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Genome-wide comparative analysis of ABC systems in the *Bdellovibrio*-and-like organisms

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Abstract

Bdellovibrio -and-like organisms (BALOs) are gram-negative, predatory bacteria with wide variations in genome sizes and GC content and ecological habitats. The ATP-binding cassette (ABC) systems have been identified in several prokaryotes, fungi and plants and have a role in transport of materials in and out of cells and in cellular processes. However, knowledge of the ABC systems of BALOs remains obscure. A total of 269 putative ABC proteins were identified in BALOs. The genes encoding these ABC systems occupy nearly 1.3% of the gene content in freshwater *Bdellovibrio* strains and about 0.7% in their saltwater counterparts. The proteins found belong to 25 ABC system families based on their structural characteristics and functions. Among these, 16 families function as importers, 6 as exporters and 3 are involved in various cellular processes. Eight of these 25 ABC system families were deduced to be the core set of ABC systems conserved in all BALOs. All *Bacteriovorax* strains have 28 or less ABC systems. On the contrary, the freshwater *Bdellovibrio* strains have more ABC systems, typically around 51. In the genome of *Bdellovibrio exovorus* JSS (CP003537.1), 53 putative ABC systems were detected, representing the highest number among all the BALO genomes examined in this study. Unexpected high numbers of ABC systems involved in cellular processes were found in all BALOs. Phylogenetic analysis suggests that the majority of ABC proteins can be assigned into many separate families with high bootstrap supports (>50%). In this study, a general framework of sequence–structure–function connections for the ABC systems in BALOs was revealed providing novel insights for future investigations.

Keywords

ABC transporter; BALOs; *Bacteriovorax*; *Bdellovibrio*; Comparative genomics

1. Introduction

The ATP-binding cassette (ABC) systems are broadly found in organisms and are involved in the transportation of a wide variety of substances and other cellular processes and

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regulation. All ABC systems share a highly conserved ATP-hydrolyzing domain or protein that is characterized by three motifs (the Walker A and Walker B motifs, indicative of the presence of a nucleotide binding site, and the signature motif, unique to ABC proteins, located upstream of the Walker B motif) (Tomii and Kanehisa, 1998; Higgins, 2001; Davidson et al., 2008). ABC systems can be divided into three main functional categories (Tomii and Kanehisa, 1998; Higgins, 2001; Davidson et al., 2008): importers, exporters and cellular processors. Importers mediate nutrient uptake in prokaryotes. The nature of the substrates that are transported is very diverse and includes mono- and oligosaccharides, organic and inorganic ions, amino acids, peptides, iron siderophores, metals, poly-amine cations, opines, and vitamins. Exporters are involved in the secretion of various molecules as peptides, lipids, hydrophobic drugs, polysaccharides and proteins, including toxins such as hemolysin. Cellular processors are not involved in transport, with some members having a role in translation of mRNA and DNA repair.

A large group of ABC systems in prokaryotic and eukaryotic organisms have recently been studied by genome-wide analysis (Tomii and Kanehisa, 1998; Higgins, 2001; Ren and Paulsen, 2005; Davidson et al., 2008; Bu et al., 2009). *Escherichia coli* was reported to have 71 ABC systems (Saurin et al., 1999; Chang and Roth, 2001), representing about 1.8% of its total number of genes (Linton and Higgins, 1998). Through comparative studies, ABC systems can be correlated to the evolutionary relationships of different organisms and to the environments in which they inhabit. For example, 19 ABC system families were deduced to constitute the core set of ABC systems conserved in all marine-living *Synechococcus* and *Prochlorococcus* (Bu et al., 2009).

*Bdellovibri*o-and-like organisms (BALOs) are gram-negative, predatory bacteria that inhabit terrestrial, freshwater and saltwater environments and the intestinal tracts of animals and humans and, belong to the α- and δ-proteobacteria (Petrović-Gegić and Baloš, 2011; Taylor et al., 1974; Kelley and Williams, 1992; Snyder et al., 2002; Davidov and Jurkevitch, 2004, 2009; Chauhan and Williams, 2006). Most δ-proteobacteria BALO species, fall into two major genera, *Bdellovibrio* (BD) and *Bacteriovorax* (Bx) (Davidov and Jurkevitch, 2004; Chen et al., 2012). *Bacteriovorax* is a saltwater genus, distinct from the freshwater/terrestrial members of BALOs by their tolerance to sodium chloride and range of % G + C ratios (Chauhan et al., 2009; Chen et al., 2011). Based on these and other differences, we hypothesized that the ABC systems could be used as a marker for distinguishing between species and genera of BALOs and also the predators from other bacteria.

In this study, we describe the distribution and functional implications of ABC systems in BALOs based on comparative genomic analyses of 8 BALO genomes (Fig. 1) from isolates with different physiological features and habitats (Fig. 1).

2. Methods

2.1. Construction of a bacteria-specific ABC system profile

The Hidden Markov Model (HMM) search profile of bacterial protein sequences of the Pfam-A 27.0 (http://pfam.sanger.ac.uk/) protein family (PF00005) was used to create a bacteria-specific ABC system HMM search profile. The Pfam ABC system profile HMM

and the HMMER 3.0 (http://hmmer.janelia.org/) program hmmsearch were used to identify the ABC system domains in the selected protein sequences. The ABC system domains, defined by the HMMER domain envelope, were aligned using MUSCLE v3.8.31 (Edgar, 2004). The alignment was manually inspected and edited by removing positions of low quality at the start and end, and the glutamate and histidine catalytic dyad residues were checked for proper alignment. Sequences lacking one or more of the conserved ATP-hydrolyzing domains were discarded. The aligned ABC_tran domains were clustered at 90% identity using the software cd-hit (Li and Godzik, 2006; Fu et al., 2012). The longest sequence in each cluster was selected to form a group of cluster representatives, in order to reduce bias due to the presence of multiple highly similar sequences in the dataset. The selected domains were aligned again, followed by manual inspection and editing, using the same approach as described above. The alignment was then used to produce the bacteria-specific ABC system HMM search profile, by applying the program hmmbuild, from the HMMER 3.0 suite, at default settings.

2.2. Identifying ABC systems in sequenced BALO genomes

FASTA files representing 8 BALO genomes: Bx BSW11 (NZ_ AUNE01000059.1), Bx DB6 (NZ_AUNJ01000508.1), Bx SJ (NR 028723.1), Bx SEQ25 (NZ_AUNI01000021.1), Bx BAL6 (NZ_ AUMC01000010.1), BD HD100 (NR 027553.1), BD Tiberius (NR 102470.1), and BD JSS (CP003537.1), were downloaded from NCBI (ftp://ftp.ncbi.nih.gov/genomes/) on March 23, 2014. ABC systems in these genomes were identified using the bacteria-specific ABC system profile HMM and HMMER 3.0 hmmsearch at default settings. Sequences with a domain independent E-value ≤ 0.01 and a score/bias ratio ≥ 10 were accepted. The ABCdb database (https://wwwabcdb.biotoul.fr/), which provides comprehensive information on ABC systems such as ABC transporter classification and predicted function (Fichant et al., 2006) was used to check predicted ABC systems.

2.3. Analyzing the domain architectures of identified ABC systems

The identified, full length ABC system sequences were subjected to a HMMER 3.0 hmmscan search at default settings, using the Pfam 27.0 protein family database of profile HMMs, supplemented with the bacteria-specific ABC system profile HMM produced earlier. In addition, structure analyses of the obtained ABC systems were performed using the SMART (Simple Modular Architecture Research Tool) (Schultz et al., 1998) and the CDD (Conserved Domains Database) (Marchler-Bauer et al., 2005), relying on Hidden Markov Models and Reverse Position-Specific BLAST, respectively.

2.4. Phylogenetic analysis

The multiple alignments of ABC_tran domain sequences and BALO 16S rRNA were conducted with the MUSCLE v3.8.31 (Edgar, 2004) using default parameters. Phylogenetic trees were constructed using NJ methods of the MEGA package (Version 4.0) (Tamura et al., 2007), and the reliability of each branch was tested by 1000 bootstrap replications.

3. Results and discussion

3.1. Uneven distribution of ABC systems in BALOs

To identify ABC systems, we used a custom bacteria-specific ABC transporter HMM profile (see Methods section) to search eight BALO genomes currently available in the NCBI database. A total of 269 putative ABC systems were found (Fig. 1 and Table S1). Interestingly, *Bacteriovorax* and *Bdellovibrio* genera which have a close phylogenetic relationship were found to have different numbers of ABC systems. *Bacteriovorax* strains encode 28 or less ABC systems. On the contrary, the freshwater *Bdellovibrio* strains have approximately 51. In the genome of *Bdellovibrio exovorus* JSS (CP003537.1), 53 putative ABC systems were detected, representing the highest of all the BALO genomes examined in this study. The relative numbers of ABC systems in *Bdellovibrio* strains were found to be significantly greater than in the *Bacteriovorax* strains (One way ANOVA, $P < 0.01$). Construction of ABC systems present in BALO genomes was also different (Fig. 2). Exporter ABC systems were higher in genomes of *Bdellovibrio* than in *Bacteriovorax*. No exporter ABC systems were detected in Bx-BAL6 genome. Interestingly, BALOs encode a larger complement of predicted ABC systems involved in cell processes (Fig. 2) than other microbial species (Fig. 2). Especially, higher number of ABC systems involved in cell processes was found in BALOs, than in the epibiotic predator *Micavibrio* spp. (Wang et al., 2011) and non-BALO predators such as *Saprospira grandis* (Saw et al., 2012). This indicates that the ABC systems involved in cell processes in the predatory BALOs may play an important role in predation and the intraplasmic growth cycle.

Compared to marine ecosystems, freshwater environments are considered to be less stable with more frequent changes in light and temperature conditions and nutrient availability (Margalef, 1978; McMichael and Butler, 2005). To survive in such an unstable environment and construct a long-term evolutionary selective advantage, freshwater bacteria may have to encode more genes within some specific gene families. For example, previous reports described variations in the numbers of ABC systems in cyanobacteria in marine and freshwater environments (Tomii and Kanehisa, 1998; Bu et al., 2009). Similar results were observed among the ABC systems in BALOs with the freshwater *Bdellovibrio* strains having about two-fold higher numbers than their saltwater counterparts. Thus, ABC systems in *Bdellovibrio* may have evolved from the environmental impacts of the freshwater environment. Another impact may be differences in the intracellular periplasmic environmental conditions between freshwater and salt water prey bacteria (Hespell et al., 1973; Ruby and McCabe, 1986).

3.2. Domain organization and core set of ABC systems in BALOs

Previous studies indicated that bacterial ABC systems could involve a great variety of additional domains with distinct functions (Tomii and Kanehisa, 1998; Paulsen et al., 2000; Davidson et al., 2008). For a more comprehensive understanding of the functional role of ABC systems in *Bacteriovorax* and *Bdellovibrio* strains, we investigated their domain architectures. Through the Pfam domain assignment, diverse domain architectures were found (Tables 1 and S1), implying versatile functions of ABC systems in *Bacteriovorax* and *Bdellovibrio*. Across the 269 putative ABC systems, the ABC system domains and other

domains, 25 organization patterns were found. Among these nearly half (126/269) are of one ABC_tran domain fused with one basic ABC transporter element. The other ABC systems are fused with various additional domains and exhibit complex architectures.

The fusion of additional domains to ABC systems can imbue them with various functional capabilities. Some functions of these additional domains have been validated by experimental approaches. For example, the HPY domain of the ABC_NikE_OppD system (number 16 in Table 1), which is involved in the transport of oligopeptides or dipeptides, is fused with the Nike_OppD domain (specific for the transport of dipeptides, oligopeptides, and nickel) involved in hydrogenase synthesis (Saier, 1998; Xu et al., 2009). Interestingly, the Carb_Monos_I domain of the ABC_Carb_Monos_I domain system (number 17 in Table 1), which represents the domain I of the carbohydrate uptake proteins that transport only monosaccharides (Monos), such as pentoses and hexoses, is found only in freshwater BALOs BD-HD 100, BD-Tiberius and BD-JSS. This suggests that freshwater BALOs can utilize external monosaccharides.

After all ABC systems were identified and grouped, a core set of ABC systems was detected in BALOs. Eight families were present in all the BALO strains, and could be assigned into two categories: import systems and cellular process systems (Table 1). Among the eight core ABC system families, seven appear to serve as import systems. The first two families (ABC_PhnC system [number 12 in Table 1] and ABC_PstB system [number 13 in Table 1]) are involved in phosphate utilization. PhnC domain is the adenosine triphosphate (ATP) binding component, responsible for direct movement of an alkylphosphonate into cells (Alicea et al., 2011). PhnC domain belongs to one of the largest superfamilies of proteins characterized by a highly conserved ATP binding cassette, which is also a nucleotide binding domain (NBD) (Holland and Blight, 1999; Rossi et al., 2006). PstB is the catalytic subunit, which couples the energy of ATP hydrolysis to the import of phosphate across cellular membranes through the Pst system (Huang et al., 2011). With these two high-affinity Pi transporters, BALOs may be able to efficiently uptake phosphate and phosphonate as phosphorus sources under Pi limited conditions. This ATP-related transport capability has been suggested to be an adaptation to the natural growth environment of BALOs within their prey (gram-negative bacteria) (Hespell et al., 1973; Rittenberg and Hespell, 1975; Ruby and McCabe, 1986).

ABC systems involved in the Fe–S cluster assembly were also found common in BALOs. This ABC system family contains two domains (FeS and ycf16). The ABC-FeS-ycf16 system (number 3 in Table 1) was reported to be important in *E. coli* and *Erwinia chrysanthemi* for Fe–S biogenesis under stressful conditions (Loiseau et al., 2003). This suggests a potential for BALOs to increase uptake and utilization of sulfate in the same way.

The ZnuC domain in ABC_DR_subfamily_A system (number 2 in Table 1) is a well-known high-affinity Mn/Zn uptake system ATP-binding protein (Higgins, 2001). The ZnuC-ABC system is detected in all the BALO genomes, suggesting that Mn/Zn ions may be essential to maintain BALO activities, such as attacking prey and penetration of prey membranes (Sockett and Lambert, 2004; Liu et al., 2006; Lambert et al., 2008, 2010).

ABC exporters in bacteria are characterized by secreting various molecules, such as peptides, lipids, hydrophobic drugs, polysaccharides, and related proteins (including hemolysin, heme-binding protein, and alkalineprotease). Six ABC systems were detected and classified as exporters in BALOs, two of them are known to export antimicrobial peptide, lipoprotein and macrolide out of cells. Only two multiple drug resistance MdlB domain related ABC systems were detected in freshwater strains (Table 1).

Besides transport, certain ABC system families are related to cellular processes (Adkins et al., 2006; Cornillot et al. 2002; Davidson et al., 2008), such as DNA repair, translation, or regulation of gene expression. Compared with other microbial species, BALOs encode a large complement of predicted ABC systems involved in cell processes (Fig. 2), such as UvrA_I domain and UvrA_II domain, which play a role in recognition and cleavage of damaged DNA. Since BALOs grow and multiply within the periplasmic space of its prey bacterium and may be exposed to bacterial restriction-modification and/or toxin–antitoxin systems, some of their ABC systems may have developed as an adaptation strategy to survive in complex microenvironments.

3.3. Phylogenetic analysis of ABC systems in BALOs

A phylogenetic tree of ABC systems from all 8 BALO strains was constructed based on domains of ABC systems using the Neighbor-Joining algorithm. From the phylogenetic tree (Fig. 3 and Table S1), it is clear that most of the ABC systems were grouped into many separate families with high bootstrap supports (>50%). However, members of each ABC system subfamily were found to have similar substrates (Table 1). Careful observation of interior branches in each cluster shows that the 8 core ABC system families are “indispensable” and represent one-third (8/25) of the total number of ABC systems. These are assumed to be associated with basic physiological functions. For example, among the 8 families, three are involved in the uptake of phosphate [ABC_PstB system, number 13 in Table 1], phosphonate [ABC_PhnC system, number 12 in Table 1] and sulfate [ABC_FeS_Assembly system, number 3 in Table 1]. Of the two families involved in cellular processes, one contains ATP-binding cassette domain of elongation factor 3 and the other with domain II of the excision repair protein UvrA. Due to limited functional information, the substrates of the ABC_YhbG system (number 15 in Table 1) conserved families remain unknown.

In addition to these commonly conserved ABC systems, many freshwater BALO specific families were observed, which were not found in salt water strains (Fig. 3). These families are able to transport substrates quite differently from salt water strains. For example, it was found that one freshwater specific cluster, ABC_FtsX_MacB_PCD system (number 22 in Table 1), detected in the three freshwater BALO genomes, has the ability to export lipoprotein and macrolide (Schmidt et al., 2004), and such ability is not found in salt water isolates. Further, this ABC system exhibited weak phylogenetic connection (bootstrap value <50%) with neighboring clusters.

Likewise, some families were only detected in *Bacteriovorax*. For example, ABC_KpsT_Wzt (number 6), involved in a polysaccharide transport system was only detected in Bx-SJ strain. This clade clustered with one of the 8 common ABC systems

(ABC_DR_subfamily_A, number 2 in Table 1) with a bootstrap value >50%. In another case, 3 genes (2 in Bx-SJ and 1 in Bx-BSW11) were grouped into a single cluster (belonging to ABC_DR_subfamily_A) which supposedly uptakes Mn/Zn that may work with the drug resistance transporter and related proteins. Interestingly, the salt water specific ABCC_MRP_like system (number 21 in Table 1) is close to the freshwater specific ABCC_MRP_DomainI system (number 19 in Table 1), which suggest that they may play similar roles.

Gene duplication events were observed in BALO specific ABC system families. For example, in a freshwater BALO specific cluster with five ABC systems [Fig. 3, 10], DB-JSS is found to have duplicate gene copies of ABC_NatA_like domain. This may result in enhanced ability to uptake Na^+ for these three freshwater BALO strains. Frequent occurrence patterns were also observed in the core set of ABC systems such as the UVR family associated with DNA repair and drug resistance, with an unknown substrate. These results indicate that specific expansion of ABC system genes combined with gene duplication appears to be the major contributors to the great divergence of the numbers of ABC systems observed between marine and freshwater BALOs. The existence of marine-specific ABC systems in salt water BALOs for certain kinds of physiological functions suggests an adaptation strategy in order to survive in marine environments.

4. Conclusions

In this study, variations in the distribution of ABC systems between marine and freshwater BALO strains were observed, and validated by phylogenetic analysis. Compared with various non-BALO species, predicted ABC systems involved in cell processes were more abundant in BALO genomes. In addition, many domains were found to fuse with ATP-binding domains, giving rise to versatile functions of ABC systems in BALOs. A conserved core of the 8 ABC systems was identified in all BALO species. More ABC systems are found in freshwater BALOs than in their saltwater counterparts. This comprehensive survey of ABC systems in BALOs provides novel insights into their physiological functions and supports the need for further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

BALOs	*Bdellovibrio*-and-like organisms
ABC	ATP-binding cassette
BD	*Bdellovibrio*

Bx	*Bacteriovorax*
HMM	Hidden Markov Model
SMART	Simple Modular Architecture Research Tool
CDD	Conserved Domain Database

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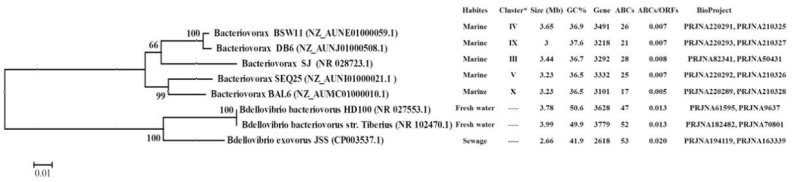

Habites	Cluster*	Size (Mb)	GC%	Gene	ABCs	ABCs/ORFs	BioProject
Marine	IV	3.65	36.9	3491	26	0.007	PRJNA220291, PRJNA210325
Marine	IX	3	37.6	3218	21	0.007	PRJNA220293, PRJNA210327
Marine	III	3.44	36.7	3292	28	0.008	PRJNA82341, PRJNA50431
Marine	V	3.23	36.5	3332	25	0.007	PRJNA220292, PRJNA210326
Marine	X	3.23	36.5	3101	17	0.005	PRJNA220289, PRJNA210328
Fresh water	----	3.78	50.6	3628	47	0.013	PRJNA61595, PRJNA9637
Fresh water	----	3.99	49.9	3779	52	0.013	PRJNA182482, PRJNA70801
Sewage	----	2.66	41.9	2618	53	0.020	PRJNA194119, PRJNA163339

Fig. 1.

Distribution of ABC systems across the phylogenetic tree of BALOs. The phylogenetic tree was constructed based on BALO 16S rRNA sequences using the Neighbor-Joining method. The reliability of the tree was evaluated with 1000 replicates of bootstrapping test and only high bootstrap value scores (>50%) were indicated on the branches. In addition, each strain is followed by its isolation habitat, total number of ORFs, as well as absolute and relative number of ABC systems and other information. *Clusters were identified by previous study (Pineiro et al., 2007). 16s rRNA sequences of strains BSW 11, DB6, SEQ25 and BAL6 were extracted from their genomic sequences according to the annotation.

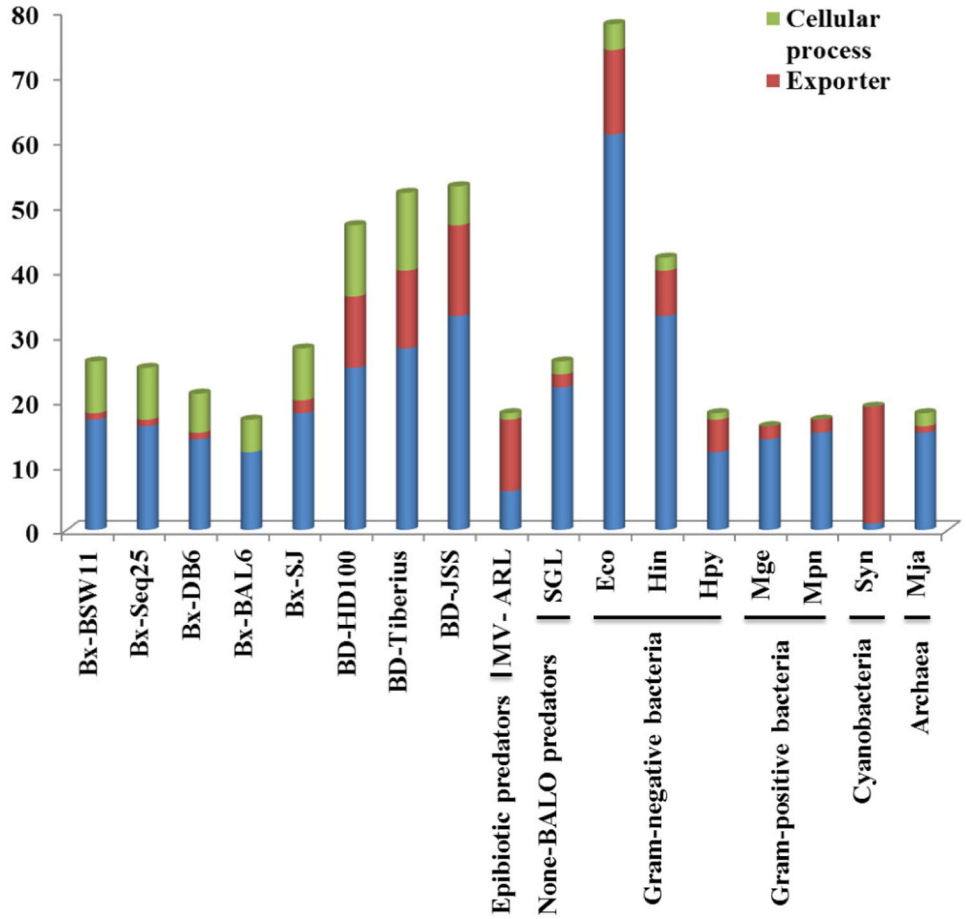

Fig. 2.

Number of predicted ABC systems present in various microbial species. Numbers of non-BALO ABC systems are reported by Dr. Kanehisa (Tomii and Kanehisa, 1998). BD, *Bdellovibrio*; Bx, *Bacteriovorax*; Eco, *Escherichia coli*; Hin, *Haemophilus influenzae*; Hpy, *Helicobacter pylori*; MV-ARL, *Micavibrio aeruginosavorus* ARL-13; SGL, *Saprospira grandis* str. Lewin; Mge, *Mycoplasma genitalium*; Mpn, *M. pneumoniae*; Syn, *Synechocystis* PCC6803 and Mja, *Methanococcus jannaschii*.

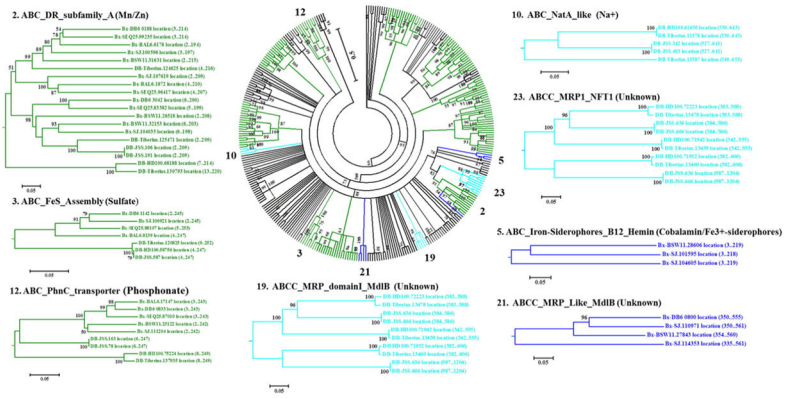

Fig. 3.

Phylogenetic tree of all of the ABC systems in BALOs. The phylogenetic tree is constructed based on the ABC system domains of ABC systems. Strain names are shortened for brevity on the phylogenetic tree using the Neighbor-Joining method. The branches of 9 common ABC system families are marked in deep green; the branches of expanded freshwater specific groups and salt water specific groups are separately marked in deep blue and light blue. Representative families were labeled with family name followed by putative substrate in bracket. BD, *Bdellovibrio* and Bx, *Bacteriovorax*. Numbers of ABC systems shown in Table 1 and S1 are made to coincide. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

Domain architectures and distribution of ABS systems in BALOs.

Order[a]	Domain architectures[b]	Bx-BSW11	Bx-Seq25	Bx-DB6	Bx-BAL6	Bx-SJ	BD-HD100	BD-Tiberius	BD-JSS	Predicted substrate[b]
	Importer									
1		0	0	0	0	0	2	2	2	Pentoses and hexoses
2		3	3	2	2	3	1	4	2	Mn/Zn
3		1	1	1	1	1	1	1	1	Sulfate
4		2	1	2	0	0	1	1	0	Histidine and glutamine
5		1	0	0	0	2	0	0	0	Cobalamin/Fe3+siderophores
6		0	0	0	0	1	0	0	0	Polysaccharide/polyol
7		0	1	0	0	0	1	1	0	Sugar
8		3	2	2	2	2	7	7	9	Antimicrobial peptide
9		1	0	0	0	1	1	1	2	Branched-chain amino acid
10		0	0	0	0	0	1	1	1	Na+
11		0	0	0	0	0	2	2	2	Nitrate/sulfonate/bicarbonate
12		1	1	1	1	1	1	1	2	Phosphonate
13		1	2	1	1	1	1	1	2	Phosphate
14		1	0	0	0	1	1	1	2	----
15		1	1	1	1	1	1	1	2	----
16		2	4	4	4	4	4	4	6	Oligopeptide/dipeptide
	Exporter									
17		0	0	0	0	0	1	1	2	Antimicrobial peptide
18		0	1	0	0	0	5	4	6	----
19		0	0	0	0	0	2	2	2	----
20		0	0	0	0	0	1	1	2	----
21		1	0	1	0	2	0	0	0	----
22		0	0	0	0	0	2	4	2	Lipoprotein and macrolide
	Cellular process									
23		0	0	0	0	0	3	4	0	----
24		5	5	3	5	5	6	6	2	----
25		3	3	3	0	3	2	2	4	----