UNIVERSITY OF CALIFORNIA, SAN DIEGO

Comparative genomic analyses of transport proteins encoded within the red algae, *Galdieria sulphuraria* and *Chondrus crispus*

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

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by

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

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Chair

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DEDICATION

This thesis of mine is dedicated to certain persons who I consider dear to me. Foremost, I would like to acknowledge my best friends Sherman, Kevin, and Wuhan. They have stuck with me throughout my college career, and they have motivated me to think outside the box. I would like to thank Carl, Sinjin, Natalie, Shounak, Betty, and Marielle for being some of the coolest people I know. I would also like to give special thanks to my buddies Chris, Joe, Trish, and Alek for keeping my morale high in our countless times of struggle. Viv, thank you for being there for me and for being an intellectual illumination. Nao, thank you for giving me the power and encouragement to do what I must. I would like to acknowledge Jalal, who I consider my brother, for being a great inspiration. Lastly, I would like to acknowledge my parents and grandparents, who have always supported me.

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ABSTRACT OF THE THESIS

Comparative genomic analyses of transport proteins encoded within the red algae, *Galdieria sulphuraria and Chondrus crispus*

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Galdieria sulphuraria is a thermo-acidophilic unicellular red alga that is capable of living in the presence of toxic heavy metals. We bioinformatically analyzed the genome of *G. sulphuraria* in order to identify and classify transport proteins related to its extensive metabolic and extremophilic capabilities. These transport proteins were compared with those of the mesophilic and multicellular red alga *Chondrus crispus* Stackhouse, or Irish moss, in order characterize the extent of their divergence. While *G. sulphuraria* has a vast array of transporters capable of importing a variety of carbon sources, *C. crispus* has relatively few. Additionally, the genome of *G. sulphuraria*

encodes many heavy metal ion transporters lacking in the genome of *C. crispus* although *C. crispus* has many monovalent ion transporters that *G. sulphuraria* lacks. The results presented in this report provide information about transport systems relevant to furthering the studies of *Galdieria sulphuraria*, *Chondrus crispus*, and other red algae with applications relevant to bioremediation.

INTRODUCTION

The ability of some algae, such as *Galdieria sulphuraria*, to not only live, but thrive in volcanic localities is quite unexpected when we consider typical eukaryotic characteristics. *G. sulphuraria* (hereafter referred to as Gsu) is a unicellular thermo-acidophilic red alga that lives in the springs, rivers, and soils of these environments, where it can represent nearly all of the biomass (Figure 1; Schonknecht et al. 2013). Extremophiles such as Gsu can live under conditions considered hostile to most other species. The specifications that define these environments can include acute high and low temperatures, extreme pH ranges, and high salt and metal ion concentrations (Canganella and Wiegel 2011). In order to survive in these drastic habitats, organisms must adapt their phenotypes, and therefore genotypes, accordingly to the surroundings. These changes might not merely allow these organisms to weather extreme conditions, they may also enable them to out-compete neighboring species (Welch et al. 2014).

Gsu is of the Cyanidiophyceae class, which diverged about 1.3 billion years ago from other rhodophytic (red algal) classes (Ciniglia et al. 2004). This alga is an asexual photosynthetic extremophile, but it is also a heterotroph that can obtain food from exogenous sources (Schonknecht et al. 2013). Gsu has been the recipient of multiple horizontal gene transfers (HGT), having acquired genes encoding non-native enzymes that promote its high metal and halo-tolerance (Schonknecht et al. 2014, Rothschild and Mancinelli 2001). Additionally, the fact that the estimated divergence of *Galdieria* from its closest characterized cousin, *Cyanidioschyzon merolae*, occurred about 1 billion years

ago, shows that Gsu is a unique rhodophyte even among its relatives (Schonknecht et al. 2014).

Like other rhodophytes, Gsu uses phycobilisomes, protein complexes that captures light energy for photosynthesis (Su et al. 2010). However, Gsu usually lives in minimal light environments that are characteristic of the murky volcanic biota. One feature that sets Gsu apart from other autotrophic red algae is that the subunits of its light harvesting complex display tighter couplings, and so are more efficient in energy retention than homologous proteins complexes from other species (Thangaraj et al. 2011). The additional fact that Gsu has heterotrophic abilities, and that it retains the metabolic pathways lost in other algae, seems to give credence to the strong evolutionary pressures that its volcanic habitats provide (Schonknecht et al. 2014). Recent genome analyses have revealed the presence of sequences encoding archaeal ATPases, bacterial antiporters, and bacterial pumps in Gsu (Schonknecht et al. 2014). These non-native proteins seem to give Gsu not only an edge in survival, but also the ability to become the majority species in its unusual environment.

Because transport proteins are integral to nutrient acquisition, waste removal, and signaling, they can be said to provide a connection between an organism's genome and its environment (Getsin et al. 2013). Focusing on their characterization in Gsu might, therefore, elucidate aspects of its physiology. They might also reveal how horizontally transferred genes were selectively retained in survival under extreme conditions. Transporter proteins evolve more complex mechanisms with varying substrate specifics,

and studying these features and comparing Gsu's transporters to homologues of non-extremophile cousins might provide a molecular insight as to how Gsu has become different from its rhodophytic relatives (Lam et al. 2011). As such, proteome analyses were performed for transporters in Gsu and the mesophilic red alga *Chondrus crispus* Stackhouse (Gigartinales) or Irish moss (hereafter referred to as Ccr). In contrast to Gsu, Ccr is a member of the Florideophyceae class, and is a macroalga. Yet, this organism has fairly small and compact gene families (Figure 1; Collen et al. 2013). A comparison between the transporters found in these two rhodophytes could assist in furthering our understanding of the adaptation of Gsu in extreme environments.

METHODS

Gsu and Ccr proteomes were retrieved from the protein database of the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov) website and were screened by our genome BLAST (GBLAST) program against the transporters tabulated, as of January 2014, in the Transporter Classification Database (TCDB; www.tcdb.org; Reddy and Saier 2012). The mitochondrial and plastidial proteomes of Gsu were not available at that time, and those were analyzed on September of same year. Each putative open-reading frame was used as a query in the BLASTP software to search for homologous proteins in TCDB under a stringent comparative e-value score cutoff of 0.001. The low complexity filter was not used as it is normally of value for larger datasets that include proteins with multiple repeat elements. The information provided included suspected query transporters, their top TC hits, TC hit accession numbers and short descriptions, protein sequence match lengths, e-values, numbers of transmembrane segments (TMSs) in both query and hit proteins, and number of TMS overlaps. The second-to-last feature was accomplished through the HMMTOP 2.0 program, which scans each open reading frame to predict the number of putative TMSs (Tusnady and Simon 1998).

Both the Ccr and Gsu lists of putative transport proteins were screened for false positives, and those that displayed 0 or 1 TMSs through the HMMTOP program were removed unless their identified protein families were shown to have membrane insertion capabilities. This would eliminate non-integral and non-multispanning membrane

proteins, but still retain 0 TMS proteins such as the β–barrel porins. In cases where TMS numbers needed to be resolved between the query and hit, the Web-based Hydropathy, Amphipathicity and Topology (WHAT) program was used with a window size of 19 residues and an angle of 100 degrees to display the protein's hydropathy plot. Though the WHAT program uses the HMMTOP program to highlight regions where predicted TMSs could be, this program sometimes predicts incorrectly, and it is ultimately up to the user to judge the actual TMS numbers. An arbitrary criterion for a TMS would be a hydrophobicity peak with a value of greater than 1 (Zhai and Saier 2001). In certain cases where the hydrophobicity plot was unclear, the TOPCONS web server program was used to provide a consensus TMS prediction. The current TOPCONS uses an algorithm that combines the prediction of five programs, which includes SCAMPI, OCTOPUS, ΔG-scale, ZPRED, and PRO/PROVID-TMHMM (Bernsel et al. 2009, Reddy et al. 2014).

When comparative e-value scores retrieved from the GBLAST program were particularly low, yet the TMS numbers and sequence locations were similar, the Global Sequence Alignment Tool (GSAT) was used to check for statistical significance. The GSAT program utilizes the EMBOSS Needleman-Wunsch (NW) algorithm to provide a global alignment and gives a standardized comparison score based on a user-defined number of shuffles (Reddy and Saier 2012). A last measure of verification is the use of the NCBI website's Conserved Domain Database (CDD) search, which produces a visualization of the conserved motifs within a query transporter, and this can be compared to the motifs in a hit protein family.

To find novel proteins, a GBLAST of the Ccr and Gsu proteomes was performed again with a poorer e-value cutoff of 0.1, and this produced a larger list of putative transporters. Only the proteins with comparative scores between 0.001 and 0.1 were scrutinized via the aforementioned steps for detecting false positives and negatives, and verified candidate transporters were entered into TCDB. For each of the proteins in the verified list of transporters, the scientific literature was checked for substrate specificities and mechanism, and annotated with a UniProt accession number in Table 3. Proteins from the mitochondrial and plastidial proteomes of Gsu were annotated with GI numbers, as those were not assigned UniProt accession numbers as of September 2014. It should be noted that protein sequences with same UniProt accession number were only counted as a single entry, and this is also reflected in the total count of proteins in the two algal proteomes.

RESULTS

Statistical analyses of transport proteins found in Ccr and Gsu

The genomes of the red algae Ccr and Gsu were scrutinized for transport proteins by using the proteomes of these organisms as separate queries in comprehensive BLAST searches against the Transporter Classification Database using the GBLAST program (TCDB; www.tcdb.org; Saier et al. 2014). The predicted TC classified transport-related proteins are recorded in Table 3, and the statistical summary of the results according to TC class and subclass is presented in Table 1. The percentages of these classes and subclasses are depicted for these two organisms in Figure 2. It appears that of Ccr's 9836 predicted proteins, only 303 proteins or 3.1% are recognized multispanning integral membrane transport proteins. In contrast, Gsu has 468 such proteins, 6.5% of its 7211 predicted proteins (Table 1). This shows that while Ccr has a larger genome, the percentage of its proteins that conform to the prediction of being known integral membrane transport proteins is less than half of that of Gsu.

Class 1 proteins in TCDB include those that either form permanent transmembrane channels, or are capable of forming transient membrane pores. The transport mechanism used by these proteins is in general, free diffusion, being energy independent (Spencer and Rees 2002, Zeth and Thein 2010). Evaluation of these transport protein numbers allows one to perceive similarities between those encoded by the genomes of Ccr and Gsu. Both have nearly the same numbers of class 1 proteins (44

and 50 respectively; Table 1). A comparison of the percentages of class 1 proteins with respect to the total number of transporters in each red alga reveals that there is about a 3.8% difference (14.5% for Ccr versus 10.7% for Gsu; Figure 2). This suggests that while passive diffusion-mediating transporters are important for red algae in general; they do not seem to confer significant benefits to the survival of Gsu in extreme environments.

Types of channel proteins are further distinguished by their subclasses, of which 1.A, 1.B, and 1.F are relevant due to their presence in the two red algal genomes. Subclass 1.A transporters are characterized by transmembrane α-helical secondary structure-forming sequences, and there appears to be only a difference of four in the numbers of these proteins between Ccr and Gsu (32 and 36 respectively; Table 1). Subclass 1.B are outer membrane porins that are typically made up of transmembrane β-barrels. They usually exhibit only 0 or 1 α-helical transmembrane segments (TMS). Both Ccr and Gsu have five of these proteins. Subclass 1.F include the vesicle fusion pore proteins, which form complexes that promote interaction of vesicles with the cell membrane for transient creations of pores, usually for solute exocytosis (Fuhrmans et al. 2014). Between Ccr and Gsu, there is a difference of two in the number of proteins of this subclass (7 and 9, respectively). Of the percentages of each aforementioned subclass relative to the total number of transport proteins between the two red algae, 1.A α-type channels have the greatest difference of 2.9%, while 1.B and 1.F have 0.6% and 0.4% differences, respectively (Figure 2).

Class 2 proteins are those that function by a secondary active transport, usually energized by ion (H^+ or Na^+) electrochemical gradients (Shi 2013). Of the class 2 transporters, only the porters of subclass 2.A, which include uniporters, antiporters, and symporters, have been found in the Ccr and Gsu genomes (Table 1). Ccr and Gsu respectively have 121 and 250 such porters, largely accounting for the differences in transport protein numbers between the two algae. The corresponding subclass 2.A percentages of 40.0% for Ccr and 53.4% for Gsu reveal that porters are a major fraction of the transporters in the two genomes. The subsequent 13.4% difference is the largest difference observed for the various subclasses (Figure 2). This suggests that catalysis function by secondary active transporter is most important for red algal growth and survival, and may confer Gsu its ability to thrive in extreme environments.

Class 3 proteins are those that function by a primary active transport mechanism in order to move solutes through the membrane against concentration gradients (Enkavi et al. 2013). Many class 3 proteins are subunits of transport complexes, where only the channel-forming integral membrane constituents are tabulated. The two algae are similar in the numbers of these transporters, with a difference of 8 proteins (91 for Ccr versus 99 for Gsu; Table 1). Class 3 proteins are the second largest transporter group in both algae, and their percentage difference of 8.8% is also the second largest (30.0% for Ccr versus 21.2% for Gsu; Figure 2). This suggests that transporters that utilize a primary energy source are of lesser importance to Gsu's survival in extreme environments than those that use a secondary source of energy.

Of the primary active transport subclasses, 3.A, 3.D, and 3.E appear in the genomes of Ccr and Gsu. Subclass 3.A include proteins that energize substrate transport by hydrolysis of pyrophosphate bonds. The relevant 3.A integral membrane subunits identified proved to be 68 for Ccr and 74 for Gsu (Table 1). Subclass 3.D includes transporters that are energized by redox reactions, and there is nearly a one-to-one ratio in the numbers of these proteins between the two red algae (19 for Ccr versus 20 for Gsu). Subclass 3.E include transport systems that use solar energy for transport, and there are also similar numbers of these proteins in Ccr and Gsu (4 and 5, respectively). Of the percentages of the class 3 subclasses between the two, 3.A P-P bond hydrolysis transporters have the greatest difference of 6.6%, while 3.D and 3.E have 2.0% and 0.2% difference respectively (Figure 2).

Class 4 proteins are those that catalyze substrate transport accompanied by substrate modification via some coupled process. Of these group translocators, only members of subclass 4.C, which utilizes Coenzyme A as an agent for carboxylic acid thioesterification, have been found in the genomes of the two red algae. The relevant Fatty Acid Transporter (FAT) Family (TC# 4.C.1) proteins are believed to allow coupled fatty acid uptake via acyl-CoA synthetases (Schneider et al. 2014). There appears to be one of such FAT proteins in Ccr and two in Gsu (Table 1; Table 3). With these proteins contributing an overall 0.3% of the total protein transporters in Ccr and 0.4% in Gsu, it seems unlikely that they play a role in providing a special benefit for Gsu's survival in extreme environments (Figure 2). An interesting feature of Gsu's FAT proteins is that

they were reportedly obtained laterally from the thermophilic bacterium *Roseiflexus castenholzii* (Schonknecht et al. 2013).

Class 5 proteins transport electrons from one side of the membrane to the other, thereby influencing the membrane potential. Ccr has 12 of these proteins in its genome, whereas Gsu has eight (Table 1). With Ccr and Gsu having respective class 5 percentages of 4.0% and 1.7%, respectively. It seems unlikely that these proteins promote the ability for Gsu to live in extreme climes (Figure 2). Gsu lacks proteins of the 5.A subclass, which transport electron pairs, and all of its class 5 proteins are of subclass 5.B, which transport single electrons. Evidently, transmembrane electron-flow processes do not play a role in fitness in extreme environments.

Class 8 includes auxiliary proteins that facilitate substrate transport by enhancing the activity of a transporter. Ccr and Gsu only have one and five of such proteins, respectively (Table 1), and these proteins belong in the 8.A subclass. The corresponding class 8 percentages are therefore small (0.3% for Ccr versus 1.1% for Gsu; Figure 2).

The class 9.A proteins of TCDB include those that are known transporters that function by an unknown mechanism, while those within subclass 9.B are putative transporters with insufficient evidence to establish a transport function. Ccr has 33 of class 9 proteins, while Gsu has 54 (Table 1). By looking at the class 9 percentages of each alga, one can see that the difference of 0.5% is minimal (11.0% for Ccr versus 11.5% for Gsu; Figure 2). It is interesting to note that there is about a one-to-two ratio in

the numbers of 9.A and 9.B proteins in both red algae (11 versus 22 proteins for Ccr, 16 versus 38 proteins for Gsu; Table 1). Thus, the subclasses have similar corresponding percentages (Figure 2).

Table 2 categorizes the substrates of transport systems identified in the genomes of Ccr and Gsu according to TC class. It can be seen that Ccr's 303 transport proteins form a total of 262 transport systems, while Gsu has 416 systems formed by its 468 transport proteins. Class 1 channels and pores of Ccr are dedicated mostly to the transport of inorganic substrates with there being about a two-to-one ratio for those transporting cations versus anions (Table 2). The same is true for the channels and pores found in Gsu, but a notable feature is that there is an increased number of systems that transport sugar and polyol compounds (5 sugar/polyol channels for Gsu versus 1 for Ccr). These belong to the Major Intrinsic Protein (MIP) Family (TC# 1.A.8) of aquaporins, which often transport both water and glycerol (Bienert et al. 2008). An interesting feature of *G. sulphuraria*'s MIP proteins is that they were reportedly obtained laterally from the mesophilic bacterium *Xanthomonas axonopodis* (Schonknecht et al. 2013).

A comparison between the numbers of class 2 secondary carriers in Ccr and Gsu shows how the latter is greater in nearly all substrate types except for peptides & conjugates, lipids, vitamins, and unknown (Table 2). Compared to Ccr, Gsu displays moderate increases of transporters for inorganic substrates, carboxylates, organoanions, amines, amides, polyamines and organocations. Surprisingly, we identified few systems for these compounds in Ccr (4 for organoanions and 2 for amines, amides, polyamines &

organocations versus 14 and 12, respectively, in *G. sulphuraria*). The greatest increases can be seen in the approximately three-to-one ratios of the sugar & polyol-specific systems, as well as the amino acids & conjugates systems (62 and 34, respectively, for Gsu versus 22 and 9, respectively, for Ccr).

The secondary active transporter family most responsible for increase in Gsu's sugar and polyol transporting systems is the Major Facilitator (MFS) Superfamily (TC# 2.A.1). This particular superfamily is extremely large and diverse with respect to substrate types (Yan 2013). Of those that transport sugars, Ccr only has three compared to the 39 for Gsu (Tables 2 and 3).

In contrast, three families account for the increase in Gsu's transport systems transporting amino acids and their conjugates (Table 3). These include the Amino Acid-Polyamine-Organocation (APC) Family (TC# 2.A.3), Amino/Acid Auxin Permease (AAAP) Family (TC# 2.A.18), and the Mitochondrial Carrier (MC) Family (TC# 2.A.29). Members of the large APC family display a common 5+5 or 7+7 TMS topology (Vastermark et al. 2014). Gsu has five of its amino acid transporters, while Ccr has one (Table 3). Members of the AAAP family transport only neutral amino acids (Fischer et al. 2002). Gsu has 14 of such symporters, while Ccr has none (Table 3). Some members of the MC family are able to import *S*-adenosylmethionine (SAM) to the mitochondrial and plastidial environments, while concurrently export *S*-adenosylhomocysteine (SAHC) from these same organelles (Haferkamp and Schmitz-Esser 2012). Ccr only has three of these SAM/SACH antiporters, compared to 10 exhibited by Gsu (Table 3).

Ccr has the capability to transport peptide conjugates, while Gsu does not (Table 2). Members of the Dispatched (TC# 2.A.6.9) Family of the Resistance-Nodulation-Cell Division (RND) Superfamily, found in Ccr, have been implicated in cholesterol-modified peptide transport (Ma et al. 2002).

There are similar numbers of protein subunits devoted to the pool of 3.A primary active transporters in Ccr and Gsu (Table 1), and the subsequent numbers of transport systems are similar as well (Table 2). Similarities can be seen for transport systems specific for cations, sugars, proteins, lipids and drugs. Differences are also observed for those that transport inorganic anions, which are exclusive to Gsu, and carboxylates and unknowns, which are exclusive to Ccr.

There are three primary active transporter families that contribute to Gsu's anion transporting capability. The first, the Sulfate/Tungstate Uptake Transporter (SuIT) Family (TC# 3.A.1.6), is a member of the ATP-binding Cassette (ABC) Superfamily, and is usually expressed in bacteria and plastids (Aguilar-Barajas et al. 2011). The second, the Nitrate/Nitrite/Cyanate Uptake Transporter (NitT) Family (TC# 3.A.1.16), allows cyanobacteria and plastids to assimilate bicarbonate, nitrate, nitrite, and cyanate (Maeda and Omata 2009). Gsu has four and one protein from each of the aforementioned ABC families, respectively (Table 3). The third family catalyzing anion transport is the Arsenite-Antimonate (ArsAB) Efflux Family (TC# 3.A.4; Yang et al. 2012). While Gsu has two such proteins, Ccr has none (Table 3). An interesting feature of *G. sulphuraria*'s

ArsAB proteins is that they were reportedly obtained laterally from the thermo-acidophilic bacterium *Leptospirillum ferriphilum* (Schonknecht et al. 2013).

All class 5 transmembrane electron carriers contribute to the total pool of systems that catalyze electron transport. It should be noted that there seems to be many more transmembrane electron carriers systems in Ccr than in Gsu (9 versus 2 respectively). The differences are mostly associated with three protein families, the Disulfide Bond Oxidoreductase D (DsbD) Family (TC# 5.A.1), the $gp91^{phox}$ Phagocyte NADPH Oxidase-associated $Cytochrome_{b558}$ (Phox) Family (TC# 5.B.1), and the Eukaryotic Cytochrome b_{651} ($Cytb_{561}$) Family (TC# 5.B.2).

Some members of the DsbD family catalyze electron transfer from cytoplasmic thioredoxins to periplasmic thioredoxins in bacteria, but other functions are known (see TCDB; Appia-Ayme and Berks 2002). Gsu lacks DsbD members (Table 3). Phox family members are respiratory burst oxidases that transfer single electrons from NADPH to O_2, thus generating superoxides for development, protection, and signaling (Cheng et al. 2001, Cheng et al. 2013). Whereas Ccr has four Phox homologues, Gsu only has one (Table 3). Lastly, $Cytb_{561}$ proteins are ferric chelate reductases that transfer single electrons from L-ascorbate to Fe^{+} for a variety of purposes (Asard et al. 2013). Plant homologues usually have an N-terminal dopamine-β-hydroxylase regulatory region (DOMON) for heme or sugar-binding (Luthje et al. 2013), and two out of the three Ccr $Cytb_{561}$ proteins have this feature. The genome of Gsu lacks members of this family (Table 3).

Class 8 auxiliary proteins found in Gsu, but not Ccr, include the Stomatin/Podocin/Band 7/Nephrosis.2/SPFH (Stomatin) Family (TC# 8.A.21) and the Tetraspanin Family (TC# 8.A.40). Stomatin homologues are capable of modulating the Mechanical Nociceptor (Piezo) Family (TC# 1.A.75) ion channels through unknown mechanisms (Poole et al. 2014). Gsu has at least three stomatin proteins. Tetraspanin family members, represented only in Gsu, create physical cell interactions and interact with each other to bind ligands and move intracellular contents (Wang et al. 2012).

Within TC subclass 9.A, Ccr and Gsu have six and 13 proteins, respectively, potentially mediating peptide transport, as well as one and eight, respectively, for lipids. Within subclass 9.B, Integral Membrane CAAX Protease-2 Family (TC# 9.B.2) members cleave certain C-terminal protein sequences in a pathway involved with substrate trafficking (Pryor et al. 2013). Ccr does not have any CAAX Protease 2 proteins, although Gsu has five (Table 3). The ability of these proteases to transport the peptide products of hydrolysis has been proposed but not established (Pryor et al. 2013).

Four families may flip lipids in Gsu, but these families are absent from Ccr (Table 2). They include the G-protein-coupled receptors (GPCR; TC# 9.A.14), which include members that can flip lipids (Menon et al. 2011, Sanyal and Menon 2009), the Outer Membrane Mitochondrial Cholesterol/Porphyrin Uptake Translocator Protein (TSPO) Family (TC# 9.A.24; Batarseh and Papadopoulos 2010), the Ca^{2+}-dependent Phospholipid Scramblase Family (TC# 9.A.306; Posada et al. 2014), and the VAMP-

associated protein (VAP) Family (TC# 9.B.17) proteins in the endoplasmic reticulum that function in vesicle biogenesis, exocytosis, and protein stabilization (Lev et al. 2008).

Figure 3 displays the percentages of transporter substrate categories in the two algae studied. A look at the major substrate groups shows that nearly half in Ccr are involved with inorganic molecules transport, while only a third are dedicated to the same substrates in Gsu (45.4% for Ccr versus 34.0% for Gsu; Figure 3). Gsu devotes larger percentages to the handling of carbon sources and amino acids and their derivatives than Ccr. Similar percentages can be seen in both algae towards transport systems that handle macromolecules, drugs, vitamins, and cofactors. Overall, it seems that Gsu is more concerned with organic substrate transport, while Ccr prioritizes inorganic molecular transport systems. It can be seen that the percentage devoted to the transport of sugars and amino acids is double for Gsu compared to Ccr. For other categories, the percentages do not differ appreciably.

Figure 4 summarizes the distribution of transporter families encoded within the genomes of Ccr and Gsu and organizes them according to occurrence in the two organisms. In all, there are 170 different transporter families represented, 20 of which are specific to Ccr and 40 of which are specific to Gsu. Some of these families have been previously described in this section. What is interesting to see here is that about 65%, or 110 out of the 170 transporter families are shared between Ccr and Gsu. Gsu has about 88% of the represented families, while Ccr has 76%.

Channel proteins in Ccr and Gsu

Table 3 presents the detailed information about multispanning transport proteins found in the two algal species analyzed. Several observations are worthy of comments and may have physiological significance. Ccr has seven paralogues of the Voltage-gated Ion Channel (VIC) Family (TC# 1.A.1), while Gsu has only one. The fact that the different paralogues in Ccr show greater sequence similarity to different TC entries suggests that these proteins did not merely arise by gene duplication events following the divergence of Ccr from Gsu. They probably serve dissimilar functions. We suggest that in the multicellular organism (Ccr), these channel proteins serve signaling functions analogous to those that are well documented in multicellular plants and animals. Such a signaling function may not be necessary in a single-celled organism such as Gsu.

The MIP family of aquaporins and glycerol facilitators has three representatives in Ccr and five in Gsu. However, the paralogues in these two organisms hit different TC entries. Thus, in Ccr, the three paralogues hit proteins in three different subfamilies of the MIP family. While, in Gsu, all five paralogues are most similar to a single member of this family. All of these paralogues show the greatest sequence similarity to the *E. coli* glycerol facilitator, which, in addition to polyols, is known to be capable of exporting arsenite and antimonite due to the negative charges on these anionic species (Meng et al. 2004). By contrast, the three MIP family members in Ccr have different ranges of specificity. For example, two dissimilar homologues respectively transport water, urea, glycerol, CO_2, NH_3, H_2O_2 and formamide (Loque et al. 2005, Bienert et al. 2007, Gattolin

et al. 2011, Saparov et al. 2007, Soria et al. 2010) and most of these compounds are not known to be transported by the *E. coli* glycerol facilitator.

Several channel protein families are represented only in Ccr or Gsu. For example, the magnesium transporters of the MgtE Family (TC# 1.A.26), and two anion-selective channel families (TC# 1.A.46 and 1.A.47) are present only in Gsu, while intracellular chloride channels of the CLIC Family (TC# 1.A.12) and formate-nitrite transporters (FNT; TC# 1.A.16) are present only in Ccr. Mechanosensitive ion channel proteins, involved in osmoregulation, are represented in both organisms, but Ccr has one member of the MscL Family (TC# 1.A.22) and one member of the MscS Family (TC# 1.A.23), while Gsu has two members of the MscS Family (Pivetti et al. 2003).

Gsu has eight members of the CorA Metal Ion Transporter (MIT) Family (TC# 1.A.35), while Ccr has only one. Five of the Gsu paralogues hit different eukaryotic CorA entries, but three show greatest sequence similarity to a single CorA entry in TCDB of the bacterium *Thermotoga maritima*. *Thermotoga maritima* CorA utilizes both Mg^{2+} and Co^{2+} as substrates, and plays a role in both Co^{2+} and Mg^{2+} homeostasis (Nordin et al. 2013). As eukaryotic paralogues can catalyze high affinity Mg^{2+} uptake and efflux (Schindl et al. 2007), Gsu may have greater versatility than Ccr over its intracellular Co^{2+} and Mg^{2+} concentrations. The only CorA protein in Ccr hits a eukaryotic member of this family. Although not suggested by (Schonknecht et al. 2013), it is possible that the Gsu paralogues were of bacterial origin and underwent intragenic duplication after transfer to this thermophilic alga.

Several beta barrel porin families were identified in the two red algae, and these include mitochondrial and plastid porins as well as the outer membrane protein insertion porin (OmpIP) constituents. Interestingly, only Ccr has a homologue of a bacterial member of the Autotransport-1 (AT-1) Family (TC# 1.B.12). However, this protein shows a low score (e^{-11}) and sequence similarity only in the serine protease domain. Although this protein appears to have an N-terminal targeting sequence, typical of AT-1 proteins, its assignment to the AT-1 Family should be considered tentative. Both organisms possess multiple constituents of the Synaptosomal Vesicle Fusion Pore (SVF-Pore) Family (TC# 1.F.1).

Secondary carriers in Ccr and Gsu

Compared to Ccr's 20 representatives of the MFS Family, Gsu has 73. Most of the increases are largely accounted for by sugar porters (SP; TC# 2.A.1.1) and phosphate:H^+ symporters (PHS; TC# 2.A.1.9). Gsu has 37 paralogues of the SP family, some hitting prokaryotic homologues with highest scores, while others hit eukaryotic homologues with highest scores in TCDB. Ccr only has one such homologue which hits a eukaryotic facilitated glucose transporter. Members of the SP family in TCDB that Gsu proteins show greatest similarity to are H^+ symporters, which can also transport arabinose, scyllo-, muco-, chiro-, and myoinositols, inositoltriphosphate and fructose (Hernandez-Montalvo et al. 2001, Schneider et al. 2008, Aouameur et al. 2007). The PHS family of phosphate symporters has 15 representatives in Gsu, but none in Ccr. The 15

Gsu paralogues show great similarity to a single member of this family, GIT1 of *Saccharomyces ceresvisiae*. This transporter has been shown to transport inorganic phosphate as well as glycerophosphoinositol or glycerophosphocholine (Almaguer et al. 2006). Finally, Gsu has six members of the Glycoside-Pentoside-Hexuronide (GPH):Cation Symporter Family (TC# 2.A.2), while Ccr has four. The presence of multiple members of these MFS families corroborates previous studies which revealed that Gsu has a large and varied carbohydrate metabolic capability (Barbier et al. 2005, Weber et al. 2007), and shows that Ccr is much more limited in this capacity. Interestingly, Ccr possesses MFS porters that can take up organic acids such as oxalate and tartrate, although these systems are lacking in Gsu. These observations suggest that while Gsu predominately utilizes sugars, Ccr may prefer organic acids. This suggestion is further substantiated by the observation that the mechanistically ill-defined Sweet Family (TC# 9.A.58) of sugar transporters is represented in Gsu, but not Ccr.

Gsu has seven members of the Amino Acid-Polyamine-Organocation (APC) Family (TC# 2.A.3), while Ccr has only one. Three of the Gsu paralogues hit eukaryotic APC entries, but four show greatest sequence similarity to a single APC homologue of the archaeon, *Thermoplasma acidophilum*. Two of the eukaryotic paralogues show maximal sequence similarity to APC homologue RMV1 of *Arabidopsis thaliana*, which uptakes polyamines and paraquat (Fujita and Shinozaki 2014). The APC homologue in *Thermoplasma acidophilum* has not been characterized, but shows similarity to a low-affinity putrescine importer of *E. coli*. While the role of APC family members in Gsu is unclear, it can be suggested that they mainly mediate uptake of polyamines. This

contrasts with the Ccr APC homologue which shows greatest similarity to a vacuolar cationic amino acid transporter of *Arabidopsis thaliana*.

Related to the APC Family, are the amino acid/auxin uptake permeases of the AAAP Family (TC# 2.A.18). 14 members of this family are represented in Gsu, although none are found in Ccr. 11 of the 14 paralogues in Gsu hit the same TC entry, which suggests intragenic duplication during evolution of Gsu. All 14 of these Gsu paralogues probably transport neutral amino acids. Since Gsu has far more APC family members, the combined results suggest that in addition to sugars, Gsu, in contrast to Ccr, uses polyamines and amino acids as primary nutrients.

Divalent cations, particular Zn^{2+} and Fe^{2+}, are taken up via ZIP Family (TC# 2.A.5) members, while these same cations as well as other divalent cations are exported via members of the CDF Family (TC# 2.A.4). Both organisms have three paralogues of the ZIP Family, but while Ccr has four members of the CDF Family, Gsu has only one. By contrast, the NRAMP Family of divalent metal ion transporters (TC# 2.A.55) has four members in Gsu, but only two in Ccr. We presume that the members of the NRAMP and ZIP families can substitute for each other. We identified only one homologue of a cholesterol-modified peptide exporter of the RND Family in Ccr, but no RND family member was found in Gsu.

The Drug/Metabolite Transporter (DMT) Superfamily (TC# 2.A.7) is well represented in both algae with about 18 members in each organism. It is interesting that

Gsu, in contrast to Ccr, has sugar phosphate uptake porters since only the former organism transports sugars as a principle carbon source. However, most members of this family in both algae transport activated sugars, exporting them from the cytoplasm to the lumen of the endoplasmic reticulum or Golgi apparatus. Many such activated sugars are known including UDP-glucose, UDP-galactose, UDP-N-acetylglucosamine, UDP-glucuronic acid, UDP-xylose, GDP-mannose, and GDP-fucose. DMT porters also take up vitamins such as thiamine pyrophosphate and divalent cations such as Mg^{2+}. All of these types are represented in both organisms.

Ccr has two proteins of the Cytochrome Oxidase Biogenesis (Oxa1) Family (TC# 2.A.9), while Gsu has six. One of the Ccr proteins hits a mitochondrial Oxa1 protein, and the other a plastidial homologue. In contrast, Gsu has five that hits the same mitochondrial Oxa1 protein corresponding to the Ccr protein, and one that also hits the same plastidial homologue.

The next large family of transporters found in both algae is the Mitochondrial Carrier (MC) Family (TC# 2.A.29). Ccr and Gsu have 19 and 37 members of this family, respectively. Thus, Gsu has twice as many as Ccr. These numbers reflect the greater numbers of ATP/ADP exchangers, Fe^{2+} transporters, vitamin transporters, and S-adenosylmethionine/S-adenosylhomocysteine exchangers in Gsu compared to Ccr. In this regard, it is interesting to note that Gsu also has a member of the ATP:ADP antiporters (AAA) Famly (TC# 1.A.12), which is lacking in Ccr. These porters are not related to the mitochondrial carriers and instead are distant members of the MFS.

Continuing the analysis of Table 3, we see that many of the families are represented in similar numbers in both organisms. However, a few families were represented exclusively in Ccr, while a much larger number of families were exclusively represented in Gsu. For example, the equilibrative nucleoside transporter (ENT) Family (TC# 2.A.57) and the Folate-Biopterin Transporter (FBT) Family (TC# 2.A.71) each has two members in Ccr, but none in Gsu. By contrast, the following families are represented in Gsu, but not Ccr: the Sulfate Permease (SulP) Family (TC# 2.A.53), two members; the Auxin Efflux Carrier (AEC) Family (TC# 2.A.69), one member; the K^+ Uptake Permease (KUP) Family (TC# 2.A.72), two members; the Organic Solute Transporter (OST) Family (TC# 2.A.82), one member; the Aromatic Acid Exporter (ArAE) Family (2.A.85), 11 members; the Autoinducer-2 Exporter (AI-2E) Family (TC# 2.A.86), one member; the Vacuolar Iron Transporter (VIT) Family (TC# 2.A.89), two members; the Acetate Uptake Transporter (AceTr) Family (TC# 2.A.96), 10 members; the Ferroportin (Fpn) Family (TC# 2.A.100), one member; the Mitochondrial Pyruvate Carrier (MPC) Family (TC# 2.A.105), one member; and the Tellurium Ion Resistance (TerC) Family (TC# 2.A.122), one member. It should be noted that all homologues of Gsu's AceTr Family were probably horizontally transferred from the bacterium *Desulfotomaculum acetoxidans* (Schonknecht et al. 2013). The prevalence of aromatic acid uptake porters in Gsu, compared to Ccr, correlates with the greater prevalence of amino acid transporters belonging to other families as noted above. Thus, this result substantiates the conclusion that Gsu uses amino acids as nutrients to a much greater extent than Ccr. The presence of 10 acetate porters in Gsu, lacking altogether in Ccr, is of unknown significance, but it is

possible that these porters function in acetate excretion as an end product of metabolism in addition to their role in acetate uptake. The significance of the occurrence of transporters of diverse function in Gsu, but not Ccr, has yet to be established. However, the occurrence of much greater diversity in Gsu could be due to the fact that this organism is unicellular, while Ccr is a multicellular organism. Single-celled microorganisms may have a much greater need to scavenge a diversity of compounds found in nature, while multicellular organisms create their own homeostatic environment with a limited number of nutrients serving as the primary sources of energy and metabolic intermediates (Li et al. 2014).

Primary Active Transporters in Ccr and Gsu

As presented in Table 3, both algae have similar number of integral membrane proteins identified as probable constituents of ABC transport systems (TC# 3.A.1; 32 Ccr versus 35 Gsu). Only seven proteins thought to be involved in solute uptake were identified, three in Ccr and four in Gsu, all probably occurring in chloroplasts. The three proteins in Ccr are possibly involved in the uptake of 1) taurine and aromatic sulfonates, 2) phthalates, and 3) lipids. However, it should be noted that the scores obtained for the first two of these, when the Ccr proteins were BLASTed against TCDB, were poor (e^{-10} and e^{-6}, respectively), although a good score was obtained with the last substrate (e^{-45}). In the case of Gsu, the four proteins may be specific for 1) sulfate and 2) inorganic anions such as bicarbonate, cyanate, nitrite, and nitrate. While the score for the first three of these proteins were excellent, suggesting that this is truly a sulfate uptake protein, the

score for the latter is poor (e^{-10}), making this substrate classification tenuous. All remaining ABC-type integral membrane proteins identified in these algae are almost certainly involved in solute or macromolecular efflux with confidence levels inversely proportional to the score (e-value). Only five of these proteins resemble bacterial transport proteins more than eukaryotic proteins, and two are present in Ccr while three are present in Gsu. The first three, one in Ccr and two in Gsu, are specific for a variety of drugs although one of the two found in Gsu may also be capable of exporting lipids. Both algae show a top hit with an iron export protein, ybbM, from *E. coli*, which functions to enhance resistance to oxidative stress (Nicolaou et al. 2013). Because the algal proteins hit the *E. coli* protein with good scores, it is probable that they function in divalent metal ion export. The cellular location of these proteins is unknown, but they appear to lack mitochondrial targeting sequences. While both proteins exhibit 6 TMSs, only the Gsu homologue has an N-terminal ATPase domain of the CbiO family. It is interesting to note that this family includes homologues from plants, one of which has been reported to be a UDP-glucose exporter (Huang et al. 2009).

All remaining ABC transporters in these algae are most similar to eukaryotic type ABC export proteins. As summarized in Table 3, the exporters that they hit in TCDB are specific for 1) multiple drugs and hydrophobic/amphipathic substances, 2) divalent cations, and 3) lipids, sterols, and hydrophobic vitamin derivatives. Most of these systems are of broad specificity with a capacity to transport many substrates. The functions in red algae are likely to similar.

Both organisms possess subunits of both F-type and V-type ATPases (TC# 3.A.2) involved in 1) energy interconversions in mitochondria and chloroplasts, and 2) intracellular vesicle acidification, respectively. However, in addition, these two organisms both contain H^+ or Na^+-translocating pyrophosphophatases of the M^+-PPase Family (TC# 3.A.10). While Ccr appears to have only one such system, catalyzing Na^+ efflux, Gsu has three such systems, all of which probably export H^+. This observation correlates with the fact that Ccr is multicellular, maintaining intercellular homeostasis, while Gsu is unicellular.

P-type ATPases generally function in the uptake or efflux of monovalent and divalent cations although one family (TC# 3.A.3.8) consists of phospholipid flippases. Two calcium ATPases (TC# 3.A.3.2) are found in each of the two algae with exceptionally good scores to those in TCDB. These function to maintain low cytoplasmic calcium concentrations in all eukaryotes and many prokaryotes. Of particular interest is the fact that while the multicellular Ccr has a homologue of mammalian Na^+/K^+-ATPases (TC# 3.A.3.1), but lacks a H^+-ATPase of the plant/fungal type (TC# 3.A.3.3), the opposite is true of Gsu. This fact correlates with our observation that only Ccr possesses multiple members of the Voltage-gated Ion Channel (VIC) Family. This confirms our suggestion that these proteins in Ccr function in signal transduction possibly by generating action potentials. In this capacity, a Na^+/K^+-ATPase would be expected to maintain low cytoplasmic concentrations of Na^+, but high concentrations of K^+. Ccr has three members of the copper (Cu^+) ATPase family (TC# 3.A.3.5) while Gsu has only two, but Gsu also has a multiple heavy metal cation exporter which is absent in Ccr.

These ATPases are thought to function in protection against heavy metal toxicity in most organisms. Finally, both organisms contain a single Mn^{2+} efflux system, likely to be present in the endoplasmic reticulum (ER). Surprisingly, while the Ccr protein is a single polypeptide chain, the Gsu system apparently consists of two polypeptide chains encoded by two different genes. One of these proteins corresponds to the N-terminal half of the Ccr protein, while the other codes for the C-terminal half. Loss of the corresponding protein in yeast results in ER stress and lowered Mn^{2+} in the ER lumen (Cohen et al. 2013).

Gsu has two arsenite/antimonite efflux (ArsB) proteins that are lacking in Ccr. These proteins function in protection against these two oxyanions by catalyzing their efflux (Yang et al. 2012).

TC class 3 includes several protein secretion systems, components of which were identified in both algae. These include the General Secretory Pathway (Sec) Family (TC# 3.A.5), the Mitochondrial Protein Translocase (MPT) Family (TC# 3.A.8), the Chloroplast Envelope Translocase (CEPT or Tic-Toc) Family (TC# 3.A.9), the Endoplasmic Reticular Retrotranslocon (ER-RT) Family (TC# 3.A.16), and the Peroxisomal Protein Importer (PPI) Family (TC# 3.A.20). Because all constituents of these systems were not always identified in the two organisms, we can not claim that all of these systems are complete or functional.

Constituents of all three of the mitochondrial H^+-translocating electron transfer complexes were identified in Gsu and Ccr, although surprisingly, only Ccr contains the H^+-translocating transhydrogenase. However, when our GBLAST searches were first run in January 2014 with the version of the Gsu proteome then available, many proteins expected to be present were not found. Subsequently, in September of the same year, many of these proteins could be identified, and a complete search was repeated to add the missing proteins to Table 3. Constituents of the light absorption-driven photosynthetic reaction center (PRC) Family (TC# 3.E.2) were also identified in both organisms.

DISCUSSION

Galdieria sulphuraria (Gsu) is a thermo-acidophilic single-celled red alga that resides in acidic hot springs frequently containing high concentrations of toxic metals. The environment in which Gsu resides can reach temperatures as high as 55 °C, have pH values as low as 1, and often involve exposure to high steam pressures (Heilmann et al. 1999). In addition to the extreme conditions under which Gsu can survive, this organism has developed the ability to grow photoautotrophically, mixotrophically, and heterotrophically on over 50 carbon sources (Barbier et al. 2005, Schonknecht et al. 2013). In contrast, *Chondrus crispus* (Ccr) is an intertidal, multicellular red alga that only grows autotrophically, through photosynthesis, along the rocky shores of the Atlantic Ocean (Collen et al. 2013, Smith and Bidwell 1989). The differences between the two rhodophytes can best be seen by contrasting Gsu's large and diverse metabolism to the much more restrictive metabolism of Ccr's.

Although Gsu is a facultative heterotroph with great metabolic flexibility, most of its red algal relatives are obligate photoautotrophs (Barbier et al. 2005). It has been reported that more than 30% of Gsu's gene sequences do not relate to those of its closest characterized relative, *Cyanidioschyzon merolae*, and many of these genes include a large number of membrane transporters and enzymes of carbohydrate metabolism (Barbier et al. 2005). The genome of Gsu encodes many different sugar kinases, including gluco-, galacto-, fructo-, xylulo-, ribo-, and glycerol kinases. These enzymes were reported to be essential to Gsu's ability to sense sugar in "feast and famine" responses, which can

influence relevant transporter gene expression (Oesterhelt and Gross 2002). Additionally, Gsu's sugar and polyol uptake genes were not induced in the absence of light, and Gsu only switches to a heterotrophic state when the relevant substrates are present in the external environment in sufficient concentrations (Oesterhelt and Gross 2002). Indeed, Gsu can efficiently grow photoautotrophically even in a minimal light environment (Thangaraj et al. 2011). True mixotrophic growth, which involves the transport of electrons in both photosystems I and II, does not occur in Gsu; heterotrophy is Gsu's preferred mode of growth (Oesterhelt et al. 2007).

Gsu has the ability to take up sugars from its surroundings through the use of its many sugar transporters. The most important families to which these transporters belong include the sugar porters of the Major Facilitator Superfamily (MFS), the related symporters of the GPH Family, and the mechanistically uncharacterized transporters of the Sweet Family. Members of these families allow the uptake of hexoses such as glucose and fructose, glucuronides, inositols and their derivatives. Gsu may use sugars to generate ATP via glycolysis to energize its multiple archaeal ATPases. There is a positive correlation between the expression of archaeal ATPases and the extent of Gsu's heat tolerance, although it is unclear as to why this is so (Schonknecht et al. 2013). The use of inositol derivatives has been found to modulate intracellular Ca^{2+} concentrations and establish monospore polarity of the red alga *Porphyra yezoensis* (Li et al. 2009). Since Gsu also produces endospores, inositol phosphates may have a similar function for this organism (Barbier et al. 2005).

Some organophosphate esters, such as glycerophosphoinositol and glycerophosphocholine, may be taken up by Gsu's PHS transporters for purposes of energy and carbon generation (Almaguer et al. 2006, Oesterhelt et al. 2008). Activated sugars are probably transported into the lumen of the ER and golgi for glycolipid and glycoprotein synthesis via Gsu's DMT transporters. The diversity and multiplicity of sugar transporters in Gsu contrasts with the minimal representation of these systems in Ccr, a probable reflection of the need of Gsu to obtain energy for its protective transport functions (e.g., H^+ and heavy metal ion expulsion). This could possibly correlate with Ccr's ability to catalyze photosynthesis and carbon fixation in order to create its own sugar products (Smith and Bidwell 1989).

Gsu's extensive metabolism also extends to other compounds such as polyamines and amino acids (APC and AAAP families). Polyamines are widely distributed among many algal species, and some of these compounds, such as norspermidine and norspemine, are present in large concentrations in Gsu (Hamana and Matsuzaki 1985). The role that polyamines play in Gsu is unclear, although these cationic compounds are known to associate with and stabilize nucleic acids (Nayvelt et al. 2010) and could therefore promote cell stability under extreme conditions. They have also been shown to enhance sporulation development in the red alga *Hydropuntia cornea* (Guzman-Uriostegui et al. 2012), and they could have a similar function in Gsu. In contrast to the great number of polyamine and amino acid transporters, Ccr has very few by comparison, and it may be that the multicellular Ccr has the capability of providing an intercellular homeostatic environment, thereby requiring a less diverse set of these transporters.

In terms of Gsu's high resistance to heavy metal and oxyanion toxicity (Schonknecht et al. 2013), several of the transporters that are likely to catalyze transport of these compounds include the members of the MIP Family of aquaporins and glycerol facilitators, some of which export arsenite and antimonite, and the divalent metal ion transporters of the CorA, MgtE, ZIP, NRAMP, and CDF Families. Through this last mentioned family, Gsu is able to effectively export divalent cations, thereby maintaining homeostasis of heavy metal ions such as Mg^{2+}, Co^{2+}, Zn^{2+}, and Fe^{2+}, which are normally taken up via the CorA, MgtE, ZIP and NRAMP porters. Though fewer in numbers, the metal ion transporters of Ccr are also able to control intracellular Mg^{2+}, Zn^{2+}, and Fe^{2+} concentrations, although the ability to which Ccr can maintain its homeostasis relative to Gsu is undoubtedly less extensive. The protection afforded by Gsu's many metal ion/oxyanion transporters reflects this single-celled organism's apparent ability to enhance and diversify gene expression via horizontal gene transfer followed by repeated gene duplication. This need would not be expected to be so great for Ccr, which does not grow under these extreme conditions and by virtue of its multicellularity, can maintain internal homeostasis.

A feature that Gsu and Ccr share is their tolerance of salty environments (Schonknecht et al. 2013). Ccr has 50% more monovalent cation transporters than Gsu, in spite of its lower number of total transporters, but Gsu has a Bestrophin anion channel (TC# 1.A.46) and a nucleotide-dependent anion-selective channel (ICln; TC# 1.A.47), which Ccr lacks. These observations suggest that these two organisms have achieved salt

tolerance by expression of different transporters. The fact that Ccr has seven members of the VIC Family compared to only one for Gsu suggests that these proteins may have signaling functions relevant to multicellularity, a feature lacking in Gsu. The predicted substrate of the single Ccr member of the M^+-PPase and one of the P-type ATPase is Na^+ although Gsu lacks enzymes of this specificity. This observation substantiates our suggestion that Ccr utilizes monovalent ions for signal transduction, possibly involving action potentials, whereas Gsu's corresponding family members are primarily concerned with H^+ transport, the concentration of which would be abundant in its heavily acidic (pH 0 to 4) environment (Schonknecht et al. 2013). Thus the specificities of the transporters appear to be explained both by their environmental stress needs and the requirements for maintenance of the multicellular state.

REFERENCES

Aguilar-Barajas, E., Diaz-Perez, C., Ramirez-Diaz, M. I., Riveros-Rosas, H. & Cervantes, C. 2011. Bacterial transport of sulfate, molybdate, and related oxyanions. *Biometals : an international journal on the role of metal ions in biology, biochemistry, and medicine,* **24:**687-707.

Almaguer, C., Fisher, E. & Patton-Vogt, J. 2006. Posttranscriptional regulation of Git1p, the glycerophosphoinositol/glycerophosphocholine transporter of Saccharomyces cerevisiae. *Current genetics,* **50:**367-75.

Aouameur, R., Da Cal, S., Bissonnette, P., Coady, M. J. & Lapointe, J. Y. 2007. SMIT2 mediates all myo-inositol uptake in apical membranes of rat small intestine. *American journal of physiology. Gastrointestinal and liver physiology,* **293:**G1300-7.

Appia-Ayme, C. & Berks, B. C. 2002. SoxV, an orthologue of the CcdA disulfide transporter, is involved in thiosulfate oxidation in Rhodovulum sulfidophilum and reduces the periplasmic thioredoxin SoxW. *Biochemical and biophysical research communications,* **296:**737-41.

Asard, H., Barbaro, R., Trost, P. & Berczi, A. 2013. Cytochromes b561: ascorbate-mediated trans-membrane electron transport. *Antioxidants & redox signaling,* **19:**1026-35.

Barbier, G., Oesterhelt, C., Larson, M. D., Halgren, R. G., Wilkerson, C., Garavito, R. M., Benning, C. & Weber, A. P. 2005. Comparative genomics of two closely related unicellular thermo-acidophilic red algae, Galdieria sulphuraria and Cyanidioschyzon merolae, reveals the molecular basis of the metabolic flexibility of Galdieria sulphuraria and significant differences in carbohydrate metabolism of both algae. *Plant physiology,* **137:**460-74.

Batarseh, A. & Papadopoulos, V. 2010. Regulation of translocator protein 18 kDa (TSPO) expression in health and disease states. *Molecular and cellular endocrinology,* **327:**1-12.

Bernsel, A., Viklund, H., Hennerdal, A. & Elofsson, A. 2009. TOPCONS: consensus prediction of membrane protein topology. *Nucleic acids research,* **37:**W465-8.

Bienert, G. P., Schussler, M. D. & Jahn, T. P. 2008. Metalloids: essential, beneficial or toxic? Major intrinsic proteins sort it out. *Trends in biochemical sciences,* **33:**20-6.

Bienert, G. P., Moller, A. L., Kristiansen, K. A., Schulz, A., Moller, I. M., Schjoerring, J. K. & Jahn, T. P. 2007. Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. *The Journal of biological chemistry,* **282:**1183-92.

Canganella, F. & Wiegel, J. 2011. Extremophiles: from abyssal to terrestrial ecosystems and possibly beyond. *Die Naturwissenschaften,* **98:**253-79.

Cheng, C., Xu, X., Gao, M., Li, J., Guo, C., Song, J. & Wang, X. 2013. Genome-wide analysis of respiratory burst oxidase homologs in grape (Vitis vinifera L.). *International journal of molecular sciences,* **14:**24169-86.

Cheng, G., Cao, Z., Xu, X., van Meir, E. G. & Lambeth, J. D. 2001. Homologs of gp91phox: cloning and tissue expression of Nox3, Nox4, and Nox5. *Gene,* **269:**131-40.

Ciniglia, C., Yoon, H. S., Pollio, A., Pinto, G. & Bhattacharya, D. 2004. Hidden biodiversity of the extremophilic Cyanidiales red algae. *Molecular ecology,* **13:**1827-38.

Cohen, Y., Megyeri, M., Chen, O. C., Condomitti, G., Riezman, I., Loizides-Mangold, U., Abdul-Sada, A., Rimon, N., Riezman, H., Platt, F. M., Futerman, A. H. & Schuldiner, M. 2013. The yeast p5 type ATPase, spf1, regulates manganese transport into the endoplasmic reticulum. *PloS one,* **8:**e85519.

Collen, J., Porcel, B., Carre, W., Ball, S. G., Chaparro, C., Tonon, T., Barbeyron, T., Michel, G., Noel, B., Valentin, K., Elias, M., Artiguenave, F., Arun, A., Aury, J. M., Barbosa-Neto, J. F., Bothwell, J. H., Bouget, F. Y., Brillet, L., Cabello-Hurtado, F., Capella-Gutierrez, S., Charrier, B., Cladiere, L., Cock, J. M., Coelho, S. M., Colleoni, C., Czjzek, M., Da Silva, C., Delage, L., Denoeud, F., Deschamps, P., Dittami, S. M., Gabaldon, T., Gachon, C. M., Groisillier, A., Herve, C., Jabbari, K., Katinka, M., Kloareg, B., Kowalczyk, N., Labadie, K., Leblanc, C., Lopez, P. J., McLachlan, D. H., Meslet-Cladiere, L., Moustafa, A., Nehr, Z., Nyvall Collen, P., Panaud, O., Partensky, F., Poulain, J., Rensing, S. A., Rousvoal, S., Samson, G., Symeonidi, A., Weissenbach, J., Zambounis, A., Wincker, P. & Boyen, C. 2013. Genome structure and metabolic features in the red seaweed Chondrus crispus shed light on evolution of the Archaeplastida. *Proceedings of the National Academy of Sciences of the United States of America,* **110:**5247-52.

Enkavi, G., Li, J., Mahinthichaichan, P., Wen, P. C., Huang, Z., Shaikh, S. A. & Tajkhorshid, E. 2013. Simulation studies of the mechanism of membrane transporters. *Methods in molecular biology,* **924:**361-405.

Fischer, W. N., Loo, D. D., Koch, W., Ludewig, U., Boorer, K. J., Tegeder, M., Rentsch, D., Wright, E. M. & Frommer, W. B. 2002. Low and high affinity amino acid H+-

cotransporters for cellular import of neutral and charged amino acids. *The Plant journal : for cell and molecular biology,* **29:**717-31.

Fuhrmans, M., Marelli, G., Smirnova, Y. G. & Muller, M. 2014. Mechanics of membrane fusion/pore formation. *Chemistry and physics of lipids*.

Fujita, M. & Shinozaki, K. 2014. Identification of polyamine transporters in plants: paraquat transport provides crucial clues. *Plant & cell physiology,* **55:**855-61.

Gattolin, S., Sorieul, M. & Frigerio, L. 2011. Mapping of tonoplast intrinsic proteins in maturing and germinating Arabidopsis seeds reveals dual localization of embryonic TIPs to the tonoplast and plasma membrane. *Molecular plant,* **4:**180-9.

Getsin, I., Nalbandian, G. H., Yee, D. C., Vastermark, A., Paparoditis, P. C., Reddy, V. S. & Saier, M. H., Jr. 2013. Comparative genomics of transport proteins in developmental bacteria: Myxococcus xanthus and Streptomyces coelicolor. *BMC microbiology,* **13:**279.

Guzman-Uriostegui, A., Robaina, R., Freile-Pelegrin, Y. & Robledo, D. 2012. Polyamines increase carpospore output and growth during in vitro cultivation of Hydropuntia cornea. *Biotechnology letters,* **34:**755-61.

Haferkamp, I. & Schmitz-Esser, S. 2012. The plant mitochondrial carrier family: functional and evolutionary aspects. *Frontiers in plant science,* **3:**2.

Hamana, K. & Matsuzaki, S. 1985. Further study on polyamines in primitive unicellular eukaryotic algae. *Journal of biochemistry,* **97:**1311-5.

Heilmann, I. I., Perera, I. Y., Gross, W. & Boss, W. F. 1999. Changes in phosphoinositide metabolism with days in culture affect signal transduction pathways in galdieria sulphuraria. *Plant physiology,* **119:**1331-40.

Hernandez-Montalvo, V., Valle, F., Bolivar, F. & Gosset, G. 2001. Characterization of sugar mixtures utilization by an Escherichia coli mutant devoid of the phosphotransferase system. *Applied microbiology and biotechnology,* **57:**186-91.

Huang, C. F., Yamaji, N., Mitani, N., Yano, M., Nagamura, Y. & Ma, J. F. 2009. A bacterial-type ABC transporter is involved in aluminum tolerance in rice. *The Plant cell,* **21:**655-67.

Lam, V. H., Lee, J. H., Silverio, A., Chan, H., Gomolplitinant, K. M., Povolotsky, T. L., Orlova, E., Sun, E. I., Welliver, C. H. & Saier, M. H., Jr. 2011. Pathways of transport protein evolution: recent advances. *Biological chemistry,* **392:**5-12.

Lev, S., Ben Halevy, D., Peretti, D. & Dahan, N. 2008. The VAP protein family: from cellular functions to motor neuron disease. *Trends in cell biology,* **18:**282-90.

Li, L., Saga, N. & Mikami, K. 2009. Ca2+ influx and phosphoinositide signalling are essential for the establishment and maintenance of cell polarity in monospores from the red alga Porphyra yezoensis. *Journal of experimental botany,* **60:**3477-89.

Li, Z. Y., Wang, Y. Z., He, L. M. & Zheng, H. J. 2014. Metabolic profiles of prokaryotic and eukaryotic communities in deep-sea sponge Lamellomorpha sp. indicated by metagenomics. *Scientific reports,* **4:**3895.

Loque, D., Ludewig, U., Yuan, L. & von Wiren, N. 2005. Tonoplast intrinsic proteins AtTIP2;1 and AtTIP2;3 facilitate NH3 transport into the vacuole. *Plant physiology,* **137:**671-80.

Luthje, S., Moller, B., Perrineau, F. C. & Woltje, K. 2013. Plasma membrane electron pathways and oxidative stress. *Antioxidants & redox signaling,* **18:**2163-83.

Ma, Y., Erkner, A., Gong, R., Yao, S., Taipale, J., Basler, K. & Beachy, P. A. 2002. Hedgehog-mediated patterning of the mammalian embryo requires transporter-like function of dispatched. *Cell,* **111:**63-75.

Maeda, S. & Omata, T. 2009. Nitrite transport activity of the ABC-type cyanate transporter of the cyanobacterium Synechococcus elongatus. *Journal of bacteriology,* **191:**3265-72.

Meng, Y. L., Liu, Z. & Rosen, B. P. 2004. As(III) and Sb(III) uptake by GlpF and efflux by ArsB in Escherichia coli. *The Journal of biological chemistry,* **279:**18334-41.

Menon, I., Huber, T., Sanyal, S., Banerjee, S., Barre, P., Canis, S., Warren, J. D., Hwa, J., Sakmar, T. P. & Menon, A. K. 2011. Opsin is a phospholipid flippase. *Current biology : CB,* **21:**149-53.

Nayvelt, I., Hyvonen, M. T., Alhonen, L., Pandya, I., Thomas, T., Khomutov, A. R., Vepsalainen, J., Patel, R., Keinanen, T. A. & Thomas, T. J. 2010. DNA condensation by chiral alpha-methylated polyamine analogues and protection of cellular DNA from oxidative damage. *Biomacromolecules,* **11:**97-105.

Nicolaou, S. A., Fast, A. G., Nakamaru-Ogiso, E. & Papoutsakis, E. T. 2013. Overexpression of fetA (ybbL) and fetB (ybbM), Encoding an Iron Exporter, Enhances Resistance to Oxidative Stress in Escherichia coli. *Applied and environmental microbiology,* **79:**7210-9.

Nordin, N., Guskov, A., Phua, T., Sahaf, N., Xia, Y., Lu, S., Eshaghi, H. & Eshaghi, S. 2013. Exploring the structure and function of Thermotoga maritima CorA reveals

the mechanism of gating and ion selectivity in Co2+/Mg2+ transport. *The Biochemical journal,* **451:**365-74.

Oesterhelt, C. & Gross, W. 2002. Different sugar kinases are involved in the sugar sensing of Galdieria sulphuraria. *Plant physiology,* **128:**291-9.

Oesterhelt, C., Schmalzlin, E., Schmitt, J. M. & Lokstein, H. 2007. Regulation of photosynthesis in the unicellular acidophilic red alga Galdieria sulphuraria. *The Plant journal : for cell and molecular biology,* **51:**500-11.

Oesterhelt, C., Vogelbein, S., Shrestha, R. P., Stanke, M. & Weber, A. P. 2008. The genome of the thermoacidophilic red microalga Galdieria sulphuraria encodes a small family of secreted class III peroxidases that might be involved in cell wall modification. *Planta,* **227:**353-62.

Pivetti, C. D., Yen, M. R., Miller, S., Busch, W., Tseng, Y. H., Booth, I. R. & Saier, M. H., Jr. 2003. Two families of mechanosensitive channel proteins. *Microbiology and molecular biology reviews : MMBR,* **67:**66-85, table of contents.

Poole, K., Herget, R., Lapatsina, L., Ngo, H. D. & Lewin, G. R. 2014. Tuning Piezo ion channels to detect molecular-scale movements relevant for fine touch. *Nature communications,* **5:**3520.

Posada, I. M., Busto, J. V., Goni, F. M. & Alonso, A. 2014. Membrane binding and insertion of the predicted transmembrane domain of human scramblase 1. *Biochimica et biophysica acta,* **1838:**388-97.

Pryor, E. E., Jr., Horanyi, P. S., Clark, K. M., Fedoriw, N., Connelly, S. M., Koszelak-Rosenblum, M., Zhu, G., Malkowski, M. G., Wiener, M. C. & Dumont, M. E. 2013. Structure of the integral membrane protein CAAX protease Ste24p. *Science,* **339:**1600-4.

Reddy, A., Cho, J., Ling, S., Reddy, V., Shlykov, M. & Saier, M. H. 2014. Reliability of nine programs of topological predictions and their application to integral membrane channel and carrier proteins. *Journal of molecular microbiology and biotechnology,* **24:**161-90.

Reddy, V. S. & Saier, M. H., Jr. 2012. BioV Suite--a collection of programs for the study of transport protein evolution. *The FEBS journal,* **279:**2036-46.

Rothschild, L. J. & Mancinelli, R. L. 2001. Life in extreme environments. *Nature,* **409:**1092-101.

Saier, M. H., Jr., Reddy, V. S., Tamang, D. G. & Vastermark, A. 2014. The transporter classification database. *Nucleic acids research,* **42:**D251-8.

Sanyal, S. & Menon, A. K. 2009. Flipping lipids: why an' what's the reason for? *ACS chemical biology,* **4:**895-909.

Saparov, S. M., Liu, K., Agre, P. & Pohl, P. 2007. Fast and selective ammonia transport by aquaporin-8. *The Journal of biological chemistry,* **282:**5296-301.

Schindl, R., Weghuber, J., Romanin, C. & Schweyen, R. J. 2007. Mrs2p forms a high conductance Mg2+ selective channel in mitochondria. *Biophysical journal,* **93:**3872-83.

Schneider, H., Staudacher, S., Poppelreuther, M., Stremmel, W., Ehehalt, R. & Fullekrug, J. 2014. Protein mediated fatty acid uptake: synergy between CD36/FAT-facilitated transport and acyl-CoA synthetase-driven metabolism. *Archives of biochemistry and biophysics,* **546:**8-18.

Schneider, S., Beyhl, D., Hedrich, R. & Sauer, N. 2008. Functional and physiological characterization of Arabidopsis INOSITOL TRANSPORTER1, a novel tonoplast-localized transporter for myo-inositol. *The Plant cell,* **20:**1073-87.

Schonknecht, G., Weber, A. P. & Lercher, M. J. 2014. Horizontal gene acquisitions by eukaryotes as drivers of adaptive evolution. *BioEssays : news and reviews in molecular, cellular and developmental biology,* **36:**9-20.

Schonknecht, G., Chen, W. H., Ternes, C. M., Barbier, G. G., Shrestha, R. P., Stanke, M., Brautigam, A., Baker, B. J., Banfield, J. F., Garavito, R. M., Carr, K., Wilkerson, C., Rensing, S. A., Gagneul, D., Dickenson, N. E., Oesterhelt, C., Lercher, M. J. & Weber, A. P. 2013. Gene transfer from bacteria and archaea facilitated evolution of an extremophilic eukaryote. *Science,* **339:**1207-10.

Shi, Y. 2013. Common folds and transport mechanisms of secondary active transporters. *Annual review of biophysics,* **42:**51-72.

Smith, R. G. & Bidwell, R. G. 1989. Mechanism of Photosynthetic Carbon Dioxide Uptake by the Red Macroalga, Chondrus crispus. *Plant physiology,* **89:**93-9.

Soria, L. R., Fanelli, E., Altamura, N., Svelto, M., Marinelli, R. A. & Calamita, G. 2010. Aquaporin-8-facilitated mitochondrial ammonia transport. *Biochemical and biophysical research communications,* **393:**217-21.

Spencer, R. H. & Rees, D. C. 2002. The alpha-helix and the organization and gating of channels. *Annual review of biophysics and biomolecular structure,* **31:**207-33.

Su, H. N., Xie, B. B., Zhang, X. Y., Zhou, B. C. & Zhang, Y. Z. 2010. The supramolecular architecture, function, and regulation of thylakoid membranes in red algae: an overview. *Photosynthesis research,* **106:**73-87.

Thangaraj, B., Jolley, C. C., Sarrou, I., Bultema, J. B., Greyslak, J., Whitelegge, J. P., Lin, S., Kouril, R., Subramanyam, R., Boekema, E. J. & Fromme, P. 2011. Efficient light harvesting in a dark, hot, acidic environment: the structure and function of PSI-LHCI from Galdieria sulphuraria. *Biophysical journal,* **100:**135-43.

Tusnady, G. E. & Simon, I. 1998. Principles governing amino acid composition of integral membrane proteins: application to topology prediction. *Journal of molecular biology,* **283:**489-506.

Vastermark, A., Wollwage, S., Houle, M. E., Rio, R. & Saier, M. H., Jr. 2014. Expansion of the APC superfamily of secondary carriers. *Proteins*.

Wang, F., Vandepoele, K. & Van Lijsebettens, M. 2012. Tetraspanin genes in plants. *Plant science : an international journal of experimental plant biology,* **190:**9-15.

Weber, A. P., Horst, R. J., Barbier, G. G. & Oesterhelt, C. 2007. Metabolism and metabolomics of eukaryotes living under extreme conditions. *International review of cytology,* **256:**1-34.

Welch, A. J., Bedoya-Reina, O. C., Carretero-Paulet, L., Miller, W., Rode, K. D. & Lindqvist, C. 2014. Polar bears exhibit genome-wide signatures of bioenergetic adaptation to life in the arctic environment. *Genome biology and evolution,* **6:**433-50.

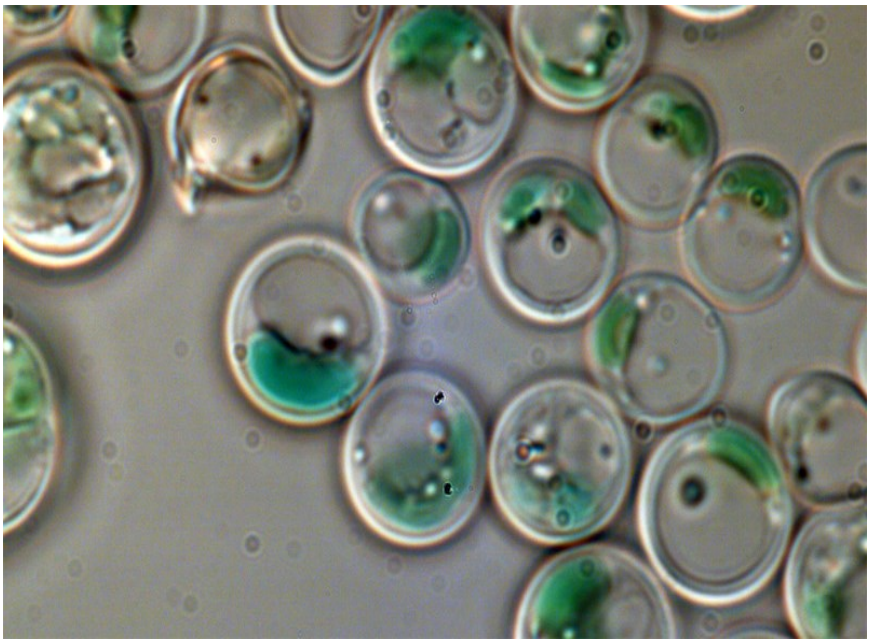
Yan, N. 2013. Structural advances for the major facilitator superfamily (MFS) transporters. *Trends in biochemical sciences,* **38:**151-9.

Yang, H. C., Fu, H. L., Lin, Y. F. & Rosen, B. P. 2012. Pathways of arsenic uptake and efflux. *Current topics in membranes,* **69:**325-58.

Zeth, K. & Thein, M. 2010. Porins in prokaryotes and eukaryotes: common themes and variations. *The Biochemical journal,* **431:**13-22.

Zhai, Y. & Saier, M. H., Jr. 2001. A web-based program (WHAT) for the simultaneous prediction of hydropathy, amphipathicity, secondary structure and transmembrane topology for a single protein sequence. *Journal of molecular microbiology and biotechnology,* **3:**501-2.

APPENDIX



Schönknecht, Gerald. *Galdieria sulphuraria*. 2013. *National Geographic*. Internet. Accessed 9/14/14.

Fenwick, David. Carraghen or Irish moss *C. crispus* – in the middlesphore pool. 2009. *aphotomarine* . Internet. Accessed 9/15/14.

Figure 1. The microalga *G. sulphuraria* in autotrophic state (top) and macroalga *C. crispus* (bottom).

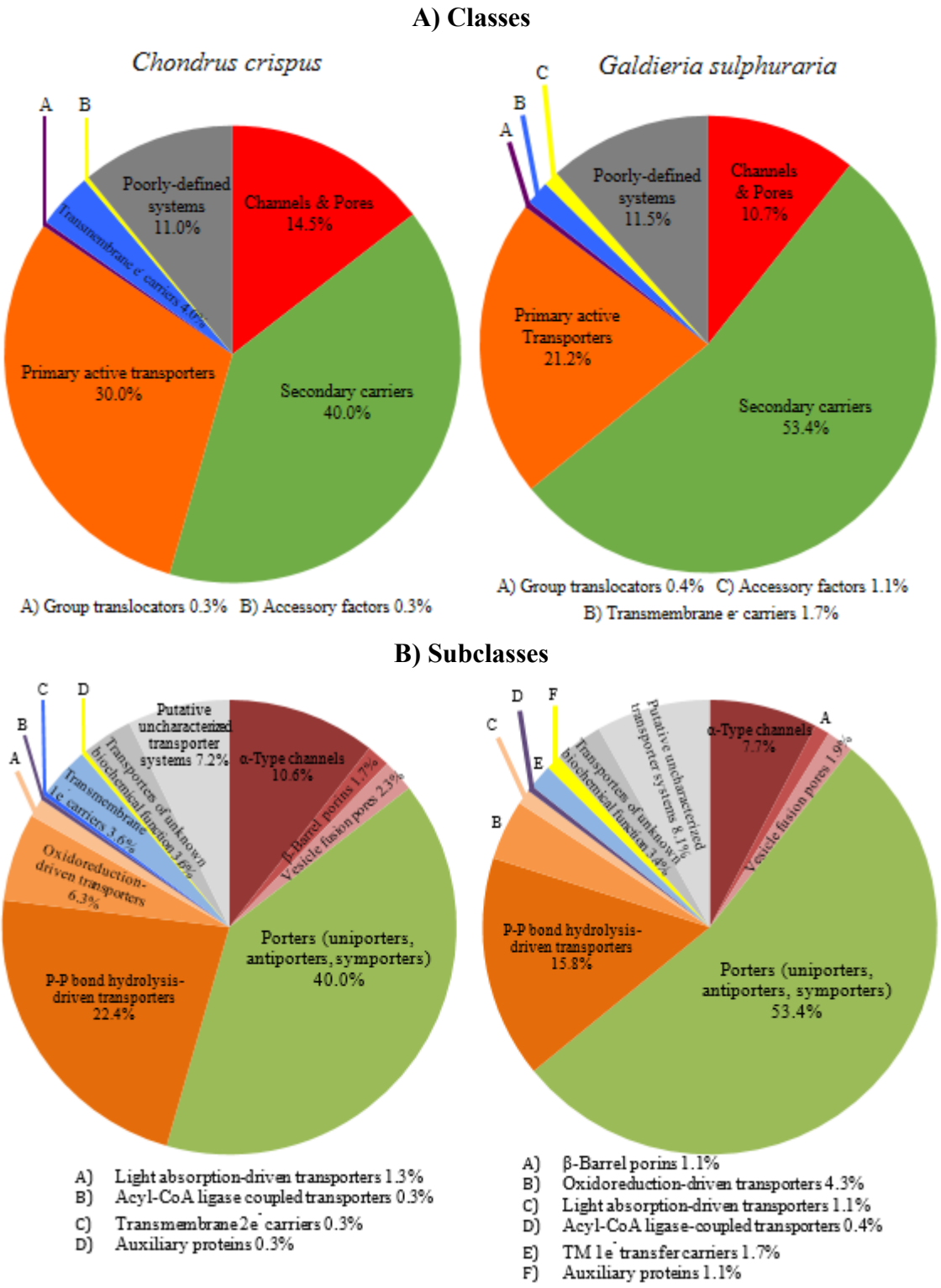


Figure 2. Distribution of transporters based on TC (A) classes and (B) subclasses in *C. crispus* and *G. sulphuraria*.

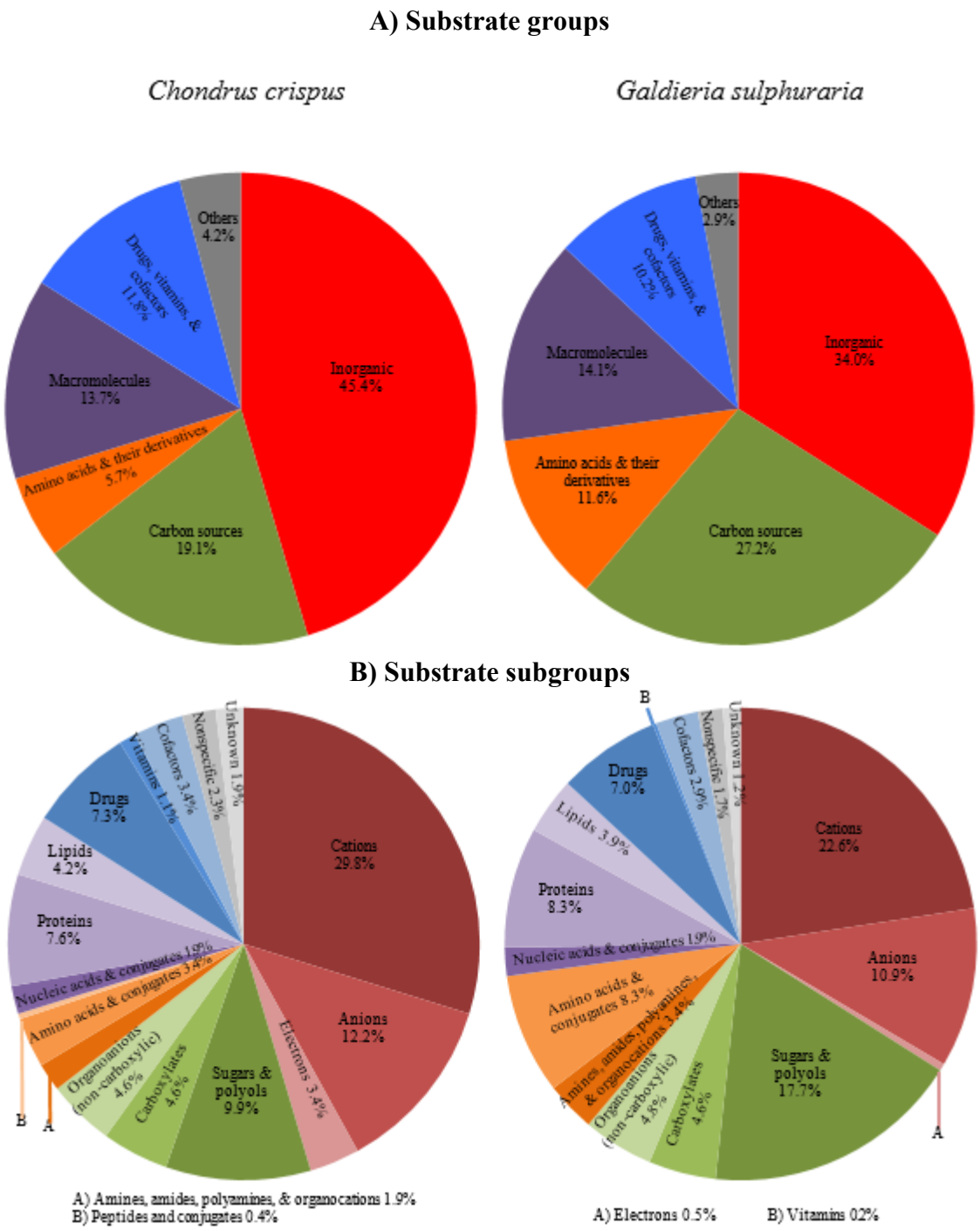


Figure 3. Distribution of transporters based on TC (A) substrate groups and (B) substrate subgroups in *C. crispus* and *G. sulphuraria*.

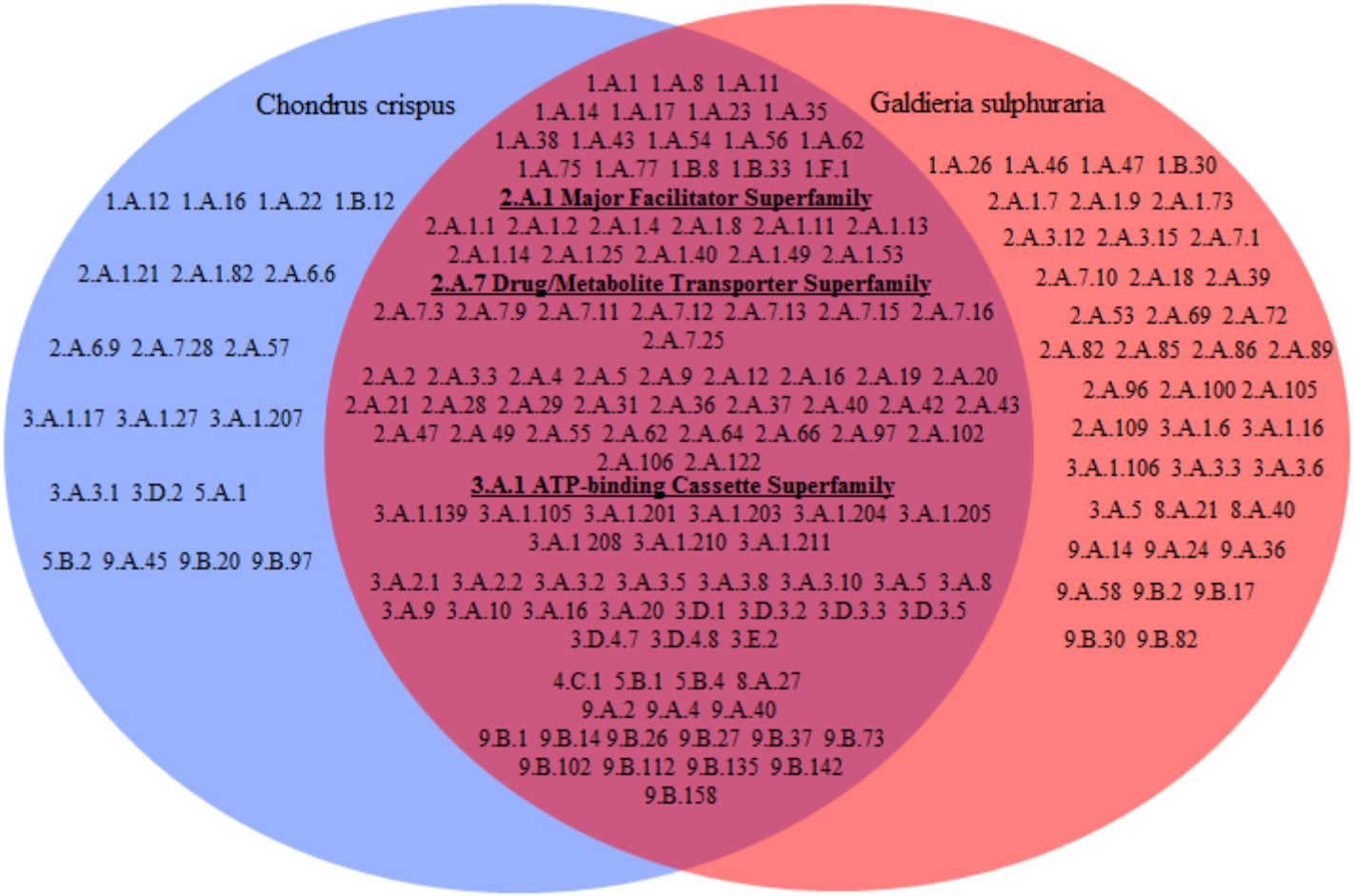


Figure 4. Recognized transporter families belonging to *C. crispus* only, *G. sulphuraria* only, or shared.

Table 1.
Overview of the *C. crispus* and *G. sulphuraria* transporter distribution based on TC class and subclass.

TC class[a]	Class descriptions	No. of transport proteins		TC subclass	Subclass description	No. of transport proteins	
		Ccr	Gsu			Ccr	Gsu
1	Channels & Pores	44	50	1.A	α-Type channels	32	36
				1.B	β-Barrel porins	5	5
				1.F	Vesicle fusion pores	7	9
2	Secondary carriers	121	250	2.A	Porters (uniporters, antiporters, symporters)	121	250
3	Primary active transporters	91	99	3.A	P-P bond hydrolysis-driven transporters	68	74
				3.D	Oxidoreduction-driven transporters	19	20
				3.E	Light absorption-driven transporters	4	5
4	Group translocators	1	2	4.C	Acyl-CoA ligase-coupled transporters	1	2
5	Transmembrane e^- carriers	12	8	5.A	Transmembrane $2e^-$ transfer carrier	1	0
				5.B	Transmembrane $1e^-$ transfer carrier	11	8
8	Accessory factors[b]	1	5	8.A	Auxiliary transport proteins	1	5
9	Unknown transporters[c]	33	54	9.A	Transporters of unknown biochemical function	11	16
				9.B	Putative uncharacterized transport systems	22	38
Total no. of transport proteins		303	468				
Total no. of proteins in genome		9836	7211				
% transporters of genome		3.1%	6.5%				

[a] Detailed class and subclass descriptions can be found at www.tcdb.org. Transporter classes 6 and 7 have not been assigned in the TC system yet and therefore are absent from this table.
[b] Accessory factors facilitate transport via established transport systems and therefore are not counted as separate systems
[c] Unknown transporters include families in TC subclass 9.A (known transporters of unknown biochemical function) and 9.B (putative uncharacterized transport systems), but not 9.C (functionally characterized transporters lacking identified sequences).

Table 2.
Substrates of transporter systems according to TC class identified in *C. crispus* (left) and *G. sulphuraria* (right). The total protein numbers that contribute to the transport systems for particular substrate subgroups are parenthesized.

Substrate category	No. of proteins of indicated type acting on substrate type in *C. crispus*								No. of proteins of indicated type acting on substrate type in *G. sulphuraria*							
	Channels & Pores	Secondary carriers	Primary active transporters	Group translocators	TM e^- carriers	Auxiliary proteins	Putative transporters	Total system #	Channels & Pores	Secondary carriers	Primary active transporters	Group translocators	TM e^- carriers	Auxiliary proteins	Putative transporters	Total system #
Inorganic																
A. Cations	18	24	20 (45)				16	78 (103)	20	38	20 (52)				15	93 (125)
B. Anions	10	20					2	32	8 (9)	34	6				1	49 (50)
C. Electrons					9 (12)			9 (12)					2 (8)			2 (8)
Carbon sources																
A. Sugars & polyols	1	22	1				2	26	5	62 (63)	1				5	73 (74)
B. Carboxylates		11	1					12		19						19
C. Organoanions (non-carboxylic)		4	7	1				12		14	4	2				20
Amino acids & their derivatives																
A. Amines, amides, polyamines, & organocations	3	2						5	2	12						14
B. Amino acids & conjugates		9						9		34						34
C. Peptides & conjugates		1						1								0
Macromolecules																
A. Nucleic acids & conjugates		5						5		8						8
B. Proteins	3	4	6 (13)			1	6	20 (27)	2	7	7 (11)			5	13	34 (38)
C. Lipids		3	7 (8)				1	11 (12)		1	7				8	16
Drugs, vitamins & cofactors																
A. Drugs		4	14				1	19		9	18				2	29
B. Vitamins		3						3		1						1
C. Cofactors		7 (8)					2	9 (10)		9					3	12
Others																
A. Nonspecific	3 (9)		2				1	6 (12)	4 (12)						3	7 (15)
B. Unknown		1	2				2	5		1					4	5
Total systems	38	120	60	1	9	1	33	262	41	249	63	2	2	5	54	416
Total proteins	44	121	91	1	12	1	33	303	50	250	99	2	8	5	54	468

Table 3.
TC classification and functional prediction of transport-related proteins found in *Chondrus crispus* and *Galdieria sulphuraria*. Sequences were retrieved using GBLAST with e-values of 0.1 or smaller. Transport proteins that were purported to be horizontally transferred are darkly shaded. Comparative e-value scores are written next to each entry in the comment section.

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
1.A. α-Type Channels												
1.A.1	Voltage-gated Ion Channel (VIC) Superfamily	1.A.1.3.1	Q03720	7	K^+	bidirectional	R7Q4W4	7	e-29			
		1.A.1.3.3	Q95V25	7	K^+	bidirectional	R7QF10	7	e-27	M2X6F1	7	e-22
		1.A.1.4.6	Q38898	7	K^+	bidirectional	R7QK72	7	e-41			
		1.A.1.7.7	Q9SVV6	4	K^+	bidirectional	R7QMR7	4	e-30			
		1.A.1.11.13	Q5QM84	11	Ca^{2+}	bidirectional	R7QFX3	11	e-76			
		1.A.1.23.1	Q4VY51	4	nonselective cations (prefers K^+)	bidirectional	R7QC79	4	e-39			
		1.A.1.24.4	B7K3R7	6	K^+	bidirectional	R7QSY6	6	e-32			
1.A.8	Major Intrinsic Protein (MIP) Family [a]	1.A.8.1.1	P0AER0	6	polyols, As(III), Sb(III)	bidirectional	R7QKP1	6	e-45	M2VS40	6	e-36
										M2XR24	6	e-40
										M2X7V5	6	e-40
		1.A.8.7.1	P43549	6	glycerol	bidirectional				M2W285	7	e-23
										M2W0W1	6	e-20
		1.A.8.10.10	Q41951	6	H_2O, urea, glycerol, CO_2, NH_3	bidirectional	R7Q3W0	6	e-15			
		1.A.8.16.1	O94778	6	H_2O, NH_3, H_2O_2, formamide	bidirectional	R7QHU0	6	e-10			
1.A.11	Ammonia Channel Transporter (Amt) Family	1.A.11.2.1	P54144	11	H^+, NH_3/ CH_3NH_2	bidirectional				M2Y7S8	11	e-106
										M2X158	11	e-104
		1.A.11.2.8	E2CWJ2	11	H+, NH_4^+	bidirectional	R7QP87	10	e-74			
1.A.12	Intracellular Chloride Channel (CLIC) Family	1.A.12.2.1	Q9FWR4	1	Cl^-	bidirectional	R7QI57	1	e-14			
							R7QRY8	1	e-14			
1.A.14	Testis-enhanced Gene Transfer (TEGT) Family	1.A.14.1.1	P55061	7	Ca^{2+}	bidirectional	R7QHS8	7	e-20	M2Y9B8	7	e-23
		1.A.14.3.4	B5X2N0	7	Na^+, K^+, Ca^{2+}	bidirectional	R7Q3S6	6	e-14			
		1.A.14.3.6	Q9HC24	7	Ca^{2+}	bidirectional				M2X7J4	7	e-43
1.A.16	Formate-Nitrite Transporter (FNT) Family	1.A.16.2.4	Q9LE25	8	NO_2^-	bidirectional	S0F3U1	8	e-32			
1.A.17	Calcium-dependent Chloride Channel (Ca-ClC) Family	1.A.17.1.10	Q8I4C3	8	Cl^-	bidirectional				M2X8B9	5	M2X8B9 (N-terminal; e-17) +
										M2XR10	3	M2XR10 (C-terminal; e-20)

([a]) The donor organism for the MIP family proteins found in *G. sulphuraria* that are homologous to the proteins with UniProt accession numbers P0AER0 and P43549 was suggested to be the mesophilic bacterium *Xanthomonas axonopodis* (Schönknecht *et al*. 2013).

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
1.A.17	Calclium-dependent Chloride Channel (Ca-ClC) Family	1.A.17.5.3	Q5T3F8	11	Cl^-	bidirectional				M2Y9A9	10	e-27
		1.A.17.5.6	Q12252	11	Cl^-	bidirectional	R7QLT8	8	e-26			
		1.A.17.5.9	Q9SY14	11	Cl^-	bidirectional	R7QGA3	9	e-33	M2W0D7	11	e-41
		1.A.17.6.1	D0NGF4	12	Cl^-	bidirectional	R7QBU5	8	e-36			
1.A.22	Large Conductance Mechanosensitive Ion Channel (MscL) Family	1.A.22.1.9	A9WMF7	2	nonselective osmolytes	bidirectional	R7QNM6	3	e-10			
1.A.23	Small Conductance Mechanosensitive Ion Channel (MscS) Family [a]	1.A.23.4.3	P0AEB5	4	nonselective osmolytes	bidirectional	R7QA09	5	e-41	M2WTW7	4	e-44
										M2X830	4	e-23
1.A.26	Mg^{2+} Transporter-E (MgtE) Family	1.A.26.1.2	Q5SMG8	5	Mg^{2+}, Co^{2+}	uptake				M2WSY5	5	e-28
1.A.35	CorA Metal Ion Transporter (MIT) Family	1.A.35.3.2	Q9WZ31	3	divalent metal cations	uptake				M2XU94	3	e-52
										M2X0G1	3	e-46
										M2XTJ8	4	e-54
		1.A.35.5.2	Q9SAH0	4	Mg^{2+}	uptake				M2X7N2	4	e-5
		1.A.35.5.3	Q058N4	4	Mg^{2+}	uptake				M2XKC1	4	e-7
		1.A.35.5.4	Q93ZD7	4	Mg^{2+}	uptake	R7QER3	3	e-26	M2XC03	4	e-13
		1.A.35.5.5	Q02783	4	Mg^{2+}	uptake				M2Y066	4	e-29
										M2Y017	4	e-25
1.A.38	Golgi pH Regulator (GPHR) Family	1.A.38.1.1	B2ZXD5	9	Cl^-	bidirectional	R7QHT6	4	e-24	M2XND6	9	e-21
1.A.43	Camphor Resistance (CrcB) Family	1.A.43.2.7	Q8RYE2	9	F^-	bidirectional	R7QP64	9	e-29	M2XJ61	8	e-19
1.A.46	Anion Channel-forming (Bestrophin) Family	1.A.46.3.1	B7K217	5	Cl^-	bidirectional				M2Y919	5	e-31
1.A.47	Nucleotide-sensitive Anion-selective Channel (ICln) Family	1.A.47.4.1	Q9LVA7	0	nonselective osmolytes	bidirectional				M2XF74	0	e-9
1.A.54	Presenilin ER Ca^{2+} Leak Channel (Presenilin) Family	1.A.54.1.1	P49768	9	Ca^{2+}	bidirectional	R7QBX8	9	e-30			
		1.A.54.1.2	P49810	9	Ca^{2+}	bidirectional				M2Y4P6	9	e-20
		1.A.54.3.3	A8K546	9	Ca^{2+}	bidirectional	R7Q6M1	9	e-33			
		1.A.54.3.4	A7KX19	9	Ca^{2+}	bidirectional	R7QNK8	10	e-27	M2W8D1	8	e-36
1.A.56	Copper Transporter (Ctr) Family	1.A.56.1.13	Q5ZD08	3	Cu^+	uptake	R7Q625	3	e-6			

([a]) The donor organism for the MscS family protein with UniProt accession number M2WTW7 found in *G. sulphuraria* was suggested to be the mesophilic bacterium *Marinomonas sp. MWYL1* (Schönknecht *et al*. 2013).

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
1.A.56	Copper Transporter (Ctr) Family	1.A.56.1.15	Q8IL79	3	Cu^{+}	uptake				M2VZF5	8	0.0026; TCDB entry 1.A.56.1.16; fused protein: N-terminal Ferric chelate reductase (5 TMS) + C-terminal Copper transporter (3 TMS)
1.A.62	Homotrimeric Cation Channel (TRIC) Family	1.A.62.1.2	Q9DAV9	3	monovalent cations	bidirectional	S0F2W2	5	0.004	M2W844	5	0.0136; TCDB entry 1.A.64.4.1
		1.A.62.2.1	A0M015	7	monovalent cations	bidirectional	R7Q7M5	7	e-16			
1.A.75	Mechanical Nociceptor, Piezo (Piezo) Family	1.A.75.1.1	Q92508	38	cations	bidirectional				M2XB31	32	e-40
		1.A.75.1.3	Q9VLS3	40	cations	bidirectional	R7Q1A5	30	e-46			
1.A.77	Mg^{2+}/Ca^{2+} Uniporter (MCU) Family	1.A.77.3.13	P33250	4	Mg^{2+}	uptake				M2Y797	4	0.0001; TCDB entry 1.A.77.4.1; fused protein: N-terminal AtpI domain (4 TMS) + C-terminal α/β-hydrolase domain (soluble)
		1.A.77.3.21	P08443	4	Mg^{2+}	uptake	R7Q4B7	4	e-5; TCDB entry 1.A.77.3.39	M2XQQ8	4	e-11; TCDB entry 1.A.77.3.45
1.B β-Barrel Porins												
1.B.8	Mitochondrial and Plastid Porin (MPP) Family	1.B.8.1.4	P46274	0	anionic metabolites	bidirectional				M2XFD6	0	e-10; TCDB entry 1.B.8.1.11
		1.B.8.1.5	P42054	0	anionic metabolites	bidirectional				M2XJL9	0	e-31; TCDB entry 1.B.8.1.10
		1.B.8.1.14	F0WZY9	0	anionic metabolites	bidirectional	R7QSJ6 R7QMN5	0 0	e-19 e-19			
1.B.12	Autotransporter-1 (AT-1) Family	1.B.12.5.1	P09489	0	serine protease	efflux	R7QAK2	0	e-11			
1.B.30	Plastid Outer Envelope Porin of 16 kDa (OEP16) Family	1.B.30.4.1	K4D2B1	4	cationic solutes, amino acids	bidirectional				M2X828	4	e-8; TCDB entry 1.B.30.5.1
1.B.33	Outer Membrane Protein Insertion Porin (OmpIP) Family	1.B.33.2.2	Q9C5J8	0	specific proteins	bidirectional	R7QVF7	0	e-8	M2XSV1	0	e-15
		1.B.33.3.3	V5IKW7	0	specific proteins	bidirectional	R7Q682	0	e-27			
		1.B.33.3.4	Q9Y512	0	specific proteins	bidirectional				M2VY12	0	e-46; TCDB entry 1.B.33.3.6

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
1.F. Vesicle Fusion Pores												
1.F.1	Synaptosomal Vesicle Fusion Pore (SVF-Pore) Family	1.F.1.1.1	Q16623	1	1.F.1 subunits form one system involved in vesicular efflux	syntaxin subunit	S0F300	1	e-18	M2XML0	1	e-24
							R7QME0	1	e-13	M2XAZ6	1	e-23
							R7QVA5	1	e-9	M2X4I9	1	e-11
			Q12846	1		syntaxin subunit				M2WV92	1	e-5
										M2VXB1	1	e-5
			P63027	1		synaptobrevin subunit				M2Y6K8	1	e-11
										M2X6T6	2	e-10
		1.F.1.1.2	P33328	1		synaptobrevin subunit	R7Q3S3	1	e-10			
		1.F.1.1.3	O16000	1		syntaxin subunit	R7QRT5	1	e-31	M2W6G9	1	e-12
							R7Q2M4	1	e-6			
			Q12241	1		syntaxin subunit				M2VSJ2	1	e-5
			O02495	1		synaptobrevin subunit	R7QHV8	1	e-7			
2.A. Porters (uniporters, symporters, antiporters)												
2.A.1	Major Facilitator Superfamily (MFS)	2.A.1.1.1	P0AEP1	12	H^+, sugars	symporter				M2XXL1	12	e-50
		2.A.1.1.2	P0AE24	12	H^+, sugars	symporter				M2XXP7	12	e-52
										M2VXG1	12	e-52
										M2W0V8	12	e-53
		2.A.1.1.25	Q96QE2	12	H^+, sugars	symporter				M2VSW1	12	e-47
										M2VYL5	12	e-37
		2.A.1.1.33	Q8NJ22	12	H^+, sugars	symporter				M2XSZ4	12	e-138
										M2W1G1	12	e-105
										M2W8G9	12	e-106
										M2XC74	12	e-108
										M2XM33	12	e-95
										M2VZF1	12	e-97
										M2WR98	12	e-128
										M2XC85	12	e-97
										M2WW79	12	e-46
		2.A.1.1.66	Q8VZR6	12	H^+, sugars	symporter				M2XBB0	12	e-51
		2.A.1.1.70	Q0ULF7	12	H^+, sugars	symporter				M2W107	12	e-118
										M2VTD3	12	e-99
										M2VTW5	12	e-96
										M2Y0A1	12	e-97
										M2XB18	12	e-66
										M2Y365	12	e-54
										M2VXM8	12	e-51
										M2W9G3	12	e-54

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
2.A.1	Major Facilitator Superfamily (MFS)	2.A.1.1.70	Q0ULF7	12	H^+, sugars	symporter				M2XZG7	12	e-61
										M2X468	12	e-49
										M2WXF5	12	e-110
										M2XVQ9	12	e-105
										M2Y5I3	12	e-56
										M2WWA9	12	e-48
										M2VSX6	12	e-44
										M2W3Y2	12	e-64
										M2VV61	12	e-49
										M2X279	12	e-56
										M2Y0D7	7	M2Y0D7 (N-terminal; e-65) + M2WZ07 (C-terminal; e-40)
										M2WZ07	4	
		2.A.1.1.89	Q9NY64	12	H^+, sugars	symporters	R7QRJ0	12	e-40			
		2.A.1.1.103	Q0WW9	12	H^+, sugars	symporter				M2Y912	12	e-78
		2.A.1.2.4	P02982	12	H^+, tetracycline	antiporter				M2VYQ3	12	e-11
		2.A.1.2.39	Q5JAK9	12	H^+, tetracycline	antiporter	R7QH96	12	e-21	M2W584	12	e-32
										M2VXR7	12	e-8; TCDB entry 2.A.1.2.80
		2.A.1.2.58	Q8RWN2	12	H^+, multidrugs (spermidine, fluoroquinolones, acriflavin, chloramphenicol, etc.)	antiporter				M2WUS0	12	e-20
		2.A.1.4.6	Q9Z7N9	12	sugar phosphates, P_i	antiporter	R7QUQ4	12	e-110	M2W1F3	12	e-110
							R7QUY4	12	e-69	M2W8V8	12	e-62
		2.A.1.7.13	Q08280	12	H^+, hexose sugars	symporter				M2WYX2	12	e-16
		2.A.1.8.4	Q9SWR8	12	H^+, NO_3^-, NO_2^-	symporter (H^+/NO_3^-), antiporter (NO_3^-/NO_2^-)	R7QFV5	12	e-64			
		2.A.1.8.8	Q9RA46	12	NO_3^-, NO_2^-	antiporter				M2XBF0	12	e-5
		2.A.1.9.7	P25346	12	P_i, glycerophosphoinositol/ glycerophosphocholine	symporter				M2Y734	12	e-92
										M2XGS3	12	e-64
										M2WW87	12	e-56
										M2XVX7	12	e-46
										M2XJA6	12	e-58
										M2X0L2	12	e-45
										M2W347	12	e-36
										M2W3I6	12	e-38
										M2Y4Y5	12	e-31

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
2.A.1	Major Facilitator Superfamily (MFS) [ab]	2.A.1.9.7	P25346	12	P_i, glycerophosphoinositol/ glycerophosphocholine	symporter				M2Y3A0 M2Y904 M2W456 M2Y6A0 M2W9V1 M2Y849	12 12 12 12 12 12	e-27 e-35 e-34 e-31 e-27 e-31
		2.A.1.11.3	P37662	12	oxalate, formate	antiporter	R7QEU2 R7QG95	12 11	0; TCDB entry 2.A.1.11.7 e-5			
		2.A.1.13.2	Q91Y77	12	H^+, aromatic amino acids/ N-methyl amino acids	antiporter	R7Q7X2	12	e-8			
		2.A.1.13.12	Q7RTX9	12	H^+, aromatic amino acids/ N-methyl amino acids	antiporters				M2XHY2	12	e-9; TCDB entry 2.A.1.13.20
		2.A.1.14.3	P70786	12	tartrate, D-galactonate, polyols	symporter	R7QQC6	12	e-75			
		2.A.1.14.22	O82390	12	Na^+, P_i	symporter	R7QFP0 S0F392 S0F3V1	11 12 12	e-27 e-56 e-105	M2XBT3	12	e-134
		2.A.1.21.10	D2PCQ8	12	drugs, H^+	antiporter	S0F383	12	0.0002			
		2.A.1.25.1	O00400	12	acetyl-CoA, CoA	antiporter	R7Q2E0 R7Q417	5 7	R7Q417 (N-terminal; e-49) + R7Q2E0 (C-terminal; e-14)	M2VTB6 M2WRJ4	12 12	e-72 e-68
		2.A.1.40.2	Q5R542	13	molybdate (putative)	uptake	R7QTD5 S0F2T1	13 12	e-66 e-66	M2XN54	13	e-57
		2.A.1.49.2	Q9H2V7	12	sphingolipid-1-phosphate or sphingolipid	uptake	R7QMS0	12	e-74			
		2.A.1.49.5	Q6NMN6	12	Similar substrates as 2.A.1.49.2	uptake				M2VXN5	11	e-10
		2.A.1.53.3	A6NID9	12	amino acids	uptake	R7QRP5	11	e-7	M2XGX9	12	e-42
		2.A.1.73.1	Q9L102	12	uncharacterized	uptake				M2XB79	12	e-5; TCDB entry 2.A.1.80.1
		2.A.1.82.3	Q8BFT9	12	copper ions	uptake	R7QQM6	12	e-89			

([a]) The donor organisms for the proteins with UniProt accession numbers M2XHY2 and M2XB79 found in G. *sulphuraria* was reported to be the mesophilic bacteria *Planococcus donghaensis* and *Geobacter sulfurreducens*, respectively (Schönknecht *et al*. 2013).

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
2.A.2	Glycoside-Pentoside-Hexuronide (GPH):Cation Symporter Family	2.A.2.4.2	Q9SXM0	12	H^+, sucrose	uniporter (H^+ only), symporter (H^+, sucrose)	R7Q7P7	12	e-11	M2Y0T8	12	e-46
							R7QD15	12	e-34	M2X344	12	e-49
										M2Y4M4	12	e-47
		2.A.2.4.3	O80605	12	H^+, sucrose	symporter	R7Q5R0	12	e-37	M2WSC6	10	e-40
		2.A.2.4.5	D1GC38	12	H^+, sucrose	symporter				M2W7L8	12	e-57
		2.A.2.4.6	Q9FE59	12	H^+, sucrose	symporter	R7Q6D4	12	e-20	M2WYC9	12	e-53
2.A.3	Amino Acid-Polyamine-Organocation (APC) Superfamily [a]	2.A.3.3.6	O43246	14	unknown	uncharacterized				M2X0G8	14	e-57
		2.A.3.3.14	Q8W4K3	14	H^+, cationic amino acids	symporter	R7Q7T9	14	e-51			
		2.A.3.12.3	Q9FFL1	12	H^+, polyamine/ paraquat	symporter				M2X1G0	12	e-54
										M2XGE3	12	e-69
		2.A.3.15.2	Q9HJ13	12	cationic amino acids (putative)	uncharacterized				M2XZP4	12	e-29
										M2WPV2	12	e-32
										M2XTB8	12	e-31
										M2X2P6	12	e-34
2.A.4	Cation Diffusion Facilitator (CDF) Family	2.A.4.3.1	Q62941	6	H^+, Zn^{2+}	antiporter				M2XJW9	6	e-43
		2.A.4.3.4	Q9ZT63	6	H^+, Zn^{2+}	antiporter	R7QCV7	6	e-32			
		2.A.4.3.7	O14863	6	H^+, Zn^{2+}	antiporter	R7QDN0	6	e-16			
		2.A.4.4.1	Q03455	16	H^+, Zn^{2+}	antiporter	R7QCS8	16	e-18			
		2.A.4.5.1	A4ZUV2	6	H^+, Mn^{2+}	antiporter	R7QE37	6	e-50			
2.A.5	Zinc (Zn^{2+})-Iron (Fe^{2+}) Permease (ZIP) Family	2.A.5.1.1	P32804	8	metal dications	symporter				M2XSB1	8	e-37
		2.A.5.1.3	O81123	8	Zn^{2+}/ Fe^{2+}	symporter				M2XU04	8	e-23
		2.A.5.3.5	P59889	8	Zn^{2+}	symporter	R7QDN4	8	e-16			
		2.A.5.4.9	Q8BZH0	8	dications	symporter	R7QTK0	7	e-10			
		2.A.5.5.5	B2UL32	8	dications	symporter	R7QJW6	8	e-43	M2X3N7	8	e-37
2.A.6	Resistance-Nodulation-Cell Division (RND) Superfamily	2.A.6.6.1	O15118	14	lipid/ sterols	antiporter	R7Q6L5	12	e-47			
							S0F2Z9	12	e-29			
		2.A.6.9.2	Q3TDN0	12	cholesterol-modified peptide (putative)	antiporter	R7QR56	12	e-37			
2.A.7	Drug/Metabolite Transporter (DMT) Superfamily	2.A.7.1.6	O31792	4	H^+, cationic lipophilic drugs	antiporter				M2X365	4	e-7
		2.A.7.3.47	Q9UYQ7	10	uncharacterized drug or metabolite	uncharacterized (efflux)	R7QC72	12	e-13	M2Y669	11	e-37; TCDB entry 2.A.7.3.50
							R7QKU6	10	e-12			
		2.A.7.9.7	P0CK96	10	glucose-6-P, P_i	antiporter				M2Y878	10	e-12
		2.A.7.9.8	Q8VCX2	10	sugar-P (putative), P_i	antiporter				M2Y7P0	10	e^{-22}

([a]) The donor organism for the APC family proteins found in *G. sulphuraria* that are homologous to the protein with UniProt accession number Q9HJ13 was suggested to be the thermo-acidophilic archaea *Thermoplasma volcanium* (Schönknecht *et al*. 2013).

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
2.A.7	Drug/Metabolite Transporter (DMT) Superfamily	2.A.7.9.15	Q7Z769	10	UDP-galactose (putative), P_i	antiporter	R7QDN5 R7Q4B2	10 10	e-47 e-46	M2XDY0	10	e-51
		2.A.7.9.16	Q9NQQ7	10	sugar-P (putative), P_i	antiporter	R7QJK9	7	e-26			
		2.A.7.10.3	Q869W7	10	UDP-N-acetylglucosamine, UDP	antiporter				M2W822	8	e-18
		2.A.7.11.1	P78383	10	UDP-galactose, UDP	antiporter	R7Q5T5	10	e-56	M2VSE0	10	e-46
		2.A.7.11.7	Q6NM25	10	UDP-galactose/ UDP-glucose, UDP	antiporter	R7QBW1	10	e-65			
		2.A.7.12.13	Q8N357	10	sugar-P, nucleotide diphosphate (both putative)	antiporter	R7QSE4	10	e-41	M2XUU8	10	e-41
		2.A.7.13.1	P40107	10	GDP-mannose, GMP	antiporter				M2X270	10	e-19
		2.A.7.13.2	Q5A477	10	GDP-mannose, GMP	antiporter	R7QNE5	10	e-16	M2XTL9	10	e-25
		2.A.7.15.2	Q18779	10	UDP-glucuronic acid/ UDP-N-acetylgalactosamine/ UDP-galactose, UDP	antiporter				M2Y6H9 M2VW66	10 9	e-34 e-7
		2.A.7.15.4	Q9NTN3	10	UDP-glucuronate/ UDP-N-acetylgalactosamine, UDP	antiporter	R7QBU8	9	e-30	M2Y1T4	10	e-34
		2.A.7.15.5	Q5M8T2	10	UDP-glucuronic acid/ UDP-N-acetylgalactosamine/ UDP-xylose (putative), UDP	antiporter	R7QGJ4	10	e-31			
		2.A.7.16.1	Q96A29	9	GDP-fucose, GMP	antiporter	R7QN60	10	e-74	M2YAH5 M2X8J4	8 8	e-59 e-59
		2.A.7.24.5	Q03730	10	thiamine pyrophosphate	uncharacterized				M2VSS0	10	e-32
		2.A.7.24.6	Q8WV83	10	thiamine pyrophosphate	uncharacterized	R7Q6H2	10	e-28			
		2.A.7.25.2	Q8N8Q9	9	Mg^{2+}	uncharacterized				M2XH80	7	e-27
		2.A.7.25.4	Q0D2K0	9	Mg^{2+}	uncharacterized				M2WPM0	9	e-28
		2.A.7.25.5	Q9LIR9	9	Mg^{2+}	uncharacterized	R7QEF8	8	e-21	M2WRW9	9	e-11
		2.A.7.25.9	Q8BMW7	9	Mg^{2+}	uncharacterized	R7Q848	8	e-10			
		2.A.7.28.8	Q2M3R5	10	sugar-P (putative)	antiporter	R7QFJ0 R7QDD5	10 10	e-9 e-22; TCDB entry 2.A.7.28.10			
2.A.9	Cytochrome Oxidase Biogenesis (Oxa1) Family	2.A.9.1.2	Q15070	4	H^+, mitochondrial proteins	insertion into mitochondrial membrane	R7Q3M2	4	e-33	M2Y260 M2X799 M2XSU1 M2Y497 M2XKW3	4 4 4 4 4	e-33 e-28 e-16 e-17 e-14
		2.A.9.2.1	Q8LBP4	4	H^+, chloroplastic proteins	insertion into chloroplastic membrane	R7QEJ8	3	e-50	M2W1T6	4	e-63

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
2.A.12	ATP:ADP Antiporter (AAA) Family	2.A.12.1.16	Q39002	12	ATP, ADP (both putative)	symporter (putative)				M2XL63	13	e-162
2.A.16	Tellurite-resistance/ Dicarboxylate Transporter (TDT) Family	2.A.16.4.3	Q2TJJ2	10	sulfite	uncharacterized (efflux)				M2XVM3	10	e-66
		2.A.16.5.2	Q9FLV9	10	anions, H_2O, CO_2	uncharacterized	R7QM48	10	e-24			
2.A.18	Amino Acid/Auxin Permease (AAAP) Family	2.A.18.4.1	P38680	11	H^+, neutral amino acids	symporter				M2XVH3	11	e-48
										M2W9B3	11	e-38
										M2Y492	11	e-31
										M2XZ35	11	e-29
										M2X215	11	e-33
										M2XYQ9	11	e-32
										M2WTM1	11	e-23
										M2W5K6	11	e-24
										M2XDA2	11	e-26
										M2XQW3	11	e-22
										M2XR07	11	e-15
		2.A.18.4.2	Q6IT47	11	H^+, neutral amino acids/ aromatic amino acids	symporter				M2XKR7	11	e-74
										M2YA49	11	e-57
		2.A.18.8.7	Q7Z2H8	11	H^+, small amino acids	symporter				M2VXF7	11	e-23
2.A.19	Ca^{2+}:Cation Antiporter (CaCA) Family	2.A.19.2.8	O59940	11	H^+, Ca^{2+}	antiporter	R7Q8L6	11	e-53	M2XIB1	11	e-56
		2.A.19.4.12	C3ZMW2	24	Na^+/ K^+, Ca^{2+}	antiporter	R7QEG4	12	e-60			
2.A.20	Inorganic Phosphate Transporter (PiT) Family	2.A.20.2.1	P15710	11	Na^+, P_i	symporter	R7QFS9	9	e-39			
		2.A.20.2.4	Q38954	12	Na^+, P_i	symporter	R7Q988	10	e-58	M2W3X5	12	e-58
							R7QNS6	10	e-56			
2.A.21	Solute:Sodium Symporter (SSS) Family	2.A.21.6.2	Q9FHJ8	15	Na^+, urea	symporter	R7QHY4	15	0	M2Y9S7	15	0
		2.A.21.6.3	Q7XBS0	15	Na^+, urea	symporter	R7Q3G5	15	0			
2.A.28	Bile Acid:Na^+ Symporter (BASS) Famly	2.A.28.2.2	E0D3H5	10	Na^+, pyruvate	symporter	R7QIK1	10	e-41			
		2.A.28.2.3	Q1EBV7	10	Na^+, pyruvate	symporter	R7QHD3	10	e-72	M2Y501	10	e-67
										M2XXJ5	10	e-50
										M2XXF4	10	e-52
2.A.29	Mitochondrial Carrier (MC) Family	2.A.29.1.2	P12235	6	ATP, ADP	antiporter				M2XWW5	6	e-111
										M2W0T3	6	e-99
		2.A.29.1.8	Q9H0C2	6	ATP, ADP	antiporter				M2XKU6	6	e-50
		2.A.29.2.8	Q03028	6	C5-C7 oxodicarboxylates	antiporter				M2XE42	6	e-8
		2.A.29.2.13	Q02978	6	2-oxoglutarate, malate	antiporter	R7Q9Q9	6	e-61	M2XTM7	6	e-96
										M2XW83	5	e-88

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
2.A.29	Mitochondrial Carrier (MC) Family	2.A.29.4.3	P23641	6	phosphonates, fatty acids, P_i	antiporter	R7Q5H3	7	e-37	M2XZM1	6	e-69
										M2XL70	7	e-43
		2.A.29.4.6	Q9FMU6	6	phosphonates, fatty acids, P_i	antiporter	R7QCH8	7	e-82			
		2.A.29.5.1	P10566	6	H^+, Fe^{2+}	symporter				M2X4S5	6	e-48
										M2XDG6	6	e-42
		2.A.29.5.6	Q96DW6	6	H^+, Fe^{2+}, glycine, 5-aminolevulinic acid (latter two are putative)	uncharacterized				M2X8W8	6	e-36
										M2WR39	6	e-36
										M2XCM2	6	e-34
		2.A.29.8.2	Q27257	6	carnitine, acyl-carnitine	antiporter				M2XKC7	6	e-47
		2.A.29.8.5	Q8BL03	7	carnitine, acyl-carnitine	antiporter	R7QBL3	7	e-33			
		2.A.29.8.9	Q8N8R3	6	carnitine, acyl-carnitine	antiporter	R7Q6T2	4	e-28			
		2.A.29.10.3	Q4A3R4	5	folate, folate derivatives	antiporter	R7Q6N0	5	e-44	M2XY94	6	e-47
		2.A.29.10.4	P38127	5	pyrimidine nucleotides	antiporter				M2Y582	5	e-49
		2.A.29.10.5	P40556	6	NAD^+, AMP, GMP	uniporter, antiporter				M2WY34	7	e-45
		2.A.29.10.6	Q96CQ1	6	pyrimidine nucleotides	antiporter	R7QQL9	6	e-49			
		2.A.29.10.8	P39953	6	NAD^+, AMP, GMP	uniporter, antiporter				M2XLN1	7	e-47
										M2W5P0	7	e-45
		2.A.29.10.10	Q8RWA5	6	NAD^+/ $NADP^+$, ADP/ AMP	antiporter	R7QQP7	5	e-45			
							R7QNT3	4	e-22			
		2.A.29.12.3	P16260	6	CoA, ADP	antiporter	R7Q957	6	e-48	M2WQU6	6	e-62
										M2XRV1	6	e-60
		2.A.29.13.1	P33303	6	succinate, fumarate	antiporter				M2XY01	6	e-27
		2.A.29.14.1	O75746	6	aspartate, glutamate	antiporter	R7Q2S5	6	e-47			
		2.A.29.14.4	Q12482	6	aspartate, glutamate	antiporter				M2VX08	6	e-47
		2.A.29.14.6	Q8TBP6	6	thiamine pyrophosphate, PP_i, nucleotides	antiporter	R7QF94	6	e-35	M2XN68	6	e-47
		2.A.29.16.1	Q9HC21	6	thiamine pyrophosphate, dNTPs, dNDPs	antiporter	R7QHL9	6	e-45			
		2.A.29.18.1	P38921	6	S-adenosylmethionine, S-adenosylhomocysteine	antiporter				M2YA78	6	e-53
										M2WVC1	5	e-13
		2.A.29.18.2	Q94AG6	6	S-adenosylmethionine, S-adenosylhomocysteine		R7QMN2	4	e-37	M2Y040	6	e-43
							R7QAE2	6	e-31	M2Y715	6	e-41
							R7Q854	6	e-33	M2XM08	7	e-35
										M2XIM3	6	e-35
										M2WUE9	7	e-33
										M2VWQ6	7	e-35
										M2Y5Y1	6	e-35
										M2XSN4	6	e-31

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
2.A.29	Mitochondrial Carrier (MC) Family	2.A.29.23.4	O04619	6	adenine nucleotides	antiporter	R7QSK4	6	e-37	M2WX85	6	e-12
		2.A.29.23.5	Q9BV35	5	adenine nucleotides	antiporter				M2Y5W0	6	e-38
		2.A.29.23.8	Q6NUK1	4	ATP, P_i	antiporter	R7Q7H8	4	e-34			
		2.A.29.29.1	Q04013	6	carboxylates	antiporter	R7Q8U3	6	e-74	M2Y753	5	e-84
2.A.31	Anion Exchanger (AE) Family	2.A.31.2.7	Q8I8G6	12	Cl^-/HCO_3^-	antiporter	R7QII1	12	e-74			
		2.A.31.4.1	Q8NBS3	12	Na^+, OH^-/ borate	antiporter (prefers borate)	R7Q0Y4	12	e-79	M2W484	12	e-42
2.A.36	Monovalent Cation:Proton Antiporter-1 (CPA1) Family [a]	2.A.36.1.9	Q9Y2E8	11	H^+, Na^+	antiporter	R7QB46	11	e-79	M2XUK4	12	e-98
		2.A.36.6.1	O29412	13	H^+, Na^+	antiporter				M2Y4T2	13	e-28
		2.A.36.6.2	Q9LCB5	13	H^+, Na^+/ Li^+	antiporter				M2Y2Y6	13	e-27
		2.A.36.6.7	Q2XWL3	13	H^+, Na^+/ Li^+	antiporter	R7QIY9	13	e-23			
		2.A.36.7.6	Q9LKW9	13	H^+, Na^+	antiporter	R7QS13	13	e-54	M2W9W0	13	e-58
										M2VUJ6	13	e-42
2.A.37	Monovalent Cation: Proton Antiporter-2 (CPA2) Family	2.A.37.1.4	Q9ZTZ7	14	H^+, K^+	antiporter	R7QHV9	12	e-98			
		2.A.37.1.6	Q9M0Z3	13	H^+, K^+	antiporter	R7QMN4	14	e-95	M2Y950	13	e-100
										M2XFK8	13	e-84
										M2VZM4	13	e-83
		2.A.37.1.8	Q8VYR9	13	H^+, K^+	antiporter	S0F3W8	13	e-43			
2.A.39	Nucleobase: Cation Symporter-1 (NCS1) Family	2.A.39.3.4	P94575	12	H^+, allantoin	symporter				M2X8U9	14	0; TCDB entry 2.A.39.3.9
2.A.40	Nucleobase/ Ascorbate Transporter (NAT) or Nucleobase: Cation Symporter-2 (NCS2) Family	2.A.40.4.1	Q07307	12	H^+, purines	symporter	R7QLA0	12	e-108			
		2.A.40.4.4	P48777	12	H^+, purines	symporter				M2XQP3	12	e-134
2.A.42	Hydroxy/ Aromatic Amino Acid Permease (HAAAP) Family	2.A.42.1.1	P0AAD4	11	H^+, tyrosine	symporter	R7Q890	10	e-25	M2X997	11	e-29
2.A.43	Lysosomal Cystine Transporter (LCT) Family	2.A.43.1.4	P57758	6	H^+, cystine	symporter	R7QRV6	6	e-27			
		2.A.43.3.1	Q60441	6	H^+, cystine	symporter	R7QG09	6	e-31	M2W618	6	e-22
							R7QRV6	6	e-28	M2X213	6	e-16
2.A.47	Divalent Anion:Na^+ Symporter (DASS) Family	2.A.47.2.2	P27514	15	Na^+, P_i/ selenite	symporter	R7QS20	11	e-47	M2XZ72	15	e-95
		2.A.47.4.5	Q9K7H7	13	Na^+, sulfate	symporter	R7QCA6	14	e-85			
2.A.49	Chloride Carrier/Channel (ClC) Family	2.A.49.2.1	P35523	11	H^+, Cl^-	bidirectional (channel)	R7Q426	11	e-40			
							R7QU10	11	e-44			
		2.A.49.2.3	P51792	10	H^+, Cl^-	antiporter (carrier)	S0F2Y5	10	e-128	M2X4P3	12	e-86

([a]) The donor organism for the CPA1 family proteins found in *G. sulphuraria* that are homologous to the proteins with UniProt accession numbers O29412 and Q9LCB5 was suggested to be the mesophilic bacterium *Cytophaga hutchinsonii* (Schönknecht *et al*. 2013).

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
2.A.49	Chloride Carrier/Channel (ClC) Family	2.A.49.2.6	P35525	10	H^+, Cl^-	antiporter (as a carrier), bidirectional (as a channel)				M2X3L1	10	e-65
		2.A.49.3.3	P51798	10	H^+, Cl^-	antiporter (carrier)				M2WY17	10	e-55
		2.A.49.6.3	Q8GX93	10	H^+, Cl^-	bidirectional (channel)				M2W5F5	10	e-39
		2.A.49.7.3	O54193	11	H^+, Cl^-	bidirectional (channel)	R7QI83	12	e-6			
		2.A.49.8.1	A3CXC7	11	H^+, Cl^-	antiporter (carrier)				M2XWR6	11	e-6; TCDB entry 2.A.49.8.2
2.A.53	Sulfate Permease (SulP) Family	2.A.53.1.7	Q9SAY1	11	H^+, sulfate	symporter				M2XG26	10	e-42
		2.A.53.2.12	G3C7W4	11	H^+, sulfate	symporter				M2XJY0	11	e-57
2.A.55	Metal Ion (Mn^{2+}-iron) Transporter (Nramp) Family	2.A.55.2.4	Q9SAH8	12	H^+, divalent metal cations	antiporter (NRAMP-1)	R7Q2I0	12	e-57	M2Y255	12	e-71
										M2Y1S4	12	e-66
										M2Y8W3	12	e-60
		2.A.55.2.10	Q553K4	12	H^+, divalent metal cations	symporter (NRAMP-2)	R7Q9R7	12	e-104	M2Y572	12	e-121
2.A.57	Equilibrative Nucleoside Transporter (ENT) Family	2.A.57.1.8	Q14542	11	Na^+, nucleosides/ nucleobases	antiporter	R7Q8W2	9	e-13; TCDB entry 2.A.57.1.9			
		2.A.57.5.3	Q59LX9	11	uncharacterized	uncharacterized	R7QGD2	10	e-29			
2.A.62	NhaD Na^+:H^+ Antiporter (NhaD) Family	2.A.62.1.1	O66163	11	H^+, Na^+/ Li^+	antiporter	R7QKN9	14	e-21			
		2.A.62.1.2	Q56EB3	14	H^+, Na^+/ Li^+	antiporter				M2XMJ9	14	e-31
2.A.64	Twin Arginine Targeting (Tat) Family	2.A.64.1.1	P69423	6	proteins	antiporter (TatC)	Q36327	6	e-7			
		2.A.64.2.1	Q9SJV5	6	proteins	antiporter (TatC)	M5DES7	6	e-48	671743558	6	e-54
2.A.66	Multidrug/ Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily	2.A.66.1.16	Q3V050	12	H^+, cationic drug	antiporter				M2Y993	12	e-52
		2.A.66.1.24	Q9SFB0	12	H^+, citrate	antiporter	R7QQM5	9	e-33	M2XWU2	11	e-31
		2.A.66.3.2	Q96AA3	12	Man5GlcNAc2-PP-Dol	flippase	R7QJY2	12	e-29			
		2.A.66.3.3	Q6V5B3	11	Man5GlcNAc2-PP-Dol	flippase				M2Y244	12	e-21
2.A.69	Auxin Efflux Carrier (AEC) Family	2.A.69.2.4	C4MAS5	10	H^+, auxin	antiporter				M2WZ72	10	e-21
2.A.71	Folate-Biopterin Transporter (FBT) Family	2.A.71.1.4	Q07492	12	H^+, folate/ biopterin	symporter	R7QCZ6	12	e-47			
		2.A.71.2.1	O68867	12	H^+, folate/ biopterin	symporter	R7QJP2	12	e-97			
2.A.72	K^+ Uptake Permease (KUP) Family	2.A.72.3.8	O22881	12	K^+	uniporter				M2XRS0	12	e-83
										M2WZK6	12	e-68

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
2.A.82	Organic Solute Transporter (OST) Family	2.A.82.1.3	Q9Y519	7	organic anions	antiporter				M2XR88	7	e-42
2.A.85	Aromatic Acid Exporter (ArAE) Family	2.A.85.2.6	Q9LPQ8	6	malate	uncharacterized (efflux)				M2W9J2	13	e-6; TCDB entry 2.A.85.11.3
		2.A.85.3.1	Q10495	12	organoanions	uncharacterized				M2Y600	11	e-7 TCDB entry 2.A.85.11.1
										M2VZN8	13	e-12; TCDB entry 2.A.85.11.2
		2.A.85.3.2	B3LHA7	10	organoanions	uncharacterized				M2X3H5	14	e-5
		2.A.85.3.3	C8Z8B0	10	organoanions	uncharacterized				M2Y6Z9	11	0.0004
										M2W7U6	12	0.003
		2.A.85.3.4	C4QYX5	11	organoanions	uncharacterized				M2XRZ1	13	0.0006
		2.A.85.11.2	M2VZN8	12	organoanions	uncharacterized				M2Y8D6	12	e-12
		2.A.85.11.3	M2W9J2	13	organoanions	uncharacterized				M2Y9I7	11	e-83
										M2XQ12	12	e-11
										M2WRS9	11	e-10
2.A.86	Autoinducer-2 Exporter (AI-2E) Family	2.A.86.1.6	D3LPG3	8	aldose	antiporter				M2VTE1	8	e-7; TCDB entry 2.A.86.1.10
2.A.89	Vacuolar Iron Transporter (VIT) Family	2.A.89.1.2	Q9ZUA5	5	Fe^{2+}	uptake				M2W143	5	e-48
										M2XH11	5	e-48
2.A.96	Acetate Uptake Transporter (AceTr) Family [a]	2.A.96.1.1	P0AC98	6	acetate/ succinate	uncharacterized (uptake)				M2Y4P8	7	e-17
		2.A.96.1.6	O14201	6	acetate	uncharacterized (uptake)				M2W6Q3	6	e-22
										M2VSJ7	7	e-9
										M2XZA4	6	e-10
										M2VU43	6	e-12
		2.A.96.1.7	P32907	6	NH_3	uncharacterized (efflux)				M2VTM8	5	e-25
										M2Y9A3	6	e-17
										M2Y856	5	e-22
										M2Y7J1	6	e-22
										M2VWV6	5	e-20
2.A.97	Mitochondrial Inner Membrane K^+/H^+ and	2.A.97.1.1	O95202	1	H^+, cations	antiporter				M2XXD3	1	e-56
										M2XWT6	1	e-56
	Ca^{2+}/H^+ Exchanger	2.A.97.1.2	Q08179	1	H^+, cations	antiporter	R7QG67	1	e-21	M2XTB4	1	0.0064
	(LetM1) Family	2.A.97.1.3	P91927	2	H^+, cations	antiporter	R7QUP8	2	e-7			
2.A.100	Ferroportin (Fpn) Family	2.A.100.1.4	Q9NP59	11	H^+, Fe^{2+}/Mn^{2+}	antiporter				M2XS45	10	e-21

([a]) The donor organism for the AceTr family proteins found in *G. sulphuraria* was suggested to be the mesophilic bacterium *Desulfotomaculum acetoxidans* (Schönknecht *et al*. 2013).

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
2.A.102	Putative 4-Toluene Sulfonate Uptake Permease (TSUP) Family [a]	2.A.102.2.1	Q0K020	8	sulfite	efflux	R7QMX4	8	e-7			
		2.A.102.4.1	Q9UYH7	9	sulfur-containing compound	efflux	R7QU47	8	0.0006	M2XWF1	8	e-6
		2.A.102.4.5	C7D7I4	9	sulfite	efflux				M2X4H1	9	e-9
		2.A.102.5.1	Q5ZAL4	12	sulfur-containing compound	efflux				M2Y449	12	e-8
										M2WRI6	12	0.001
2.A.105	Mitochondrial Pyruvate Carrier (MPC) Family	2.A.105.1.4	Q949R9	2	pyruvate	uncharacterized (uptake)				M2Y850	2	e-36
2.A.106	Ca^{2+}:H^+ Antiporter-2 (CaCA2) Family	2.A.106.1.1	P52876	6	H^+, Ca^{2+}	antiporter				M2WRF4	7	e-27
		2.A.106.2.1	P52875	6	H^+, Ca^{2+}	antiporter				M2XN18	6	e-28
		2.A.106.2.2	Q9HC07	5	H^+, Ca^{2+}	antiporter	R7QVN8	6	e-30	M2WTA0	6	e-11
		2.A.106.2.4	Q10320	7	H^+, Ca^{2+}	antiporter				M2WA50	7	e-43
2.A.109	Tellurium Ion Resistance (TerC) Family	2.A.109.1.6	I1HMH4	8	tellurium dianion (Te^{2-})	uncharacterized (effux)				M2WX97	8	e-36
2.A.122	LrgB/CidB holin-auxilary protein (LrgB/CidB) Family	2.A.122.2.1	Q9FVQ4	12	glycolate, glycerate	symporter	R7QCB2	12	e-35	M2X9P0	11	e-44
3.A P-P-bond Hydrolysis-driven Transporters												
3.A.1	ATP-binding Cassette (ABC) Superfamily	3.A.1.6.7	Q6QJE2	6	sulfate	uptake (chloroplastic)				M2XXV7	10	e-53
										671743627	7	e-50
										671743626	7	e-53
		3.A.1.16.3	Q55106	6	bicarbonate, cyanate, nitrate, nitrite	uptake (chloroplastic)				M2XYQ7	13	e-10
		3.A.1.17.1	Q47539	6	taurine, aromatic sulfonates	uptake (chloroplastic)	R7QS55	13	e-10; TCDB entry 3.A.1.17.9			
		3.A.1.17.5	C0LZR8	6	phthalate	uptake (chloroplastic)	R7QG39	6	e-6			
		3.A.1.27.2	Q8L4R0	6	lipids (Trigalactosyl-diacylglycerol), γ-hexachlorocyclohexane	uptake (chloroplastic)	M5DDJ0	6	e-45			
		3.A.1.105.3	Q70J76	6	multidrugs (chromomycin, mithramycin, oleandomycin)	efflux	M5DCU7	6	e-5	671743504	6	e-10

([a]) The donor organism for the TSUP family protein with UniProt accession number M2X4H1 found in *G. sulphuraria* was suggested to be the thermophilic bacterium *Thermodesulfovibrio yellowstonii* (Schönknecht *et al*. 2013).

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
3.A.1	ATP-binding Cassette (ABC) Superfamily	3.A.1.106.1	P60752	5	phospholipid, LPS, lipid A, multidrugs (azidopine, daunomycin, vinblastine, Hoechst 33342, ethidium)	efflux				M2Y7P7	5	e-71
		3.A.1.139.2	P77307	7	iron	efflux	R7QED5	6	e-18	M2X1H2	7	e-34
		3.A.1.201.1	P08183	12	multidrugs (xenobiotics, long-chain fatty acids, tetramethylrosamine analogues, peptides, phospholipids, cholesterols)	efflux	R7Q5S3 R7QRK4 R7QKD7	10 10 10	0 0 0	M2VZ40	8	e-117
		3.A.1.201.7	O80725	12	indole acetic acid, indole-3-propionic acid, vanillic acid, auxin	efflux				M2WX40	12	e-180
		3.A.1.201.17	Q9NRK6	5	multidrugs (peptides, auxins, xenobiotics)	efflux	R7QPE9 S0F366	5 6	e-73 e-107	M2VVT6	6	e-120
		3.A.1.203.1	P28288	5	long-chain fatty acyl-CoA	uptake	R7QFQ6 R7Q5S4	5 5	e-142 e-98	M2XY28	5	e-151
		3.A.1.203.8	Q6NLC1	5	long-chain fatty acyl-CoA	uptake	R7QQ95 R7QP51 R7Q832	5 6 6	e-85 e-126 e-84	M2W6N8 M2Y8S2	5 6	e-132 e-110
		3.A.1.203.9	O14678	5	long-chain fatty acyl-CoA	uptake	S0F357	6	e-48	M2X071	5	e-44
		3.A.1.204.1	P10090	6	multidrugs (3-hydroxykynurenine, sterols, mitoxantrone, falvopiridol, methotrexate, 7-hydroxymethotrexate, methotrexate diglutamate, topotecan, resveratrol, folates, mitoxantrone, daunorubicin, doxorubicin, glutathione, phospholipids, calcein-AM, bodipy-verapamil, bodipy-vinblastine)	efflux	R7QTJ3	5	e-61	M2XN84 M2VZV8 M2VRW9	6 6 8	e-88 e-85 e-63
		3.A.1.204.8	Q8RXN0	7	Similar substrates as 3.A.1.204.1	efflux				M2WYF8	7	e-96
		3.A.1.204.11	A9SCA8	7	Similar substrates as 3.A.1.204.1	efflux	R7Q270 R7QQ28 R7Q994	6 7 7	e-83 e-89 e-88	M2WUR1 M2Y704	7 7	e-95 e-97
		3.A.1.204.15	I0DHI9	6	Similar substrates as 3.A.1.204.1	efflux				M2XLM7 M2XXM4 M2Y6J5 M2Y549	6 7 6 6	e-87 e-90 e-89 e-87

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
3.A.1	ATP-binding Cassette (ABC) Superfamily	3.A.1.205.6	Q8X0Z3	12	multidrugs (anilinopyrimidine, benzimidazole, phenylpyrrole, phenylpyridylamine, strobirulin, azoles, dicarboximides, quintozene, acriflavin, rhodamine 6G, camptothecin, stilbene phytoalexin, resveratrol, brefeldin, actinomycin D,cerulenin, cytochalasin B)	efflux	R7Q8Z8	12	e-130			
		3.A.1.205.11	P41820	13	Similar substrates as 3.A.1.205.6	efflux				M2XHH8 M2X8Y6	13 13	e-168 e-117
		3.A.1.207.2	Q22NS1	10	uncharacterized	uncharacterized	R7Q8M1	10	e-14			
		3.A.1.207.3	Q8ST07	11	uncharacterized	uncharacterized	R7Q4B9	9	e-28			
		3.A.1.208.5	Q42093	15	multidrugs (folates, antifolates, dianionic bile salts, cysteinyl leukotrienes, anthracyclines, epipodophyllotosine, cisplatin, methotrexate, protease inhibitors, cyclic nucleotides, purines, prostaglandins, estradiols, nucleobases, arsenicals, antimonals, mercurials)	efflux	R7QLB5	13	0			
		3.A.1.208.7	O15439	10	Similar substrates as 3.A.1.208.5	efflux	R7QTL3	12	0			
		3.A.1.208.8	P33527	14	Similar substrates as 3.A.1.208.5	efflux	R7QCI4	12	e-108	M2XPP1 M2VT30	14 12	0 0
		3.A.1.210.3	Q9ZDW0	5	Fe^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, phytochelins	efflux	R7QMH2 R7QKH4	5 5	e-98 e-94			
		3.A.1.210.4	O75027	6	Similar substrates as 3.A.1.210.3	efflux				M2XKZ6	6	e-180
		3.A.1.210.8	Q9LVM1	7	Similar substrates as 3.A.1.210.3	efflux	R7QEA3 R7QF92	6 6	e-162 e-106	M2XD51	6	e-138
		3.A.1.210.9	Q1LRE9	6	Similar substrates as 3.A.1.210.3	efflux				M2W1K3 M2WZN5	6 8	e-130 e-128
		3.A.1.211.7	Q8T5Z7	7	lipids and protein surfactants, sterols, vitamin A derivatives, phosphatidylethanolamine,	efflux	R7QS59	8	e-62			
		3.A.1.211.12	Q9FLT8	7	Similar substrates as 3.A.1.211.12	efflux	R7QSS4	6	e-84	M2X541 M2XRG0 M2XUE0	8 8 6	e-68 e-103 e-85

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
3.A.2	H^+- or Na^+-translocating F-type, V-type and A-type ATPase (F-ATPase) Superfamily	3.A.2.1.2	P21904	1	Na^+	B subunit, efflux	M5DDJ2	1	e-9	671743577	1	0.0016
										671743576	1	e-7
			P21905	2		C subunit	M5DDH1	2	e-14	671743575	2	e-11
			P21903	6		A subunit	M5DBX5	5	e-17	671743574	5	e-11
		3.A.2.1.3	P61829	2	H^+	C subunit, efflux	P48880	2	e-12	671743668	2	e-11
			P00854	7		A subunit	P48878	6	e-37	671743661	7	e-12
		3.A.2.1.6	F8L1Z7	6	Na^+	A subunit, efflux				M2VYY4	9	e-11
										M2XEV2	2	e-11
		3.A.2.2.3	Q3E7B6	2	H^+	E subunit, efflux	R7QD22	2	e-5			
		3.A.2.2.5	P59227	4	H^+	C1/C3/C5 subunit, efflux				M2XUB3	4	e-36
										M2Y749	4	e-14
		3.A.2.2.6	Q91V37	5	H^+	C subunit, efflux	R7QV90	5	e-36			
			Q9Z1G4	9		A subunit form 1	R7QSQ8	7	e-170	M2Y228	9	e-171
							R7QJJ0	7	e-151			
			Q920R6	9	H^+	A subunit form 4				M2X2W4	9	e-168
3.A.3	P-type ATPase (P-ATPase) Superfamily	3.A.3.1.1	P05023	8	Na^+/ K^+	α_1 subunit, Na^+ efflux, K^+ uptake	R7Q7P6	11	0			
			P50993	10		α_2 subunit	R7QIT7	9	0			
		3.A.3.2.11	Q37145	12	Ca^{2+}	efflux	R7QJ90	11	e-169			
		3.A.3.2.19	Q9SY55	9	Ca^{2+}, Mn^{2+}	efflux				M2XDK9	9	0
		3.A.3.2.32	Q49LV5	11	Ca^{2+}	efflux	R7QED9	10	0	M2Y4V4	11	0
		3.A.3.3.8	Q9SH76	10	H^+	efflux				M2Y128	10	e-23
		3.A.3.5.3	P35670	11	Cu^+, Ag^+, and putatively Fe^{2+}	efflux				M2XSA5	10	e-98
		3.A.3.5.32	Q9S7J8	8	Cu^+	efflux	R7QF87	7	e-108	M2X397	8	e-93
							R7QDI7	7	e-92			
							R7QIQ7	9	e-113			
		3.A.3.6.6	Q9M3H5	7	Cu^+, Cu^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Ca^{2+}	efflux				M2XZB5	6	e-78
		3.A.3.8.1	Q29449	7	phospholipids	uptake	R7Q823	10	e-111	M2X329	8	e-127
		3.A.3.8.6	Q9XIE6	9	phospholipids	uptake				M2W841	10	e-179
										M2XR68	10	e-73
		3.A.3.8.10	Q5KP96	10	lipids	uptake	R7QCD9	4	R7QCD9			
							R7QAN1	6	(N-terminal; e-59) + R7QAN1 (C-terminal; e-108)			
		3.A.3.8.13	Q9Y2Q0	7	phospholipids	uptake	R7QFL0	9	e-137			
							R7QBM1	10	e-168			

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
3.A.3	P-type ATPase (P-ATPase) Superfamily	3.A.3.10.1	B9RHM6	12	Mn^{2+}, Ca^{2+}	efflux	R7Q862	10	0	M2VZA1 M2XF79	6 5	M2XF79 (N-terminal; e-82) + M2VZA1 (C-terminal; e-41)
3.A.4	Arsenite-Antimonite (ArsAB) Efflux Family [a]	3.A.4.1.1	P0AB93	13	arsenite, antimonite	efflux				M2WZL1 M2VUF6	12 13	e-115 e-110
3.A.5	General Secretory Pathway (Sec) Family	3.A.5.4.1	P0A4H1	10	unfolded proteins	SecY, uptake	M5DDE1	10	e-87	M2XYF0 671743517	10 10	e-77 e-85
		3.A.5.9.1	P60059	1	unfolded	Sec61 γ subunit, uptake	R7QAF6	1	e-21	M2Y4C1	1	e-21
			Q9UGP8	3	proteins	Sec63 subunit	R7Q9F5	4	e-46	M2X8H4	3	e-25
			Q9H9S3	10		Sec61 α_2 subunit	R7QCN6	12	0			
			P61619	12		Sec61 α_1 subunit				M2VUM6	12	e-176
3.A.8	Mitochondrial Protein Translocase (MPT) Family	3.A.8.1.1	P32897	3	proteins	Tim23 subunit, uptake (mitochondrial)	R7QGL9 R7QH43	3 3	e-5 e-5	M2XSI8	3	e-11
			P39515	4		Tim17 subunit (mitochondrial)	R7QSY8	3	e-34			
			Q12328	3		Tim22 subunit (mitochondrial)				M2XRN2	3	e-11
3.A.9	Chloroplast Envelope Protein Translocase (CEPT or Tic-Toc) Family	3.A.9.1.1	O49931	3	proteins	Tic55 subunit, uptake (chloroplastic)	R7QBL8 R7QNM3 R7QD05	2 2 4	e-34 e-28 e-31			
			Q9ZST8	3		Tic20 subunit (chloroplastic)	M5DEQ8	5	e-7	M2Y1M3	5	e-11
3.A.10	H^+, Na^+-translocating Pyrophosphatase (M^+-PPase) Family	3.A.10.1.3	Q2RIS7	16	Na^+	efflux	R7QL36	15	e-14			
		3.A.10.2.3	Q56ZN6	17	H^+	efflux				M2Y519 M2Y1L8 M2W5M5	16 16 17	0 0 0
3.A.16	Endoplasmic Reticular Retrotranslocon (ER-RT) Family	3.A.16.1.1	Q9BUN8	4	misfolded proteins	Derlin-1 subunit, efflux				M2Y3F8 M2VTI4	4 4	e-21 e-24
		3.A.16.1.3	Q8ILM8	7	misfolded proteins	uncharacterized subunit, efflux	R7QRC3	7	e-32			
3.A.20	Peroxisomal Protein Importer (PPI) Family	3.A.20.1.2	Q9M841	4	proteins	Pex12 subunit, uptake	R7QCH6	3	e-34			
			Q9FE40	2	proteins	Pex14 subunit, uptake				M2XC77	1	e-7

([a]) The donor organism for the ArsAB family proteins found in *G. sulphuraria* was reported to be the thermo-acidophilic bacterium *Leptospirillum ferriphilum* (Schönknecht *et al*. 2013).

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
3.D Oxidoreduction-driven Transporters												
3.D.1	H^+ or Na^+-translocating NADH Dehydrogenase (NDH) Family	3.D.1.1.1	P33607	18	3.D.1 subunits form one system involved in bidirectional H^+ transport	NuoL subunit	R7Q3W2	12	e-96			
		3.D.1.2.1	P29919	3		NQO7 subunit	P48910	3	e-33	671743667	3	e-16
			P29922	5		NQO10 subunit	P48924	5	e-28	671743672	4	e-9
			P29923	3		NQO11 subunit				671743669	2	e-8
			P29925	14		NQO13 subunit	P48915	14	e-96	671743664	12	e-58
			P29926	15		NQO14 subunit	P48903	14	e-45	671743665	13	e-15
		3.D.1.6.1	P03887	8		NuoH subunit	P48898	8	e-82	671743666	8	e-51
		3.D.1.6.2	P05509	2		NuoK subunit	P48930	3	e-13			
			P05510	19		NuoL subunit	P48920	21	e-146	671743663	14	e-50
3.D.2	Proton-translocating Transhydrogenase (PTH) Family	3.D.2.3.1	P11024	16	H^+	bidirectional	R7QN92	14	0			
3.D.3	Proton-translocating Quinol:Cytochrome c Reductase (QCR) Superfamily	3.D.3.2.1	P08067	1	3.D.3 subunits form one system involved in bidirectional e^- and H^+ transport	Rieske subunit	R7QDK5	1	e-55	M2XSR4	2	e-53
			P00157	9		Cytochrome B	P48875	9	e-123	671743673	8	e-70
		3.D.3.3.1	P07143	2		Cytochrome c_1	R7QIJ3	2	e-76	M2X3L5	1	e-72
		3.D.3.5.1	P26290	1		Riekse subunit	S0F3J4	1	e-53	M2W8R1	1	e-47
			P26287	2		Cytochrome c_{533}	M5DCZ8	2	e-90	M2XXZ8	5	e-56
										671743557	2	e-74
		3.D.3.5.2	P56774	3		Cytochrome b_6/f complex subunit 4	M5DER2	3	e-72	M2XYD8	5	e-69
										671743488	3	e-72
			P56773	5		Cytochrome b_6	M5DCT3	4	e-114	671743487	5	e-112
			Q9ZR03	1		Rieske subunit				M2WYB8	2	e-61
3.D.4	Proton-translocating Cytochrome Oxidase (COX) Superfamily	3.D.4.7.1	P00415	6	3.D.4 subunits form one system involved in bidirectional H^+ transport	COX3, efflux	P48872	7	e-77	671743675	7	e-72
		3.D.4.8.1	P00410	2		Cytochrome c oxidase subunit 2, efflux	P48869	2	e-73	671743674	3	e-48
			P00401	12		Cytochrome c oxidase subunit 1	P48866	13	0	671743659	12	e-141
3.E Light Absorption-driven Transporters												
3.E.2	Photosynthetic Reaction Center (PRC) Family	3.E.2.2.1	P11004	6	3.E.2 subunits form one system involved in H^+ efflux	photosystem II CP43	M5DDF7	7	e-11			
							M5DCS4	6	0			
			P11005	6		photosystem II D2	M5DDD2	6	0	671743477	7	0
			P04997	8		photosystem II D1	M5DCR8	6	e-173	D4NY65	8	e-180
		3.E.2.2.2	P09193	8		photosystem II CP43				M2WWU0	12	0
										671743478	6	0
										D4NY66	6	e-11

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
4.C Acyl-CoA Ligase-coupled Transporters												
4.C.1	Proposed Fatty Acid Group Translocation (FAT) Family [a]	4.C.1.1.4	P69451	2	fatty acids	uptake	R7Q4U4	2	e-28	M2YAG2 M2Y8J1	5 3	e-84 e-70
5.A Transmembrane 2-electron Transfer Transporters												
5.A.1	Disulfide Bond Oxidoreductase D (DsbD) Family	5.A.1.2.4	Q8S3X4	6	e^-	uptake	M5DBL1	6	e-9			
5.B Transmembrane 1-electron Transfer Transporters												
5.B.1	gp91^{phox} Phagocyte NADPH Oxidase-associated Cytochrome b_{558} (Phox) Family	5.B.1.1.5 5.B.1.3.1	Q96PH1 O81209	7 5	e^- e^-	export export	R7QIV3 R7QFF3 R7QMM5 R7QP63	9 9 10 10	e-33 e-30 e-26 e-31	M2Y4Q6	11	e-38
5.B.2	Eukaryotic Cytochrome b_{561} ($Cytb_{561}$) Family	5.B.2.2.1 5.B.2.2.2	G7ZYU6 Q0WRW8	5 5	e^- e^-	import import	R7QS52 R7Q827 R7QFL5	5 5 5	e-12 e-15 e-5			
5.B.4	Plant Photosystem I Supercomplex (PSI) Family	5.B.4.1.1	Q9SUI4 Q9SHE8 P56767 P56766	2 3 10 11	5.B.4 subunits form one system involved in e^- uptake	PsaL subunit, uptake PsaF subunit PsaB subunit PsaA subunit	M5DD33 M5DEQ3 M5DES3 M5DBT1	2 2 12 11	e-31 e-50 0 0	M2XEV6 671743586 E3UIU5 E3UIU1 M2WWW9 671743539 M2XYF9	3 3 3 12 8 12 4	e-24 e-33 e-43 0 0 0 e-93
8.A Auxiliary Transport Proteins												
8.A.21	Stomatin/Podocin/ Band 7/ Nephrosis.2 /SPFH (Stomatin) Family	8.A.21.1.1 8.A.21.2.1	Q27433 O59180	2 1	negative regulator of univalent cation permeability	accessory protein accessory protein				M2Y123 M2Y0Q8 M2XVU9	2 1 1	e-48 e-44 e-29
8.A.27	CDC50 P-type ATPase Lipid Flippase β-Subunit (CDC50) Family	8.A.27.1.4	Q9LTW0	2	associated with lipid transport capability	accessory protein	R7QEE4	2	e-35	M2Y5E4	2	e-50
8.A.40	Tetraspanin (Tetraspanin) Family	8.A.40.1.13	G6DTR0	4	associated with signaling	accessory protein				M2XT95	4	e-7; TCDB entry 8.A.40.1.14

([a]) The donor organisms for the proteins with UniProt accession numbers M2YAG2 and M2Y8J1 found in G. *sulphuraria* were suggested to be the thermophilic bacteria *Roseiflexus castenholzii* and *Thermaerobacter marianensis* respectively (Schönknecht *et al*. 2013).

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
9.A Recognized Transporters of Unknown Biochemical Mechanism												
9.A.2	Endomembrane protein-70 (EMP70) Family	9.A.2.1.2	Q9LIC2	10	Copper ions	uptake	R7QGP2	8	e-142	M2X5E1	10	e-179
							R7QGJ3	9	e-103	M2Y3P3	10	e-164
							R7QQY9	10	e-147	M2W8F9	10	e-175
							R7Q9U3	10	e-127			
		9.A.2.1.5	Q84LF6	10	Copper ions	uptake				M2XSZ3	9	e-73
							R7QDU4	10	e-113			
9.A.4	YggT or Fanciful K^+ Uptake-B (FkuB; YggT) Family	9.A.4.2.1	Q81WE1	2	K^+	uptake	R7Q9R6	2	e-5	M2WX31	3	e-8; TCDB entry 9.A.4.2.3
							R7QMH5	3	e-6	M2WYR6	2	0.003; TCDB entry 9.A.4.2.2
9.A.14	G-protein-coupled receptor (GPCR) Family	9.A.14.5.1	P13773	6	lipid	efflux				M2X2Z8	7	e-8
9.A.24	Mitochondrial Cholesterol/Porphyrin Uptake Translocator Protein (TSPO) Family	9.A.24.1.2	Q3J192	5	cholesterol, porphyrin	uptake				M2Y7W1	3	e-5; TCDB entry 9.A.24.1.2
										M2VTF1	4	e-21
9.A.36	Ca^{2+}-dependent Phospholipid Scramblase (Scramblase) Family	9.A.36.1.2	P47140	0	phospholipids	flippase				M2X4U5	0	e-36
9.A.40	HlyC/CorC (HCC) Family of Putative Transporters	9.A.40.2.2	Q0PBV6	4	auxiliary protein to CorA channels	accessory protein	R7QJ18	4	e-30	M2Y203	4	e-44
		9.A.40.3.1	Q3TWN3	5	divalent metal cations	uptake	R7Q6H3	5	e-38	M2VV80	4	e-103
							R7QDU9	3	e-40	M2XZK3	5	e-43
										M2WW42	3	e-40
9.A.45	Magnesium Transporter1 (MagT1) Family	9.A.45.1.4	Q54N33	5	Mg^{2+}	uptake	R7QTT2	5	e-10			
9.A.58	Sweet; PQ-loop; Saliva; MtN3 (Sweet) Family	9.A.58.1.12	Q7JVE7	7	sugars	bidirectional				M2WQ48	6	e-13
		9.A.58.1.14	Q54JW5	7	sugars	bidirectional				M2X865	7	e-15

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
9.B Putative Transport Proteins												
9.B.1	Integral Membrane CAAX Protease (CAAX Protease) Family	9.B.1.1.2	Q8RX88	7	protein, protein fragments	uncharacterized	R7QBI2	7	e-75	M2XZV0	7	e-84
		9.B.1.2.5	Q8GW19	8	protein, protein fragments	uncharacterized	R7Q5V9	7	e-14			
		9.B.1.2.6	C4LVT7	7	protein, protein fragments	uncharacterized				M2Y8U2	7	e-13
9.B.2	Integral Membrane CAAX Protease-2 (CAAX Protease2) Family [a]	9.B.2.1.8	F9DWD5	7	protein, protein fragments	uncharacterized				M2X807	9	e-5
		9.B.2.1.9	Q93GK6	7	protein, protein fragments	uncharacterized				M2XV39	7	0.0003; TCDB entry 9.B.2.2.1
										M2XVS1	6	0.004
		9.B.2.1.12	A1U2M7	5	protein, protein fragments	uncharacterized				M2Y846	5	e-10; TCDB entry 9.B.2.1.13
		9.B.2.1.14	Q1IPL5	10	protein, protein fragments	uncharacterized				M2X6B9	10	e-11; TCDB entry 9.B.2.1.15
9.B.14	Putative Heme Handling Protein (HHP) Family	9.B.14.2.4	Q9I3N5	6	heme	efflux (putative)				671743670	5	e-13
		9.B.14.3.2	Q7VCA3	8	heme	efflux (putative)				M2XEQ6	8	e-71
		9.B.14.3.3	P48269	9	heme	efflux (putative)	M5DD44	8	e-71	671743643	9	e-76
9.B.17	VAMP-associated protein (VAP) Family	9.B.17.1.1	O95292	2	lipid regulation	accessory protein				M2W825	2	e-17
										M2WTN1	2	e-12
9.B.20	Putative Mg^{2+} Transporter-C (MgtC) Family	9.B.20.1.2	O07221	4	Mg^{2+} (putative)	uptake (putative)	R7Q846	5	e-8			
9.B.26	Regulator of ER stress and autophagy TMEM208 (TMEM208) Family	9.B.26.1.2	F0ZPF9	3	ER stress, autophagy (both putative)	uncharacterized	R7Q2R0	3	e-9	M2W1J3	3	e-12; TCDB entry 9.B.26.1.3
9.B.27	DedA or YdjX-Z (DedA) Family	9.B.27.1.1	P76219	6	selenite, oxalate (putative)	uncharacterized	R7QL49	6	e-12	M2XQH5	7	e-19
		9.B.27.5.3	I1L089	6	selenite, oxalate (putative)	uncharacterized	S0F376	6	e-6			
9.B.30	Hly III (Hly III) Family	9.B.30.1.1	P54176	7	nonselective osmolytes (putative)	pore-forming (putative)				M2WR23	7	e-20
										M2VSX1	11	e-23

([a]) The donor organisms for the protein with UniProt accession number M2X6B9 found in G. *sulphuraria* were suggested to be the mesophilic bacteria *Solibacter usitatus* (Schönknecht *et al*. 2013).

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS#	Comment
9.B.37	Huntington-interacting Protein 14 (HIP14) Family	9.B.37.1.1	Q8IUH5	6	divalent metal cations	efflux	R7QHA0	4	e-23			
		9.B.37.1.2	Q9VUW9	6	divalent metal cations	efflux				M2XRM1	4	e-17
		9.B.37.2.1	Q8R173	4	divalent metal cations	efflux	R7QI71	4	e-13	M2W6D5	4	e-15
							R7QC90	4	e-15	M2X872	4	e-19
							R7QDT7	4	e-9			
9.B.73	Chloroplast Envelope/ Cyanobacterial Membrane Protein (CemA) Family	9.B.73.1.2	P75028	4	H_+ (putative)	efflux (putative)	M5DET3	4	e-78	M2VYY8	4	e-84
										671743585	4	e-84
9.B.82	Endoplasmic Reticulum Retrieval Protein1 (Rer1) Family	9.B.82.1.3	O48670	4	heavy metals (putative)	efflux (putative)				M2Y7A2	4	e-40
9.B.97	Acyltransferase-3/Putative Acetyl-CoA Transporter (ATAT) Family	9.B.97.5.1	B9DIS8	11	acyl derivatives (putative)	uncharacterized	R7QC82	11	e-24			
9.B.102	YedE/YeeE (YedE/YeeE) Family	9.B.102.5.2	E7BBJ1	4	prodigiosin (putative)	efflux (putative)				M2VZH1	9	e-22; TCDB entry 9.B.102.5.3
										M2Y1K0	9	e15; TCDB entry 9.B.102.5.4
		9.B.102.5.3	M2VZH1	9	prodigiosin (putative)	efflux (putative)	R7Q7X1	9	e-42			
9.B.104	Rhomboid Protease Family	9.B.104.1.3	F0Z2G1	6	proteins	efflux	R7Q8K5	7	e-22	M2W031	6	e-7
							R7QSU1	7	e-10	M2X9Z3	7	e-15
		9.B.104.1.5	QBF2A9	5	proteins	efflux				M2Y467	6	e-7
										M2Y3S8	6	e-7
		9.B.104.4.1	Q8TEB9	4	proteins	efflux	R7Q9I5	5	e-19	M2XLS9	5	e-15
9.B.112	Stress-inducible Transmembrane Protein (TMPIT1) Family	9.B.112.1.1	G0ZL54	4	unknown	accessory protein (putative)	R7QLX5	4	e-24	M2Y0E9	5	e-20
9.B.135	Membrane Trafficking Yip (Yip) Family	9.B.135.1.1	P53039	5	vesicle biogenesis	accessory protein	R7Q878	5	e-23	M2Y198	5	e-16
		9.B.135.1.2	O64614	5	vesicle biogenesis	accessory protein				M2WTE6	5	e-32

Table 3. *(continued)*

Transporter Classification (TC)							*Chondrus crispus* (Ccr)			*Galdieria sulphuraria* (Gsu)		
Family TC#	Family Name	Hit TCID	Hit Accession #	Hit TMS#	Substrate(s)	Comment	Accession #	Query TMS#	Comment	Accession #	Query TMS #	Comment
9.B.142	Integral membrane Glycosyltransferase family 39 (GT39) Family	9.B.142.2.1	C6NTX4	13	glycosyl moeity (putative)	efflux (putative)				M2X0E8	14	e-26
		9.B.142.3.3	B3S136	13	glycosyl moeity (putative)	efflux (putative)	R7QKI9	11	0	M2W036	13	0
		9.B.142.5.1	I1WBQ5	11	glycosyl moeity (putative)	efflux (putative)	R7QJX1	12	e-13	M2W581	11	e-5
9.B.158	4 TMS Putative DMT2 (DMT2) Family	9.B.158.1.1	F8L081	4	unknown	uncharacterized				M2XHH4	4	0.0011
		9.B.158.1.3	K9ZMM5	4	unknown	uncharacterized				M2XLC6	4	e-8; TCDB entry 9.B.158.1.7
		9.B.158.1.7	M2XLC6	4	unknown	uncharacterized	S0F3T4	4	e-5			