochrome Oxidase Biogenesis (Oxa1) Family	1	42525232	539	5	YidC [*E. coli*]
2.A.17	Proton-dependent Oligopeptide Transporter (POT) Family	1	42523325	451	11	DtpT [*L. lactis*]
2.A.19	Ca^{2+}:Cation Antiporter (CaCA) Family	1	42523602	371	10	ChaA [*E. coli*]
2.A.22	Neurotransmitter:Sodium Symporter (NSS) Family	1	42523422	455	11	MJ1319 [*M. jannaschii*]
2.A.27	Glutamate:Na^+ Symporter (ESS) Family	1	42524043	394	12	GltS [*E. coli*]
2.A.35	NhaC Na^+:H^+ Antiporter (NhaC) Family	1	42521884	427	9	MleN [*B. subtilis*]
2.A.37	Monovalent Cation:Proton Antiporter-2 (CPA2) Family	5	42521965	294	10	GrmA [*B. subtilis*]
			42522245	650	11	KefC [*E. coli*]
			42523556	746	16	MagA [*Magnetospirillum*]+TrkA_C
			42523635	388	10	NhaS3-like [*Synechocystis*]
			42523724	739	16	GrmA [*B. megaterium*]+TrkA_C
			42525048	192	0	YheR [*E. coli*]
2.A.46	Benzoate:H^+ Symporter (BenE) Family	1	42521934	387	12	BenE [*A. calcoaceticus*]
2.A.47	Divalent Anion:Na^+ Symporter (DASS) Family	2	42523367	488	11	SodiTl [*S. oleracea*]
			42523612	612	10	SdrP [*Synechocystis*]
2.A.50	Glycerol Uptake (GUP) Family	1	42523167	470	9	GUP1 [*S. cerevisiae*]
2.A.51	Chromate Ion Transporter (CHR) Family	1	42523317	378	9	SrpC [*Synechococcus*]
2.A.58	Phosphate:Na^+ Symporter (PNaS) Family	1	42523679	535	8	YjbB [*E. coli*]
2.A.63	Monovalent Cation (K^+ or Na^+):Proton Antiporter-3 (CPA3) Family	1	42523736	109	3	MnhG [*S. aureus*]
			DNA	NA		MnhF [*S. aureus*]
			42523737	112	2	MnhE [*S. aureus*]
			42523738	486	13	MnhD [*S. aureus*]
			42523739	98	3	MnhC [*S. aureus*]
			42523740	853	24	MnhAB [*S. aureus*]
2.A.64	Twin Arginine Targeting (Tat) Family	1	42523658	79	1	TatE [*E. coli*]
			42523995	81	0	TatA [*E. coli*]
			42525186	253	6	TatC [*E. coli*]
			42525187	118	0	TatB [*E. coli*]
2.A.66	MOP Superfamily					

TC Family	Family Name	Number of transport systems[a]	Bba Protein ID	Size	# of TMSs[b]	Best blast-hit in TCDB and/or comments
2.A.66.1	Multi Antimicrobial Extrusion (MATE) Family	1	42523788	404	11	NorM [*B. vietnamiensis*]
2.A.66.2	Polysaccharide Transport (PST) Family	1	42523185	422	12	MTH347 [*M. thermautotrophicus*]
2.A.66.4	Mouse Virulence Factor (MVF) Family	2	42523761	520	11	MviN [*S. typhimurium*]
			42522981	523	12	MviN [*S. typhimurium*]
2.A.68	p-Aminobenzoyl-glutamate Transporter (AbgT) Family	2	42524987	514	12	MtrF/AbgT
			42521870	454	10	Putative AtoE
2.A.72	K^+ Uptake Permease (KUP) Family	1	42523474	669	12	KUP [*E. coli*]
2.A.85	The Aromatic Acid Exporter (AraE) Family	1	42524263	344	8	YccS [*E. coli*]
2.C.1	TonB-ExbB-ExbD/TolA-TolQ-TolR (TonB) Family of Auxiliary Proteins for Energization of Outer Membrane Receptor (OMR)-mediated Active Transport	6	42521812	461	0	TolB [*E. coli*]
			42521813	286	1	TonB-like [*E. coli*]
			42521814	138	1	ExbD/TolR [*E. coli*]
			42521815	245	3	TolQ [*E. coli*]
			42522010	316	1	TonB-like ?
			42522032	165	1	ExbD/TolR-like ?
			42522033	149	1	ExbD-like [*E. coli*]
			42522034	248	3	ExbB [*E. coli*]
			42522085	209	3	TolQ [*E. coli*]
			42522086	134	1	TolR-like [*E. coli*]
			42522223	168	0	Pal [*E. coli*]
			42522225	223	0	YbgF-like [*E. coli*]
			42522342	295	1	TonB-like ?
			42522410	221	3	TolQ [*E. coli*]
			42522411	158	1	TolR [*E. coli*]
			42522990	167	1	ExbD/TolR-like [*E. coli*]
			42522991	185	0	TolR-like [*E. coli*]
			42522992	236	3	TolQ-like [*E. coli*]
			42523572	224	1	YbgF-like [*E. coli*]
			42523821	175	1	ExbD/TolR-like ?
			42523822	151	1	ExbD-like [*E. coli*]

TC Family	Family Name	Number of transport systems[a]	Bba Protein ID	Size	# of TMSs[b]	Best blast-hit in TCDB and/or comments
			42523823	244	3	ExbB [*E. coli*]
			42523907	175	0	YbgF-like [*E. coli*]
			42524267	244	0	TonB-like ?
			42524795	341	0	TolB-like ?
			42524919	209	1	TonB-like [*E. coli*]
			42525096	471	1	TonB-like ?
3.A.1	ABC Superfamily [c]					
3.A.1.1	Carbohydrate Uptake Transporter-1 (CUT1) Family	1	42522756	347	0	[C] MalK
			42522757	272	6	[M] MalG2
			42522758	733	7	[R+M] MalE1+MalF1
3.A.1.2	Carbohydrate Uptake Transporter-2 (CUT2) Family	1	42522051	291	8	[M]
			42522052	342	8	[M] FrcC-like [*R. meliloti*]
			42522053	494	0	[C]
			42522054	339	0	[R]
3.A.1.3	Polar Amino Acid Uptake Transporter (PAAT) Family	1	42522399	247	0	BgtA [C]
			42522400	247	4	BgtB_C [M]
			42522401	262	0	BgtB_N [R]
3.A.1.4	Hydrophobic Amino Acid Uptake Transporter (HAAT) Family	1	42524744	373	0	LivK [R]
			42524745	300	8	LivH [M]
			42524746	323	8	LivM [M]
			42524747	270	0	LivG [C]
			42524748	237	0	LivF [C]
3.A.1.5	Peptide/Opine/Nickel Uptake Transporter (PepT) Family	3	42521975	540	0	DppE [R]
			42521976	343	6	DppB [M]
			42521977	339	6	DppC [M]
			42523449	487	0	MppA [R]
			42523651	322	0	OppD [C]
			42523652	329	0	OppF [C]
			42523653	564	0	OppA [R]

TC Family	Family Name	Number of transport systems[a]	Bba Protein ID	Size	# of TMSs[b]	Best blast-hit in TCDB and/or comments
			42523654	323	6	OppB [M]
			42523655	404	6	OppC [M]
			42524127	274	6	DppC [M]
			42524128	310	7	DppB [M]
3.A.1.7	Phosphate Uptake Transporter (PhoT) Family	1	42523158	333	0	PstS-like [R]
			42523159	312	6	PstC [M]
			42523160	305	8	PstA [M]
			42523161	255	0	PstB [C]
3.A.1.9	Phosphonate Uptake Transporter (PhnT) Family	1	42524492	322	7	PhnE [M]
			42524493	262	0	PhnC [C]
			42524494	297	0	PhnD [R]
3.A.1.11	Polyamine/Opine/Phosphonate Uptake Transporter (POPT) Family	1	42522934	344	0	PotD [R]
			42522935	292	0	PotA [C]
			42522936	277	6	PotB [M]
			42522937	277	6	PotC [M]
3.A.1.15	Manganese/Zinc/Iron Chelate Uptake Transporter (MZT) Family (Similar to 3.A.1.12 and 3.A.1.16)	1	42524734	291	0	ZnuA [R]
			42524735	189	0	ZnuC [C]
			42524736	270	7	ZnuB [M]
3.A.1.16	Nitrate/Nitrite/Cyanate Uptake Transporter (NitT) Family	1	42523495	310	0	[R]
			42523496	253	5	[M] CmpB-like [*Synechococcus*]
			42523497	266	0	[C]
3.A.1.17	Taurine Uptake Transporter (TauT) Family (Similar to 3.A.1.12 and 3.A. 1.16)	1	42524162	266	0	TauB [C]
			42524163	275	6	TauC [M]
			42524164	333	1	TauA [R]
3.A.1.19	Thiamin Uptake Transporter (ThiT) Family (Most similar to 3.A.1.10, 3.A. 1.6 and 3.A.1.8 in that order)	1	42522187	346	1	ThiB [R]
			42522188	487	11	ThiP [M]
			42522189	208	0	ThiQ [C]
3.A.1.102	Lipooligosaccharide Exporter (LOSE) Family	1	42521775	308	0	NodI [C]
			42521776	262	6	NodJ [M]
3.A.1.106	Lipid Exporter (LipidE) Family	3	42521794	573	5	MsbA [M+C]

TC Family	Family Name	Number of transport systems[a]	Bba Protein ID	Size	# of TMSs[b]	Best blast-hit in TCDB and/or comments
			42523036	588	4	MsbA [M+C]
			42524487	567	5	MsbA [M+C]
3.A.1.115	Na^+ Exporter (NatE) Family	1	42523356	250	0	[C]
			42523357	246	0	[C]
			42523358	368	6	[M] NatB-like
			42523359	358	6	[M]
3.A.1.122	Macrolide Exporter (MacB) Family	1	42522295	650	4	MacB [C+M]
3.A.1.125	Lipoprotein Translocase (LPT) Family	2	42522999	405	4	LolE [M]
			42523000	220	0	LolD [C]
			42523263	416	4	LolC [M]
			42524549	402	4	LolE [M]
			42524550	231	0	LolD [C]
3.A.1.208	Conjugate Transporter Family (ABCC)	2	42523967	597	5	AtMRP2_N [*A. thaliana*]
			42523968	620	6	AtMRP2_C [*A. thaliana*]
			42524005	1228	10	MRP3 [*H. sapiens*]
3.A.1.x	Orphan members of the ABC Superfamily	15	42521819	252	0	[C]
			42521882	251	0	[R] [3.A.1.3 ?]
			42521925	305	0	[C]
			42521962	221	0	[C]
			42521963	849	10	[M] [duplicated LolE-like]
			42522096	254	0	[R] [3.A.1.3; TcyA *B. subtilis*]
			42522111	273	1	[R] [3.A.1.3; ArtJ *E. coli*]
			42522191	246	0	[R] [3.A.1.3 ?]
			42522255	496	0	[R] [duplicated 3.A.1.3 ?]
			42522275	274	1	[R] [3.A.1.3; ArtJ-like *E. coli*]
			42522313	243	1	[R] [3.A.1.3; ArgT-like *E. coli*]
			42522356	242	0	[R] [3.A.1.3 ?]
			42522414	240	0	[C]
			42522416	274	0	[R] [3.A.1.3; GlnH *E. coli*]
			42522431	296	0	[R] [3.A.1.9; PhnD *E. coli*]

TC Family	Family Name	Number of transport systems[a]	Bba Protein ID	Size	# of TMSs[b]	Best blast-hit in TCDB and/or comments
			42522432	247	0	[C]
			42522433	253	6	[M] [NosY-like] [PliI ?]
			42522485	252	0	[C]
			42522556	603	0	[C+C]
			42522582	314	0	[C]
			42522583	257	6	[M] ?
			42522584	511	4	[M] [NA; ABC ?]
			42522612	292	0	[R] [3.A.1.3 ?]
			42522628	330	0	[r] [3.A.1.10; FutA1 *Synechocystis*]
			42522659	229	0	[C]
			42522660	421	3	[M] [LolE-like]
			42522753	569	0	[C]
			42522767	245	0	[R] [3.A.1.3; HisJ *S. typhimurium*]
			42522834	285	0	[R] [3.A.1.3 ?]
			42522852	556	0	[C+C]
			42522853	277	5	[M] [NA; ABC ?]
			42522868	247	0	[R] [3.A.1.3 ?]
			42522895	582	0	[R] [3.A.1.2+3; RbsB *E. coli* + HisJ *S. typhimurium*]
			42522931	543	0	[C+C]
			42522945	254	1	[R] [3.A.1.3; ArtJ-like *E. coli*]
			42523223	248	6	[M] [3.A.1.105]
			42523224	287	0	[C] [3.A.1.105]
			42523258	274	6	[M] [9.B. 11; Tgd1 *A. thaliana*]
			42523259	236	0	[C] [3.A.5 FtsE-like ATPase]
			42523260	317	1	[R] [Ttg2C]
			42523261	261	0	[R] [3.A.1.3 ?]
			42523262	265	0	[R] [3.A.1.3 ?]
			42523274	247	0	[R] [3.A.1.3; ArgT-like *E. coli*]
			42523464	237	1	[R] [3.A.1.3 ?]

TC Family	Family Name	Number of transport systems[a]	Bba Protein ID	Size	# of TMSs[b]	Best blast-hit in TCDB and/or comments
			42523514	246	1	[R] [3.A.1.3; GlnH-like *E. coli*]
			42523547	600	1	[R] [duplicated; 3.A.1.2 ?]
			42523561	257	0	[R] [3.A.1.3; ArgT *E. coli*]
			42523595	257	6	[M] [3.A.1.102]
			42523596	305	0	[C] [3.A.1.102]
			42523831	267	0	[R] [3.A.1.13; ButF *S. typhimurium*]
			42523888	239	0	[R] [3.A.1.3 ?]
			42524071	246	0	[R] [3.A.1.3; CysX *E. coli*]
			42524132	347	0	[R] [3.A.1.2]
			42524161	149	4	[M] [NA; ABC ?]
			42524193	260	0	[R] [3.A.1.3; TcyA-like *B. subtilis*]
			42524262	287	0	[R] [3.A.1.3]
			42524284	254	0	[R] [3.A.1.3 ?]
			42524507	459	1	[R] [Ttg2C]
			42524508	254	0	[C]
			42524509	275	5	[M] [9.B.11; Tgd1 *A. thaliana*]
			42524523	264	4	[M] [NA; ABC ?]
			42524770	262	5	[M] [9.B.11; Tgd1 *A. thaliana*]
			42524771	248	0	[C]
			42524772	272	1	[R] [Ttg2C]
			42524780	474	0	[R] [3.A.1.3 ? + SLT]
			42524915	622	0	[C+C]
			42524918	515	0	[C+C]
			42524922	265	0	[R] [3.A.1.3; ArtI *E. coli*]
			42524935	276	0	[C]
			42524936	270	7	[M] ?
			42524937	266	6	[M] [NA; ABC ?]
			42524980	554	0	[C+C]
			42525129	258	1	[R] [Ttg2C]
			42525130	239	5	[M] [9.B.11; Tgd1 *A. thaliana*]

TC Family	Family Name	Number of transport systems[a]	Bba Protein ID	Size	# of TMSs[b]	Best blast-hit in TCDB and/or comments
			42525131	259	3	[M] [9.B. 11; Tgd1 *A. thaliana*]
			42525132	243	0	[C]
			42525133	252	0	[C]
			42525140	237	0	[C]
			42525141	786	9	[M] ?
			42525194	556	0	[R] [3.A.1.4; ? NatB *Synechocystis*]
3.A.2	H^+ or Na^+ translocating F-type, V-type and A-type ATPase (F-ATPase) Superfamily	1	42521659	229	6	F0-A
			42521660	105	2	F0-C
			42525217	140	0	F1-ε
			42525218	468	0	F1-β
			42525219	295	0	F1-γ
			42525220	507	0	F1-α
			42525221	182	0	F1-δ
			42525222	186	2	F0-B
			42525223	144	1	F0-B
3.A.3	P-type ATPase (P-ATPase) Superfamily	5	42522486	825	9	pMA1, Ca^{2+}-ATPase
			42523248	141	2	KdpA_N, K^+-ATPase
			42523249	160	3	KdpA_C, K^+-ATPase
			42523682	724	8	CopA, Cu^{2+}-ATPase
			42523748	692	8	CopB, Cu^{2+}, Cu^+, Ag^+ ATPase
			42524029	798	8	CopA, Cu^{2+}-ATPase
3.A.5	General Secretory Pathway (Sec) Family	1	42521801	220	0	FtsE
			42521895	889	0	SecA
			42522594	161	2	SecG
			42522713	402	1	FtsY
			42523591	450	0	Ffh
			42523691	312	6	SecF
			42523692	564	5	SecD
			42523693	117	1	YjaC
			42524357	442	10	SecY

TC Family	Family Name	Number of transport systems[a]	Bba Protein ID	Size	# of TMSs[b]	Best blast-hit in TCDB and/or comments
			42524389	125	3	SecE
3.A.6	Type III (Virulence-related) Secretory Pathway (IIISP) Family	1	42524690	699	7	FlhA
			42524691	353	3	FlhB
			42524692	259	7	FliR
			42524693	90	2	FliQ
			42524694	252	5	FliP
			42524695	224	1	FliO
			42524696	122	0	FliN
			42524697	333	0	FliM
			42524760	442	0	FliI
			42524761	261	0	FliH
			42524763	549	2	FliF
3.A.10	H^+ translocating Pyrophosphatase (H^+PPase) Family	1	42523211	688	16	V-PPase [*A. thaliana*]
3.A.12	Septal DNA Translocator (S-DNA-T) Family	1	42521689	797	5	SpoIIIE of [*B. subtilis*]
3.A.15	Outer Membrane Protein Secreting Main Terminal Branch (MTB) Family	2	42522434	259	6	PilD
			42522813	190	1	PilA
			42523016	566	0	PilB
			42523017	347	0	PilT
			42523018	405	3	PilC
			42523092	411	0	PulK ?
			42523093	294	1	PulJ ?
			42523094	166	1	PulI ?
			42523095	194	1	FimT ? /PulH ?
			42523096	136	1	PulG
			42523100	405	3	PulF
			42523101	564	0	PulE
			42523102	765	0	PulD [1.B.22]
			42523103	304	1	PulC
			42523986	289	1	FimU ?
			42524677	433	0	PilQ-like [1.B.22]

TC Family	Family Name	Number of transport systems[a]	Bba Protein ID	Size	# of TMSs[b]	Best blast-hit in TCDB and/or comments
			42525175	367	0	PilU/PilT-like
3.B.1 (?)	Na^+ transporting Carboxylic Acid Decarboxylase (NaT-DC) Family		42524925	127	0	Gamma [*A. fermentans*]
			42524928	535	0	Alpha [*P. abyssi*]
			42525181	522	0	Alpha [*V. parvula*]
			42525183	175	0	Gamma [*P. abyssi*]
3.D.1	Proton-translocating NADH Dehydrogenase (NDH) Family	1	42524471	174	1	Nqo9
			42524472	504	0	Nqo3
			42524473	431	0	Nqo1
			42524474	159	0	Nqo2
			42524475	560	0	Nqo5-Nqo4
			42524476	187	0	Nqo6
			42525207	486	12	Nqo14
			42525208	517	13	Nqo13
			42525209	643	16	Nqo12
			42525210	107	3	Nqo11
			42525211	178	5	Nqo10
			42525212	385	8	Nqo8
			42525213	127	3	Nqo7
3.D.4	Proton-translocating Cytochrome Oxidase (COX) Superfamily	3	42521909	318	2	Cox2
			42521910	535	12	Cox1
			42521911	221	5	Cox3
			42521912	112	3	Cox4
			42521913	292	8	CoxX
			42524021	441	12	Cox1-like
			42524022	215	0	Cox2-like
			42524031	706	13	CcoN-CcoO
5.A.1	Disulfide Bond Oxidoreductase D (DsbD) Family	1	42524631	653	9	DsbD [*E. coli*]
5.A.3	The Prokaryotic Molybdopterin-containing Oxidoreductase (PMO) Family	1	42523112	1033	0	DmsAB [*H. salinarium*]

TC Family	Family Name	Number of transport systems[a]	Bba Protein ID	Size	# of TMSs[b]	Best blast-hit in TCDB and/or comments
			42523113	453	10	DmsC [*H. salinarium*]
			42524233	151	1	TorC_N [*E. coli*]
8.A.1	Membrane Fusion Protein (MFP) Family		42522294	321	1	MacA [for 3.A.1.122]
			42523355	204	0	MFP [for 3.A.1.115]
			42522395	582	1	Probably with [NA SapB-like 9.B.20]
			42525142	297	1	AcrA-like [for 3.A.1.x]
8.A.21	Epithelial Na^+ Channel (ENaC) Family		42523755	250	0	Stomatin homolog [*P. horikoshii*]
			42523756	424	5	NfeD protease [*P. horikoshii*]
			42524093	307	1	Stomatin homolog-like protein
9.A.8	Ferrous Iron Uptake (FeoB) Family	1	42523346	76	0	[FeoA] FeoB2_N [*P. gingivalis*]
			42523347	638	8	FeoB [*L. biflexa*]
9.A.19	Mg^{2+} Transporter-E (MgtE) Family	1	42523969	447	4	MgtE [*B. firmus*]
9.A.23	Ferroportin (FP) Family	1	42523494	440	8	Fpn1 [*M. musculus*] Putative MFS?
9.B.3	Putative Bacterial Murein Precursor Exporter (MPE) Family	2	42523896	374	9	RodA [*E. coli*]
			42524580	380	9	FtsW [*E. coli*]
9.B.17	Putative Fatty Acid Transporter (FAT) Family		42521902	498	0	FadD [*E. coli*]
			42522110	562	0	CaiC [*E. coli*]
			42522828	645	0	CaiC [*E. coli*]
			42523290	554	0	FadD [*E. coli*]
			42524534	593	0	FadD [*E. coli*]
			42524536	805	0	X+FadD [*E. coli*]
9.B.22	Putative Permease (PerM) Family	2	42523616	345	7	Yct2 [*B. subtilis*]
			42523150	371	8	PerM-like ? [*E. coli*]
9.B.26	PF27 (PF27) Family	2	42523213	90	2	Y615-like [C-half]
			42524601	183	5	Y615 [*Synechocystis*]
9.B.27	YdjX-Z (YdjX-Z) Family	1	42521725	224	4	YdjZ [*E. coli*]
9.B.30	Hly III (Hly III) Family	2	42522243	215	7	HlyIII [*B. cereus*]
			42525120	208	7	HlyIII [*B. cereus*]

TC Family	Family Name	Number of transport systems[a]	Bba Protein ID	Size	# of TMSs[b]	Best blast-hit in TCDB and/or comments
9.B.37	HlyC/CorC (HCC) Family	2	42522543	346	3	YrkA [*B. subtilis*]
			42523633	343	3	YrkA [*B. subtilis*]
9.B.53 ?	Unknown IT-6 (UIT6) Family	1	42524007	429	12	Putative transporter ? [*L. interrogans*]
9.B.63	9 TMS Putative Metabolite Efflux (9-PME) Family	1	42522953	325	8	YeiH [*E. coli*]

[a]Proteins of certain families are known to function either as part of multi-component transport systems or are accessory proteins. Therefore this information is considered when calculating the number of transport systems.

[b]The numbers of putative α-helical transmembrane segments (TMSs) were calculated using the TMHMM program. Unfortunately, in the case of outer membrane porins (TC #1.B), the numbers do not reflect the numbers of β-strands and therefore are of limited value.

[c]The various protein components of ABC transporters are labeled as [M]: Integral membrane protein, [C]: Cytoplasmic ATP-hydrolyzing protein, and [R]: Extracytoplasmic (periplasmic) solute-binding receptor. Duplication of domains (e.g., [C+C]) or fusion of two or more protein domains (e.g., [R+M]) are also indicated.

Table 3

Unusual compositions of protein complexes in Bba.

Complex	Component	# of Homologues in Bba	# of Homologues in *E. coli*
A. Bacterial Motor Complexes			
	MotA	3	1
	MotB	3	1
B. OMR Energizers			
	TolA/TonB	6	2
	TolQ/ExbB (like MotA)	6	2
	TolR/ExbD (like MotB)	9	2
	TolB	2	1
	Pal	1	1
	YbgF	3	1
C. Outer Membrane Assembly Complex			
	YaeT	4	2
	OstA-L	1	1
	OstA-S	0	1
	YfgL	2	1
	OfiO	1	1
	NlpB	0	1