

SHORT GENOME REPORT

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# Draft genome sequences for the obligate bacterial predators *Bacteriovorax* spp. of four phylogenetic clusters

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## Abstract

*Bacteriovorax* is the halophilic genus of the obligate bacterial predators, *Bdellovibrio* and like organisms. The predators are known for their unique biphasic life style in which they search for and attack their prey in the free living phase; penetrate, grow, multiply and lyse the prey in the intraperiplasmic phase. *Bacteriovorax* isolates representing four phylogenetic clusters were selected for genomic sequencing. Only one type strain genome has been published so far from the genus *Bacteriovorax*. We report the genomes from non-type strains isolated from aquatic environments. Here we describe and compare the genomic features of the four strains, together with the classification and annotation.

**Keywords:** Predatory bacteria, *Bdellovibrio* and like organisms, *Bacteriovorax*, Marine, Gram-negative, Motile

## Introduction

As a member of the highly diverse *Deltaproteobacteria* class, the obligate bacterial predators *Bdellovibrio* and like organisms possess unique ecological features that are worth exploring. They are the only known predatory bacteria that exhibit a life cycle alternating between an extracellular free-living phase and an intraperiplasmic phase and are capable of invading the periplasmic space of prey cells, resulting in the lysis of the prey and release of new progeny [1]. Based on their small size, about 1/5th that of a typical bacterium cell, BALOs have been called “the world’s smallest hunters”. Nevertheless, their genomes are larger than expected, more than 3.98 Mb in *Bdellovibrio. bacteriovorus* Tiberius [2], 3.78 Mb in *B. bacteriovorus* HD100 [1] and 3.44 Mb in *Bacteriovorax marinus* SJ [3]. Despite the uniqueness [4], and increasing understanding, of the potential of these organisms in various applications [5-7], their phylogeny and unique predatory features are only beginning to be understood.

Systematics has played a most important role in advancing the study of the BALOs. Based on systematic genomic molecular techniques, the original BALO genus,

*Bdellovibrio*, has been subdivided into four genera: *Bdellovibrio*, *Bacteriolyticum*, *Peredibacter*, and *Bacteriovorax* [8-10]. Being an exclusive saltwater genus, *Bacteriovorax* is distinct from the freshwater/terrestrial members of BALOs in many ways. It is ubiquitous in salt-water environments [10], requires at least 0.5% NaCl for growth, prefers saltwater prey [11], thrive at a lower temperature range [12] and has a lower % GC ratio of ca. 37% [13] compared to the 50.65% of the freshwater *Bdellovibrio bacteriovorus* HD100. Currently, *Bacteriovorax marinus* SJ is the only strain from the genus *Bacteriovorax* of which the complete genome has been sequenced and reported.

To date, variations in the 16S rRNA sequences have yielded approximately eight *Bacteriovorax* clusters or OTUs. The previously sequenced *Bacteriovorax marinus* SJ<sup>T</sup> is one of the representatives that belong to phylogenetic Cluster III. This classification scheme has enabled for the first time the detection of specific *Bacteriovorax* strains in environmental/ecological studies. The validity of using the 16S rRNA gene was tested by comparison with the *rpoB* gene [10]. The results of recent studies monitoring the activities and distribution of specific phylogenetic

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clusters have yielded new discoveries on the distribution, predation patterns, prey preferences, and ecology of this bacterial predator [14-16].

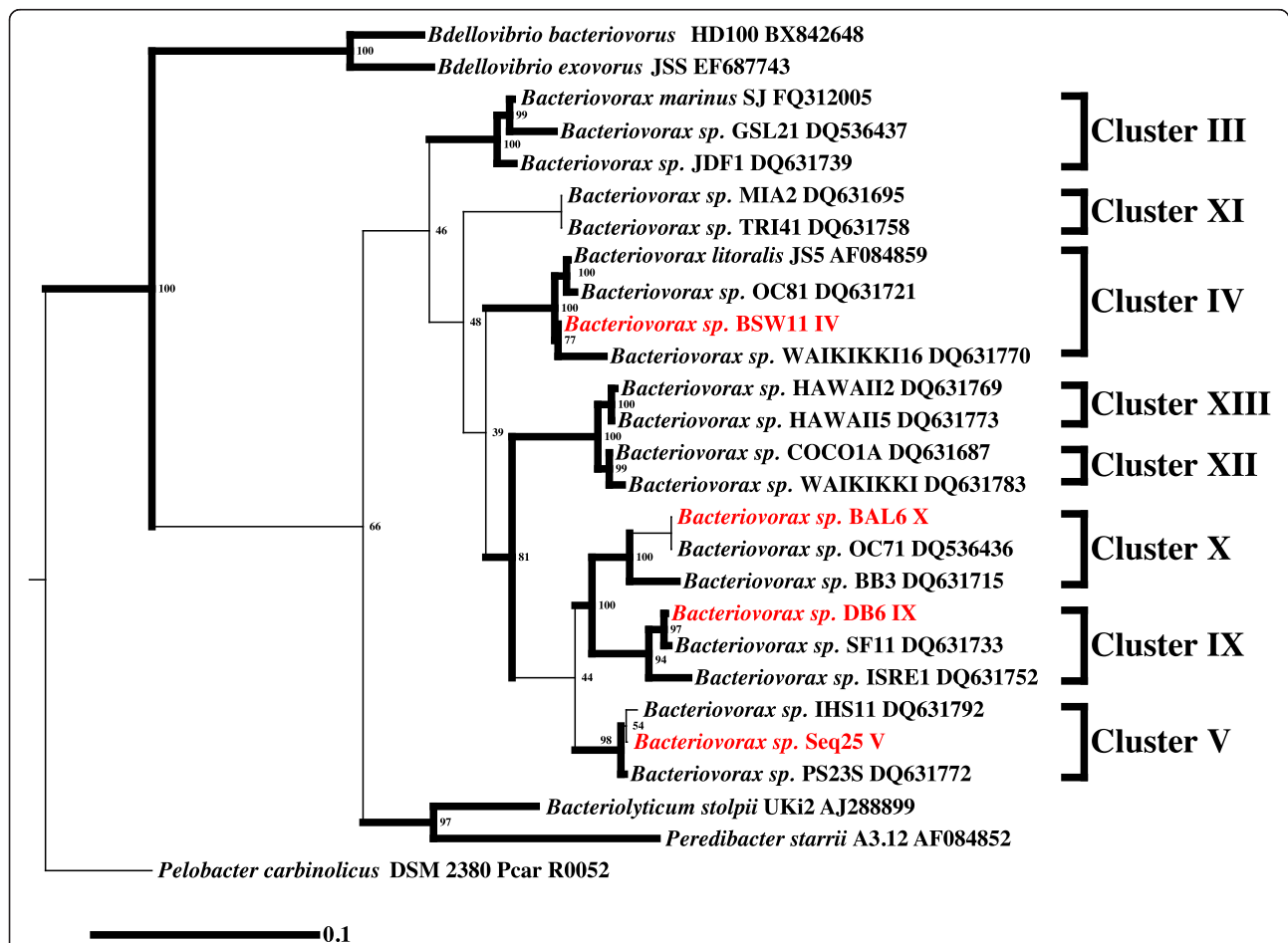
Here we present a description of the draft genomes of *Bacteriovorax* isolates of four phylogenetic clusters isolated from estuarine systems, together with the description of the genomic sequencing and annotation.

### Organism information

A 16S rRNA phylogenetic tree was constructed showing the phylogenetic neighborhood of the four newly

sequenced *Bacteriovorax* strains within the family of *Bdellovibrionaceae* (Figure 1). As expected, *Bacteriovorax* sp. strain BSW11\_IV was grouped together with cluster IV, strain SEQ25\_V with cluster V, Strain DB6\_IX with Cluster IX and lastly strain BAL6\_X with cluster X.

General features of *Bacteriovorax* spp. are summarized in Table 1. Individual features of *Bacteriovorax* isolates have not been sufficiently explored and are largely unknown. Micrographs generated by both transmission electron microscopy and scanning microscopy (Figure 2) suggest that *Bacteriovorax* spp. employ similar predation



**Figure 1** Phylogenetic tree highlighting (red) the position of four newly sequenced *Bacteriovorax* strains relative to the type strains within the family *Bdellovibrionaceae* and two non-type strains of each *Bacteriovorax* phylogenetic clusters. The tree was constructed using 16S rRNA gene sequences aligned by the RDP aligner, and was inferred using RaxML 7.25 [17] with the GTRGAMMA model of sequence evolution. The strains and their corresponding GenBank accession numbers for 16S rRNA genes were (type = T): *Bacteriovorax* sp. BSW11\_IV; *Bacteriovorax* sp. SEQ25\_V; *Bacteriovorax* sp. DB6\_IX; *Bacteriovorax* sp. BAL6\_X; *Bdellovibrio bacteriovorus* HD100<sup>T</sup> (BX842648); *Bacteriolyticum stolpii* UKi2<sup>T</sup> (AJ288899); *Bacteriovorax marinus* SJ<sup>T</sup> (FQ312005); *Peredibacter starrii* A3.12<sup>T</sup> (AF084852); *Bx* sp. BB3 (DQ631715); *Bx* sp. OC71 (DQ536436); *Bacteriovorax* sp. PS23S (DQ631772); *Bx* sp. IHS11 (DQ631792); *Bacteriovorax* sp. SF11 (DQ631733); *Bacteriovorax* sp. ISRE1 (DQ631752); *Bacteriovorax* sp. GSL21 (DQ536437); *Bacteriovorax* sp. JDF1 (DQ631739); *Bacteriovorax* sp. MIA2 (DQ631695); *Bacteriovorax* sp. TRI41 (DQ631758); *Bx* sp. COCO1A (DQ631687); *Bacteriovorax* sp. WAIKIKKI (DQ631783); *Bx* sp. OC81 (DQ631721); *Bacteriovorax* sp. WAIKIKKI16 (DQ631770); *Bacteriovorax* sp. HAWAII2 (DQ631769); *Bx* sp. HAWAII5 (DQ631773). *Deltaproteobacterium*, *Pelobacter carbinolicus* DSM2380 (CP000142), was used as an out-group. The numbers along the branches reflect the proportion of times the groups cluster together based on 100 bootstrapped replicates. Thick branches represent those with greater than 75% bootstrap support. Phylogenetic clusters of *Bacteriovorax* based on 96.5% or greater 16S rRNA gene sequence similarity are denoted by brackets on the right of the tree. Clusters were numbered consistently with previous reports [9,10,18].

**Table 1 Classification and general features of *Bacteriovorax* strains according to the MIGS recommendations [19]**

MIGS ID	Property	Term	Evidence code <sup>a</sup>	
	Current classification	Domain	<i>Bacteria</i>	TAS [20]
		Phylum	<i>Proteobacteria</i>	TAS [21]
		Class	<i>Deltaproteobacteria</i>	TAS [22,23]
		Order	<i>Bdellovibrionales</i>	TAS [24]
		Family	<i>Bacteriovoracaceae</i>	TAS [25]
		Genus	<i>Bacteriovorax</i>	TAS [3]
		Species	Cluster IV, Cluster V, Cluster IX, Cluster X	TAS [18]
		Strains:	BSW11_IV, SEQ25_V, DB6_IX, BAL6_X	IDA
	Gram stain	Negative	TAS [26]	
	Cell shape	comma-shaped, 0.35-1.2 μm	TAS [26]	
	Motility	motile (one single, polar, sheathed flagellum)	TAS [26]	
	Sporulation	Non-sporulating	NAS	
	Temperature range	10-35°C	TAS [13]	
	Optimum temperature	15-30°C	TAS [3]	
	Carbon source	Peptides, proteins	TAS [13]	
	Energy source	Chemo-organotroph	TAS [13]	
	Terminal electron receptor	Unknown	IDA	
MIGS-6	Habitat	marine, estuarine	TAS [18]	
MIGS-6.3	Salinity	>0.5‰	TAS [3]	
MIGS-22	Oxygen	Aerobic	NAS	
MIGS-15	Biotic relationship	free living/ parasitic	TAS [3]	
MIGS-14	Pathogenicity	Not reported	TAS [3]	
MIGS-4	Geographic location	Breton Sound, LA (BSW11_IV);	IDA	
		Barataria Bay, LA (SEQ25_V);		
		Apalachicola Bay, FL (DB6_IX, BAL6_X)		
MIGS-5	Sample collection time	April, 2011 (BSW11_IV);	IDA	
		June, 2011 (SEQ25_V);		
		October, 2010 (DB6_IX, , BAL6_X)		
MIGS-4.1 MIGS-4.2	Latitude – Longitude	29.63 -89.66 (BSW11_IV);	IDA, TAS [14]	
		29.38 -89.98 (SEQ25_V);		
		29.67 -85.09 (DB6_IX, BAL6_X)		
MIGS-4.3	Depth	not reported (BSW11_IV, SEQ25_V);	NAS,TAS [14]	
		1.74 m (DB6_IX, BAL6_X)		
MIGS-4.4	Altitude	not reported	IDA	

<sup>a</sup>Evidence codes - IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [27].

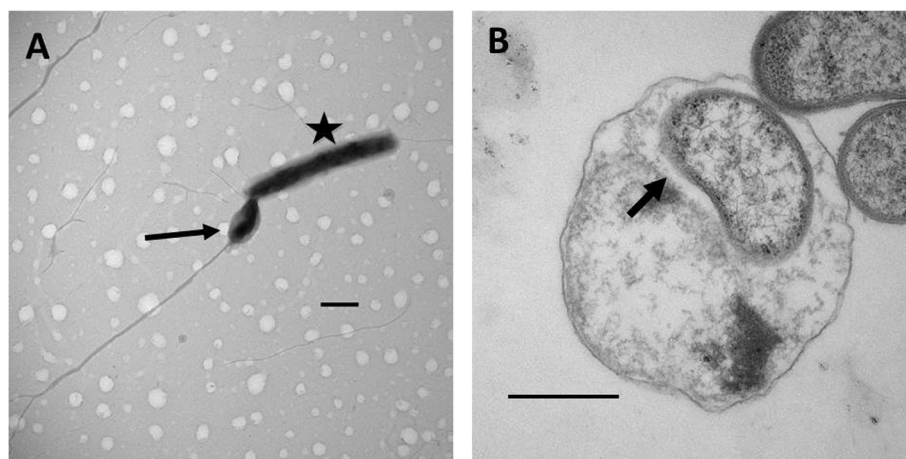
strategies as other BALO members to attack and reside in the periplasmic space of its prey.

## Genome sequencing information

### Genome project history

The four genomes were selected for sequencing on the basis of their phylogenetic position and isolation source. Low salt *Bacteriovorax* sp. BSW11\_IV was isolated from Breton Sound, Louisiana (salinity 0.6 ppt; Temperature

26.4°C) and SEQ25\_V was obtained from water samples of Barataria Bay, Louisiana (salinity 5.2 ppt; Temperature 19.2°C). High salt DB6\_IX (Salinity 32.4 ppt; Temperature 24.1°C) and BAL6\_X (Salinity 30.9 ppt; Temperature 25.2°C) were obtained from Apalachicola Bay, Florida. The genome sequences were deposited in GenBank. Sequencing and annotation were performed at the J. Craig Venter Institute. Table 2 presents the project information and its association with MIGS version 2.0 compliance [19].



**Figure 2** Electron micrographs showing (A) *Bx* sp. DB6\_IX (arrow) attached to the polar end of the prey cell *V. vulnificus* (Star); (B) thin sections of bdelloplast, the post-BALO infection structure with the predator (arrow) residing inside the prey cell. Scale Bar represents 500 nm.

### Growth conditions and DNA isolation

*Bacteriovorax* cultures were grown separately in 70% artificial sea water (ASW) (Instant Ocean, Aquarium Systems, Inc., Mentor, Ohio) (pH 8, salinity 22 ppt.) amended with prey, *Vibrio. vulnificus* CMCP6 (for *Bx* sp. BSW11\_IV and SEQ25\_V), or *V. parahaemolyticus* RIMD 2210633 (for *Bacteriovorax* sp. DB6\_IX and BAL6\_X). The genomes of both prey bacteria have been sequenced previously [28,29]. When cultures became clear (2–3 days after inoculation of the prey), which indicated the majority of the prey cells were lysed by the predators, 300 ml suspensions were filtered consecutively through 0.45 and 0.22  $\mu\text{m}$  sterile syringe filters (Corning, NY, USA) to remove any remaining prey. Filtrates containing high concentrations of *Bacteriovorax* cells (ca.  $4 \times 10^8$  PFU ml<sup>-1</sup>) were centrifuged at  $27,485 \times g$  for 20 min at 4°C. The

pellets were then re-suspended in 1 ml of ASW respectively. To test that the concentrated *Bacteriovorax* suspensions were free of prey cell contamination, aliquots of 0.1 ml of the filtrate were spread-plated onto LB agar and incubated at 37°C for two days.

Subsequently, total DNA from the cell pellets were extracted using the QIAGEN Kit (QIAamp DNA Mini Kit), according to the manufacturer's protocol. The concentration and purity of DNA was measured by a NanoDrop Spectrophotometer (ND 1000, Thermo Fisher Scientific, DE). To reconfirm the phylotype of the isolations, the DNA was PCR amplified using *Bacteriovorax* specific primers, Bac-676 F (5'-ATT TCG CAT GTA GGG GTA-3') and Bac-1442R (5'-GCC ACG GCT TCA GGT AAG-3') [30] by puReTaq Ready-To-Go PCR Beads (GE Healthcare Bio-Sciences). PCR products were purified

**Table 2** Genome sequencing project information

MIGS ID	Property	BSW11_IV	SEQ25_V	DB6_IX	BAL6_X
MIGS-31	Finishing quality	improved-high-quality draft	improved-high-quality draft	improved-high-quality draft	improved-high-quality draft
MIGS-28	Libraries used	3 KB 454 PE, 327 bp avg. insert Illumina fragment	3 KB 454 PE, 335 bp avg. insert Illumina fragment	3 KB 454 PE, 346 bp avg. insert Illumina fragment	3 KB 454 PE, 316 bp avg. insert Illumina fragment
IGS-29	Sequencing platforms	Illumina GAll, 454 GS FLX Titanium	Illumina GAll, 454 GS FLX Titanium	Illumina GAll, 454 GS FLX Titanium	Illumina GAll, 454 GS FLX Titanium
MIGS-31.2	Fold coverage	700x hybrid coverage	85x hybrid coverage	583x hybrid coverage	81x hybrid coverage
MIGS-30	Assemblers	Newbler 2.6	CLC 5.0	CA 7.0	CA 7.0
MIGS-32	Gene calling method	Glimmer 3.02	Glimmer 3.02	Glimmer 3.02	Glimmer 3.02
	Genome Database release	August 16, 2013	August 16, 2013	August 16, 2013	August 5, 2013
	Genbank ID	PRJNA210325	PRJNA210326	PRJNA210327	PRJNA210328
	Genbank Date of Release	August 16, 2013	August 16, 2013	August 16, 2013	August 5, 2013
	GOLD ID	Gi0051698	Gi19265	Gi0051699	GOi005170
MIGS-13	Project relevance	Environment	Environment	Environment	Environment

**Table 3 Summary of genomes**

Label	Size (Mb)	Topology	INSDC identifier
BSW11_IV	3.65	Circular	PRJNA210325
SEQ25_V	3.45	Circular	PRJNA210326
DB6_IX	2.97	Circular	PRJNA210327
BAL6_X	3.23	Circular	PRJNA210328

with the QIAquick PCR-Purification Kit (QIAGEN) and sequenced with Bac-676 F primer at the DNA Sequencing Laboratory at Florida State University. 16S DNA sequences were analyzed with the Basic Local Alignment Search Tool (BLAST) server from the National Center of Biotechnology Information [31].

#### Genome sequencing and assembly

Genome sequencing of the four *Bacteriovorax* isolates was conducted at the J. Craig Venter Institute employing a combination of Illumina and 454 sequencing platforms. The 454 data consisted of a half plate of 454 FLX per genome from 3 KB mate paired libraries. The Illumina data consisted of one-quarter lane of 2 × 100 bp Illumina HiSeq data per genome. On average, 300,000 454 reads (average length trimmed 300 bp) and 10 million Illumina sequences (average length trimmed 100 bp) were generated per genome. To incorporate a hybrid assembly using both 454 and Illumina sequence libraries, one million reads were randomly sampled (with their mates) from the Illumina library using Celera [32], which was sufficient to provide high coverage in the initial assemblies.

#### Genome annotation

Genes were identified using GLIMMER3 [33] as part of the JCVI prokaryotic annotation pipeline followed by manual curation using the Manatee annotation-editing platform. The JCVI automated pipeline incorporates HMM3 [34] searches against Pfam [35] and TIGRFAMS [36] and BLASTP against UniProt [37], JCVI's database of experimentally characterized proteins CharProt DB [38], and PIR [39].

#### Genome properties

The *Bacteriovorax* sp. BSW11\_IV draft genome contains 3,650,096 bp with a GC content of 37%. The hybrid assembly was scanned for contamination using BlastP and the appropriate contigs were filtered out. The final assembly comprised of 3 scaffolds, 30 RNAs and 3457 CDS. For the CDSs, 2591 (75%) proteins had a BLASTP hit with an e-value of 1e-9 or better to *Bacteriovorax marinus* SJ, and an additional 151 (4%) CDSs had a hit within the genus *Bdellovibrio*.

The *Bacteriovorax* sp. SEQ25\_V draft genome contains 3,450,786 bp with a GC content of 37%. The sequences were assembled into 29 contigs comprised of 35 RNAs and 3,292 CDSs. Among the CDSs, 2,456 (75%) of proteins had a BLASTP hit with an e-value of 1e-9 or better to *Bacteriovorax marinus* SJ, and an additional 131 (4%) CDSs had a hit within the genus *Bdellovibrio*.

The *Bacteriovorax* sp. DB6\_IX draft genome contains 2,969,235 bp with a GC content of 38%. Sequences were assembled into 10 scaffolds with 30 RNAs and 3192 CDSs. Among the CDSs, 2,253 (71%) proteins had a BLASTP hit with an e-value of 1e-9 or better to *Bx marinus* SJ, and an additional 97 (3%) CDSs had a hit within the genus *Bdellovibrio*.

The *Bacteriovorax* sp. BAL6\_X draft genome contains 3,233,679 bp with a GC content of 36%. The reads were assembled into 9 contigs with 37 RNAs and 3,065 CDSs. Among the CDSs, 2,298 (72%) proteins had a BLASTP hit with an e-value of 1e-9 or better to *Bacteriovorax marinus* SJ, and an additional 92 (3%) CDSs had a hit within the genus *Bdellovibrio*.

It is noteworthy to point out that three phage tail fiber proteins were identified within the *Bx* sp. BSW11\_IV genome but were absent from all the other BALO genome including the completed *Bacteriovorax marinus* SJ and *Bdellovibrio bacteriovorus* HD100 genomes. A staphylococcal phi-Mu50B-like prophage element was present in both SJ and HD 100 genomes but was not found in the genomes of the four newly sequenced *Bacteriovorax* isolates. The properties and the statistics of the genome are summarized in Tables 3, 4 and 5 and (Additional file 1: Table S1).

**Table 4 Nucleotide content and gene count levels of the genome**

	BSW11_IV		SEQ25_V		DB6_IX		BAL6_X	
	Value	% of total <sup>a</sup>	Value	% of total <sup>a</sup>	Value	% of total <sup>a</sup>	Value	% of total <sup>a</sup>
Genome size (bp)	3,650,096	100.00%	3,450,786	100.00%	2,969,235	100.00%	3,233,679	100.00%
G + C content (bp)	1,347,908	36.93%	1,243,844	36.05%	1,117,420	37.63%	1,179,198	36.47%
Total genes	3,487	100.00%	3,327	100.00%	3,222	100.00%	3,102	100.00%
RNA genes	30	0.86%	35	1.05%	30	0.93%	37	1.19%
Protein-coding genes	3,457	99.14%	3,292	98.95%	3,192	99.07%	3,065	98.81%
Proteins assigned to COGs	2,144	62.02%	2,045	62.12%	1,911	59.87%	1,815	59.22%
Proteins with transmembrane helices	708	20.48%	650	19.74%	578	18.11%	661	21.57%

<sup>a</sup>The total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome.

**Table 5 Number of genes associated with the 25 general COG functional categories**

Code	BSW11_IV		SEQ25_V		DB6_IX		BAL6_X		Description
	Value	% of total <sup>a</sup>	Value	% of total	Value	% of total	Value	% of total	
J	166	4.80	167	5.07	137	4.29	163	5.31	Translation
A	0	0.00	0	0.00	0	0.00	0	0.00	RNA processing and modification
K	126	3.64	121	3.67	109	3.41	108	3.52	Transcription
L	115	3.32	103	3.12	100	3.13	112	3.65	Replication and repair
B	1	0.03	1	0.03	2	0.06	1	0.03	Chromatin structure and dynamics
D	24	0.69	27	0.82	23	0.72	29	0.94	Cell cycle control and mitosis
Y	0	0.00	0	0.00	0	0.00	0	0.00	Nuclear structure
V	45	1.3	34	1.03	31	0.97	40	1.30	Defense mechanisms
T	229	6.62	205	6.22	219	6.86	126	4.11	Signal transduction mechanisms
M	155	4.48	161	4.89	129	4.04	140	4.56	Cell wall/membrane/biogenesis
N	95	2.74	82	2.49	68	2.13	71	2.31	Cell motility
Z	2	0.06	3	0.09	2	0.06	3	0.10	Cytoskeleton
W	21	0.61	22	0.67	16	0.50	19	0.62	Extracellular structures
U	43	1.42	43	1.31	38	1.19	44	1.43	Intracellular trafficking and secretion
O	125	3.61	118	3.58	97	3.03	118	3.84	Posttranslational modification, protein turnover, chaperones
C	117	3.38	123	3.73	106	3.32	112	3.65	Energy production and conversion
G	72	2.08	80	2.43	67	2.09	53	1.72	Carbohydrate transport and metabolism
E	192	5.55	166	5.04	166	5.20	138	4.50	Amino Acid transport and metabolism
F	53	1.53s	51	1.54	65	2.03	49	1.59	Nucleotide transport and metabolism
H	76	2.19	83	2.52	72	2.25	73	2.38	Coenzyme transport and metabolism
I	108	3.12	91	2.76	104	3.25	96	3.13	Lipid transport and metabolism
P	90	2.60	84	2.55	88	2.75	87	2.83	Inorganic ion transport and metabolism
Q	54	1.56	60	1.82	69	2.16	48	1.56	Secondary metabolites biosynthesis, transport and catabolism
R	308	8.90	296	8.99	274	8.58	262	8.54	General Functional Prediction only
S	156	4.51	150	4.55	124	3.88	142	4.63	Function Unknown
-	1372	39.68	1293	39.27	1355	42.44	1244	40.58	Not in COG

<sup>a</sup>The total is based on the total number of protein coding genes in the annotated genome.

## Insights from the genome sequences

### Genome Comparisons between BALO Members

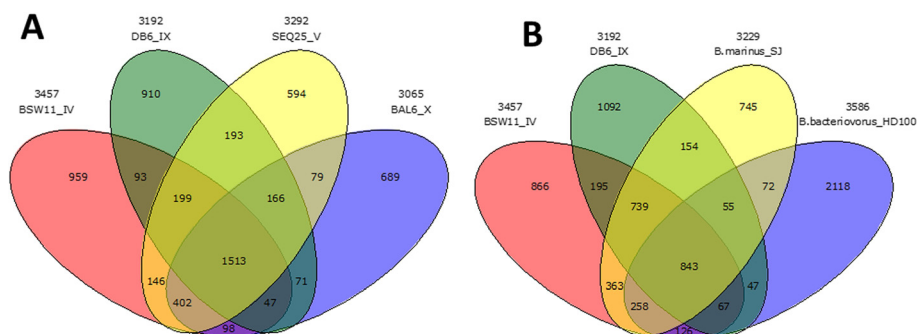
Crossman et al., [40] reported that the genomic sequences of *Bacteriovorax marinus* SJ were unique with about one third of predicted genes over 500 bp in length having no significant hit in the databases. No genomic synteny was found between SJ and its closest whole genome sequenced relative at that time, *Bdellovibrio bacteriovorus* HD100.

We found that even within the genus *Bacteriovorax*, the genomic sequences were highly divergent with an average identity of 70%. A Venn diagram summarizing the comparison of the four *Bacteriovorax* isolates is presented in Figure 3. As shown in the diagram, a core of 1,513 proteins is shared by all four *Bacteriovorax* genomes and each encodes many proteins without orthologs in the other three (Figure 3A). When compared to the freshwater/

terrestrial *Bdellovibrio bacteriovorus* HD 100, only a total of 843 genes were shared between all BALO members (Figure 3B). The calculated ANI [41] for BALO members (Additional file 2: Table S2) is below 75%, which is the threshold for the scores to be reliable. The AAI among the five *Bacteriovorax* genomes ranged between 50% to 60% (Additional file 3: Table S3), also significantly lower than the typical values found for species within a genus (73%-99.5%) [42,43]. Currently, several proposals to clarify and revise the systematics of BALOs are under consideration.

### Comparisons of BALOs and non-predatory bacteria

Phylogenetically, most genera of BALOs (including *Bacteriovorax*) are classified as *Deltaproteobacteria*. Members of this class are found in diverse environments with various lifestyles such as *Myxococcus xanthus* which is



**Figure 3** Venn diagram of shared and unique genes in (A) the four newly sequenced *Bacteriovorax* isolates, (B) three *Bacteriovorax* strains and *Bdellovibrio bacteriovorus* HD100. Orthology was assumed using the best reciprocal BLASTP matches (cutoff P value = 10<sup>-9</sup>).

characterized by its gliding motility and wolf pack predatory strategy to prey on other bacteria [44], *Pelobacter carbinolicus* which grows by using iron and sulfur as electron acceptors [45], and the focus of this study, the obligate predators *Bacteriovorax* spp. which replicate within the periplasmic space of prey bacteria. Although their ecological features are distinct, the genomes of *Deltaproteobacteria* were found to exhibit some common characteristics. For example, most *Deltaproteobacteria*, including the *Bdellovibrio bacteriovorus* HD 100, typically possess two giant S1 ribosomal protein genes and high numbers of TonB receptors and ferric siderophore receptors which facilitate metal uptake and removal [46]. In contrast, only one giant S1 protein was found in the *Bacteriovorax marinus* SJ genome [40], and our study confirmed that this is the case for the other four *Bacteriovorax* genomes. *Bacteriovorax* genomes also encode multiple TonB receptor proteins (6–11 copies) and ferric siderophore receptors (2–4 copies) that they may use for predation.

Using a reciprocal best match analysis with e-value cutoff of 10<sup>-9</sup>, 843 core genes were found to contain orthologs in all six BALO genomes including previously sequenced SJ and HD 100 genomes (see center of Figure 3B). Fifty nine of these genes (Additional file 4: Table S4) have no homologs with an E-value of 10<sup>-9</sup> or lower to proteins from any non-predatory bacterium in the NCBI “nr” database. These genes, including periplasmic proteins, a radical activating enzyme and an outer membrane channel protein, may represent a core set of unique genes involved in the predatory process and prey interactions such as locating the prey, degradation and consumption of prey cellular content, formation of bdelloplast, synchronous nonbinary septation or release of progeny from the ghost cell.

## Conclusion

The genomes of four *Bacteriovorax* phylogenetic clusters isolated from the environment were sequenced. The genome sizes of the four strains were comparable with

*Bacteriovorax* SJ but were slightly smaller than the two freshwater BALOs, *Bdellovibrio bacteriovorus* Tiberius and *B. bacteriovorus* HD100. Fifty-nine genes were identified that are conserved among BALOs, but not present in other organisms, that may be responsible for their predatory life style. The unique genomic features of *Bacteriovorax* that are essential for their ecological function were also reported.

## Additional files

**Additional file 1: Table S1.** Associated MIGS record.

**Additional file 2: Table S2.** Comparison of the average nucleotide identity (ANI) for the BALO genomes. ANI was calculated using ANI.pl script (<https://github.com/chjp/ANI/blob/master/ANI.pl>). All values are in percentages.

**Additional file 3: Table S3.** Percentage of average amino acid identity (AAI) between BALO genomes. AAI calculation of all two-way BLAST conserved genes was computed using AAI.rb script (<http://enveomics.blogspot.com/2013/10/airb.html>).

**Additional file 4: Table S4.** Annotation of genes that are present in all BALO members but have no homologs from any non-predatory bacterium in NCBI’s “nr” database (E-value < 10<sup>-9</sup>). Genes are listed by the protein number in BALO genomes.

## Abbreviations

BALOs: *Bdellovibrio* and Like Organisms; ANI: Average nucleotide identity; AAI: Average amino acid identity.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

JHB and HNW initiated and supervised the study. HC draft the manuscript, conducted wetlab work and performed electron microscopy. HC, LMB, DSL, TLD and NG annotated the genome. HC, PM, LMB and JHB worked on genome sequencing and assembly. HC, NL, JHB, PM and HNW discussed, analyzed the data and revised the manuscript. All authors read and approved the final manuscript.

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#### References

1. Rendulic S, Jagtap P, Rosinus A, Eppinger M, Baar C, Lanz C. A predator unmasked: life cycle of *Bdellovibrio bacteriovorus* from a genomic perspective. *Science*. 2004;303:689–92.
2. Hobley L, Lerner TR, Williams LE, Lambert C, Till R, Milner DS, et al. Genome analysis of a simultaneously predatory and prey-independent, novel *Bdellovibrio bacteriovorus* from the River Tiber, supports in silico predictions of both ancient and recent lateral gene transfer from diverse bacteria. *BMC Genomics*. 2012;13:670.
3. Baer ML, Ravel J, Pineiro SA, Guether-Borg D, Williams HN. Reclassification of salt-water *Bdellovibrio* sp. as *Bacteriovorax marinus* sp. nov. and *Bacteriovorax litoralis* sp. nov. *Int J Syst Evol Microbiol*. 2004;54:1011–6.
4. Chen H, Williams HN. Sharing of prey: coinfection of a bacterium by a virus and a prokaryotic predator. *MBio* 2012;3(2):e00051-12.
5. Atterbury RJ, Hobley L, Till R, Lambert C, Capeness MJ, Lerner TR, et al. Effects of orally administered *Bdellovibrio bacteriovorus* on the well-being and *Salmonella* colonization of young chicks. *Appl Environ Microbiol*. 2011;77:5794–803.
6. Dashiff A, Junka R, Libera M, Kadouri D. Predation of human pathogens by the predatory bacteria *Micavibrio aeruginosavorus* and *Bdellovibrio bacteriovorus*. *J Appl Microbiol*. 2011;110:431–44.
7. Cao H, Hou S, He S, Lu L, Yang X. Identification of a *Bacteriovorax* sp. isolate as a potential biocontrol bacterium against snakehead fish-pathogenic *Aeromonas veronii*. *J Fish Dis*. 2013;37(3):283–289.
8. Baer ML, Ravel J, Chun J, Hill RT, Williams HN. A proposal for the reclassification of *Bdellovibrio stolpii* and *Bdellovibrio starrii* into a new genus, *Bacteriovorax* gen. nov. as *Bacteriovorax stolpii* comb. nov. and *Bacteriovorax starrii* comb. nov., respectively. *Int J Syst Evol Microbiol*. 2000;50 Pt 1:219–24.
9. Davidov Y, Jurkevitch E. Diversity and evolution of *Bdellovibrio*-and-like organisms (BALOs), reclassification of *Bacteriovorax starrii* as *Peredibacter starrii* gen. nov., comb. nov., and description of the *Bacteriovorax-Peredibacter* clade as *Bacteriovoracaceae* fam. nov. *Int J Syst Evol Microbiol*. 2004;54:1439–52.
10. Pineiro SA, Williams HN, Stine OC. Phylogenetic relationships amongst the saltwater members of the genus *Bacteriovorax* using *rpoB* sequences and reclassification of *Bacteriovorax stolpii* as *Bacteriolyticum stolpii* gen. nov., comb. nov. *Int J Syst Evol Microbiol*. 2008;58:1203–9.
11. Schoefield AJ, Williams HN. Efficiencies of Recovery of *Bdellovibrios* from Brackish- Water Environments by Using Various Bacterial Species as Prey. *Appl Environ Microbiol*. 1990;56:230–6.
12. Williams HN. Cultural, immunologic and ecologic studies of marine bdellovibrios isolated from the Atlantic Ocean and the Chesapeake Bay. Baltimore, MD, USA: University of Maryland; 1979.
13. Marbach A, Varon M, Shilo M. Properties of marine bdellovibrios. *Microb Ecol*. 1976;2:284–95.
14. Chen H, Young S, Berhane T-K, Williams HN. Predatory *Bacteriovorax* Communities Ordered by Various Prey Species. *PLoS ONE*. 2012;7:e34174.
15. Chauhan A, Cherrier J, Williams HN. Impact of sideways and bottom-up control factors on bacterial community succession over a tidal cycle. *Proc Natl Acad Sci U S A*. 2009;106:4301–6.
16. Chen H, Athar R, Zheng G, Williams HN. Prey bacteria shape the community structure of their predators. *ISME J*. 2011;5:1314–22.
17. Stamatakis A, Hoover P, Rougemont J. A rapid bootstrap algorithm for the RAxML web servers. *Syst Biol*. 2008;57:758–71.
18. Pineiro S, Stine O, Chauhan A, Steyert S, Smith R, Williams H. Global survey of diversity among environmental saltwater *Bacteriovoracaceae*. *Environ Microbiol*. 2007;9:2441–50.
19. Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, et al. The minimum information about a genome sequence (MIGS) specification. *Nat Biotechnol*. 2008;26:541–7.
20. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains *Archaea*, *Bacteria*, and *Eucarya*. *Proc Natl Acad Sci U S A*. 1990;87:4576–9.
21. Garrity GM, Holt JG. The Road Map to the Manual. In: Garrity GM, Boone DR, Castenholz RW, editors. *Bergey's Manual of Systematic Bacteriology*, vol. 1. 2nd ed. New York: Springer; 2001. p. 119–69.
22. Validation List No. 107: List of new names and new combinations previously effectively, but not validly, published. *Int J Syst Evol Microbiol*. 2006;56:1–6.
23. Kuever J, Rainey FA, Widdel F. Class VI. *Deltaproteobacteria* class nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM, editors. *Bergey's Manual of Systematic Bacteriology*. Volume 2, part C. 2nd ed. New York: Springer; 2005.
24. Garrity GM, Bell JA, Lilburn T. Order VII. *Bdellovibrionales* ord. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM, editors. *Bergey's Manual of Systematic Bacteriology*. Volume 2, Part C. 2nd ed. New York: Springer; 2005. p. 1040.
25. Garrity GM, Bell JA, Lilburn T. Family I. *Bdellovibrionaceae* fam. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM, editors. *Bergey's Manual of Systematic Bacteriology*. Volume 2, Part C. 2nd ed. New York: Springer; 2005. p. 1040–1.
26. Williams H, Baer M, Tudor J. *Bdellovibrio Stolp* and *Starr* 1963, 243<sup>AL</sup>. In: *Bergey's Manual of Systematic Bacteriology*. 2005. p. 1041–53.
27. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet*. 2000;25:25–9.
28. Makino K, Oshima K, Kurokawa K, Yokoyama K, Uda T, Tagomori K, et al. Genome sequence of *Vibrio parahaemolyticus*: a pathogenic mechanism distinct from that of *V. cholerae*. *Lancet*. 2003;361:743–9.
29. Kim YR, Lee SE, Kim CM, Kim SY, Shin EK, Shin DH, et al. Characterization and pathogenic significance of *Vibrio vulnificus* antigens preferentially expressed in septicemic patients. *Infect Immun*. 2003;71:5461.
30. Davidov Y, Friedjung A, Jurkevitch E. Structure analysis of a soil community of predatory bacteria using culture-dependent and culture-independent methods reveals a hitherto undetected diversity of *Bdellovibrio*-and-like organisms. *Environ Microbiol*. 2006;8:1667–73.
31. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol* 1990;215(3):403–410.
32. Miller JR, Delcher AL, Koren S, Venter E, Walenz BP, Brownley A, et al. Aggressive assembly of pyrosequencing reads with mates. *Bioinformatics*. 2008;24:2818–24.
33. Delcher AL, Harmon D, Kasif S, White O, Salzberg SL. Improved microbial gene identification with GLIMMER. *Nucleic Acids Res*. 1999;27:4636–41.
34. Eddy SR. Accelerated profile HMM searches. *PLoS Comput Biol*. 2011;7:e1002195.
35. Finn RD, Clements J, Eddy SR. HMMER web server: interactive sequence similarity searching. *Nucleic Acids Res*. 2011;39:W29–37.
36. Haft DH, Selengut JD, Richter RA, Harkins D, Basu MK, Beck E. TIGRFAMs and genome properties in 2013. *Nucleic Acids Res*. 2013;41:D387–95.
37. Magrane M. UniProt Knowledgebase: a hub of integrated protein data. *Database: J Biol Dat Curation*. 2011;2011.
38. Madupu R, Richter A, Dodson RJ, Brinkac L, Harkins D, Durkin S, et al. CharProtDB: a database of experimentally characterized protein annotations. *Nucleic Acids Res*. 2011;40(D1):D237.
39. Barker WC, Garavelli JS, Huang H, McGarvey PB, Orcutt BC, Srinivasarao GY, et al. The Protein Information Resource (PIR). *Nucleic Acids Res*. 2000;28:41–4.
40. Crossman LC, Chen H, Cerdeño-Tárraga A-M, Brooks K, Quail MA, Pineiro SA, et al. A small predatory core genome in the divergent marine *Bacteriovorax marinus* SJ and the terrestrial *Bdellovibrio bacteriovorus*. *ISME J*. 2012;7:148–60.
41. Konstantinidis KT, Tiedje JM. Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci U S A*. 2005;102:2567–72.
42. Konstantinidis K, Tiedje J. Towards a genome-based taxonomy for prokaryotes. *J Bacteriol*. 2005;187:6258.
43. Thompson C, Vicente A, Souza R, Vasconcelos A, Vesth T, Alves N, et al. Genomic taxonomy of vibrios. *BMC Evol Biol*. 2009;9:258.
44. Reichenbach H. The ecology of the myxobacteria. *Environ Microbiol*. 1999;1:15–21.
45. Lovley DR, Phillips E, Lonergan DJ, Widman PK. Fe (III) and S<sup>0</sup> reduction by *Pelobacter carbinolicus*. *Appl Environ Microbiol*. 1995;61:2132.
46. Karlin S, Brocchieri L, Mrázek J, Kaiser D. Distinguishing features of  $\delta$ -proteobacterial genomes. *Proc Natl Acad Sci U S A*. 2006;103:11352.

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