Nanopore long reads enable the first complete genome assembly of a Malaysian *Vibrio parahaemolyticus* isolate bearing the pVa plasmid associated with acute hepatopancreatic necrosis disease

Han Ming Gan[1,2,3*] and Christopher M. Austin[1,2,3]

[1] Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, 3220 Geelong, Victoria, Australia

[2] Deakin Genomics Centre, Deakin University, 3220 Geelong, Victoria, Australia

[3] School of Science, Monash University Malaysia, 47500 Petaling Jaya, Selangor, Malaysia

*Corresponding author

Email: han.gan@deakin.edu.au

Address: Building KA4, School of Life and Environmental Sciences, 75, Pigdons Road, Deakin University, Waurn Ponds, 3216 Geelong, Victoria, Australia

Abstract

Background : *Vibrio parahaemolyticus* MVP1 was isolated from a Malaysian aquaculture farm affected with shrimp acute hepatopancreatic necrosis disease (AHPND). Its genome was previously sequenced on the Illumina MiSeq platform and assembled *de novo* producing a relatively fragmented assembly. Despite identifying the binary toxin genes in the MVP1 draft genome that were linked to AHPND, the toxin genes were localized on a very small contig precluding proper analysis of gene neighbourhood.

Methods: The genome of Vibrio parahaemolyticus MVP1 was sequenced on the Nanopore MinION device to obtain long reads that can span longer repeats and improve genome contiguity. *De novo* genome assembly was subsequently performed using long-read only assembler (Flye) followed by genome polishing as well as hybrid assembler (Unicycler).

Results: Long-read only assembly produced three complete circular MVP1 contigs consisting of chromosome 1, chromosome 2 and the pVa plasmid that *pirAB*^{vp} binary toxin genes. Polishing of the long read assembly with Illumina short reads was necessary to remove indel errors. The complete assembly of the pVa plasmid could not be achieved using Illumina reads due to the presence of identical repetitive elements flanking the binary toxin genes leading to multiple contigs. Whereas these regions

were fully spanned by the Nanopore long reads resulting in a single contig. In addition, alignment of Illumina reads to the complete genome assembly indicated there is sequencing bias as read depth was lowest in low-GC genomic regions. Comparative genomic analysis revealed the presence of a gene cluster coding for additional insecticidal toxins in chromosome 2 of MVP1 that may further contribute to host pathogenesis pending functional validation. Scanning of all publicly available *V. parahaemolyticus* genomes revealed the presence of a single AinS-family quorum-sensing system in this species that can be targeted for future microbial management.

Conclusions: We generated the first chromosome-scale genome assembly of a Malaysian *pirAB*^{Vp}-bearing *V. parahaemolyticus* isolate. Structural variations identified from comparative genomic analysis provide new insights into the genomic features of *V. parahaemolyticus* MVP1 that may be associated with host colonization and pathogenicity.

Introduction

Vibrio parahaemolyticus is a marine gram-negative *Enterobacteriaceae* that has been recognized as an important pathogen affecting commercially-relevant shrimp species such as the giant tiger prawn (*Penaeus monodon*) and the Pacific white shrimp (*Litopenaeus vannamei*) [1-3]. In recent years, *V. parahaemolyticus* has been linked to acute hepatopancreatic necrosis disease (AHPND)[4, 5]. An AHPND-causing *V. parahaemolyticus* isolate is thought to produce a homolog of the insecticidal *Photorhabdus* insect-related (Pir) binary toxin that induces prolonged damage to the shrimp tissue[2]. AHPND preferentially affects shrimp post-larvae or juveniles with nearly 100% mortality rate within 30-35 days post-stocking[6]. Studies suggest that AHPND originated from China in 2009 and subsequently spread to South East Asia[7]. In Malaysia, AHPND was first reported in 2011 where it still persists in shrimp farms[8].

The implementation of effective microbial management in aquaculture requires genomic surveillance data that can provide accurate information regarding the geographical origin, virulence factors and antibiotic resistance of a sequenced microbial strain[9]. In Malaysia, this remains challenging due to the paucity of available genomic resources for the relevant pathogens. To date, a majority of the microbial genomic studies in Malaysia consist of single draft genome reports mostly focusing on clinical pathogens without substantial comparative genomics[10-13]. However, recent years have seen a modest increase in the number of studies with more comprehensive sequencing dataset and analysis[14-16]. For example, Yan *et al* sequenced and assembled the draft genomes of 40 Malaysian *V. parahaemolyticus* isolates associated with shrimp aquaculture and performed comparative genomics of more than Asian 100 *V.*

60 *parahaemolyticus* genomes from public databases. *In-silico* multi-locus sequencing typing and
61 phylogenomic analyses indicate that several Malaysian *V. parahaemolyticus* isolates belong to
62 previously undescribed sequence types and genomic lineages[17] and recommended further studies be
63 undertaken.

64 The *pirAB*^{vp} genes coding for AHPND-causing binary toxins are localized on a 70-kb pVa plasmid[2].
65 Surprisingly, despite the small size of the plasmid relative to the chromosomal genome, a complete *de*
66 *novo* assembly of the pVa plasmid using Illumina-only short reads has not been achieved to date. Recent
67 studies suggested that this is due to the presence of repetitive elements on the pVa plasmid that are
68 longer than the Illumina read length[18]. As a result, *pirAB*-containing contigs that were assembled from
69 Illumina reads are generally short with limited gene content, precluding detailed analysis of the *pirAB*
70 gene neighborhood and their stability in the plasmidome[19]. *V. parahaemolyticus* isolate MVP1 is one of
71 the first *pirAB*^{Vp} -harboring *V. parahaemolyticus* isolated from Malaysia to have its genome sequenced[11].
72 Isolate MVP1 was previously sequenced on an Illumina MiSeq followed by *de novo* genome assembly
73 using the SPAdes assembler. While the *pirAB*^{vp} genes could be identified in the draft genome, the
74 assembly was problematic as these were localized on a short contig flanked by small fragments of a
75 transposase gene.

76 Recent years have seen the democratization of long-read sequencing enabled by Nanopore technology.
77 In contrast to another popular long-read sequencing platform, PacBio, Nanopore sequencing requires
78 minimal lab footprint and very low capital investment. Importantly, it is the first sequencing technology
79 that allows native sequencing, thus eliminating sequencing bias associated with the activity of *Taq*-
80 polymerase. Long reads have been utilized for *de novo* genome assembly in two distinct ways. First, long
81 reads can be used directly in genome assembly followed by polishing with Illumina short reads to
82 improve consensus accuracy[20]. An alternative approach utilizes long-reads to reorder and link contigs
83 that were initially assembled from Illumina reads[21, 22]. While the final assembly produced with this
84 hybrid approach is highly accurate, the contiguity of the assembly is dependent on the quality of the
85 initial Illumina assembly[23].

86 Leveraging on the availability of Illumina dataset for isolate MVP1, we aimed to improve on the original
87 genome assembly[11] through the addition of 40× Nanopore read coverage. We first performed a
88 Nanopore-led assembly using the long-read Flye assembler[24] followed by polishing with Illumina reads.
89 In addition, we generated a hybrid assembly using Unicycler assembler[23]. Although both methods
90 produced a complete circular pVa plasmid sequence with intact *pirAB*^{vp}, complete chromosome

assembly was only achieved with the Nanopore-led Flye assembly. This superior assembly provides new insight into the genomic features of *V. parahaemolyticus* MVP1 and will allow a greater understanding of host pathogenicity through comparative genomic analysis with selected *V. parahaemolyticus* isolates.

Methods

Nanopore sequencing

Sample collection, gDNA isolation and Illumina sequencing of *V. parahaemolyticus* isolate MVP1 have been previously reported[11]. For Nanopore sequencing, 1 µg of unfragmented gDNA was processed using the now obsolete Nanopore SQK-NSK007 library preparation kit. Sequencing was subsequently performed on an R9 flowcell attached to a MinION device for 48 hours. Base-calling of the produced raw fast5 files used Guppy v3.1 (high accuracy mode) that only is available to Oxford Nanopore Technology (ONT) customers via the ONT community site (https://community.nanoporetech.com).

De novo genome assembly and genome polishing

Illumina-only assembly and hybrid assembly incorporating high-quality (q>7) Nanopore long reads were performed using Unicycler v.0.4.7[23]. Only contigs equal or larger than 1,000 bp were retained for subsequent analyses. A long-read only *de novo* assembly was also performed with Flye v2.4.2 utilizing similar Nanopore sequencing data[24]. Illumina polishing of the Flye assembly used the unicycler polish tool in Unicycler v.0.4.7 that performed multiple rounds of bwa-mem-based Illumina read alignment to the assembly followed by polishing with Pilon v1.22[25]. Genome completeness was assessed with BUSCO v3 (Gammaproteobacteria odb9) based on the whole proteome from each genome assembly that was predicted using Prodigal v 2.6.3 (default setting) [26, 27].

Comparative Genomic analysis and protein homology modeling

Automated annotation of the whole-genome was carried out using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP)[28]. The translated coding sequences generated from PGAP were subject to additional functional annotation using InterProScan. Intra-chromosomal structural variations and sequence similarity were visualized with BLAST ring image generator (BRIG) using the blastN (e-value = 0.001 cutoff). Sequencing depth was also calculated in BRIG based on the BAM alignment file generated from the bwa-mem mapping of Illumina paired-end reads to the whole-genome[29]. Structural modeling

of the putative insecticidal toxin proteins identified in MVP1 used the online Phyre2 webserver (http://www.sbg.bio.ic.ac.uk/phyre2)[30].

Identification and phylogenetic analysis of LuxM, OpaM and AinS proteins

Vibrio proteins containing the InterPro signature IPR035304, corresponding to the AinS-family N-acyl-homoserine-lactone autoinducer synthase, were downloaded from the UniProt database [31] on the 7th of November 2019 and clustered at 98% amino acid identity cutoff using cd-hit v4.6 [32]. The protein clusters were subsequently aligned with MAFFT v7.31 [33] ("--maxiterate 1000 –localpair" setting) followed by maximum likelihood tree construction using FastTree v2.1 [34]. Calculation and visualization of pairwise protein sequence divergence used SDT v1.2 [35]. In addition, to confirm the absence of the LuxI-type autoinducer synthase homolog in the *Vibrio parahaemolyticus* proteomes, additional domain search specifically for PF00765 (LuxI autoinducer synthase domain) was performed followed by subsequent InterProScan validation as previously described [36].

Results and discussion

Chromosomal assembly enabled by Nanopore long reads

A total of 231 megabases of Nanopore data contained in 30,315 reads (N50 = 10,908 bp) were produced from a single Nanopore MINION run on the discontinued R9 flowcell. The base-called Nanopore fastq file has been deposited in the SRA database under the accession code SRX6759854. An initial Unicycler assembly using previously generated Illumina reads (SRX6759853) yielded a relatively fragmented genome (198 contigs; N50 length = 54,109 bp). Supplementing the assembler with approximately 40 x coverage of Nanopore long reads led a substantial improvement in the Unicycler assembly contiguity (9 contigs; N50 length = 1,738,848 bp). Of the 9 assembled contigs, one of them was flagged as "complete" with a contig length of 70kb. On the other hand, the Flye assembly using only Nanopore reads produced three contigs all flagged as "complete and circular". Despite generating the most contiguous assembly, the initial Flye assembly has the worst BUSCO score with 37.4% BUSCO single-copy and 45.1% fragmented BUSCO genes (Table 1). Polishing of the Flye assembly with Illumina paired-end reads restored the genome completeness to a level that is comparable to the hybrid and Illumina-only assemblies (Table 1 and *Extended data:* Supplemental File 1). The high percentage of fragmented genes in long-read-only assembly is usually a result of erroneous frameshifts from indel sequencing errors in the long reads[20]. Although higher coverage of long read may lead to an increase the consensus accuracy,

150 its accuracy will unlikely match that of an Illumina-polished assembly due to Nanopore-specific
151 systematic errors. As a result, polishing of long-read assembly using Illumina short reads can be
152 considered as a cost-effective but less convenient approach for producing highly accurate and
153 contiguous microbial genome assembly.

154 ***Reduced Illumina sequencing depth in several low-GC genomic regions***

155 The alignment of Illumina reads to the final chromosomal assembly revealed substantial lower
156 sequencing depths in genomic regions with low GC-content (<30%) (Figure 1). Some of these regions are
157 unique to MVP1 and may risk being overlooked in the Illumina-only assembly at lower sequencing
158 coverage. For example, less than 5x sequencing depth was observed in the region 3,240 to 3,280 kb that
159 contain various putative carbohydrate metabolism genes that are unique to isolate MVP1 in this current
160 genomic comparison. The incorporation of PCR during library preparation is known to reduce the
161 representation of high-AT genomic regions due to the polymerase amplification bias towards GC-
162 balanced fragments[37]. Reduced Illumina sequencing depth in high-AT genomic regions affecting
163 biological interpretation has also been reported in several crustacean mitogenomic libraries as both the
164 phylogenetically informative control region and 16S rRNA genes are relatively AT-rich[38, 39]. Depending on
165 the amount of DNA available for sequencing, a few modifications to the Illumina library preparation step
166 can be considered, such as PCR-free preparation for sample with high starting DNA (> 100 ng) and
167 reduced PCR cycle coupled with higher (>100× genome coverage) sequencing depth for samples with
168 low starting DNA.

169 ***Multiple localization of IS5 family transposase genes in the complete genomes of pVa-carrying V.***
170 ***parahaemolyticus***

171 In the Illumina-only assembly, the pVa plasmid was assembled into three contigs (Contig24: 63.4 kb,
172 Contig164: 3.4kb; Contig198: 1 kb). Contig198 contains a 921-bp gene that codes for an intact IS5 family
173 transposase and could be aligned equally well to two genomic regions flanking the $pirAB^{Vp}$ genes in the
174 complete plasmid assembly (Figure 2). This observation can be explained by the inability of Illumina
175 reads to fully span the repeats thus causing them to be collapsed into a single contig. As a result, the
176 localization of $pirAB^{Vp}$ on the pVa plasmid cannot be confirmed from an Illumina-only assembly. In
177 contrast the entire $pirAB^{Vp}$ genes and their flanking transposase genes were fully covered by a number
178 of Nanopore long reads enabling the complete assembly of the pVa plasmid in addition to supporting
179 the localization of $pirAB^{Vp}$ genes on the pVa plasmid. Nanopore read depth compared to Illumina, is

180 relatively even across the aligned genomic region, which is consistent with the absence of PCR bias in
181 the Nanopore library preparation.

182 The minor structural variations previously reported in *pirAB*^{Vp} -containing region suggest that these
183 genes are not stably maintained and are prone to transposition mediated by the flanking IS5 family
184 transposase genes[40]. Interestingly, the localization of this IS5 family transposase gene is not specific to
185 just the pVa plasmid. Local nucleotide similarity search of this transposase gene against the complete
186 MVP genome revealed three additional perfect hits (100% query coverage and 100% nucleotide identity)
187 in each of the chromosomal genome (Figure 1). The localization of these additional IS5 family
188 transposase in both chromosome 1 and chromosome 2 further raises the possibility of the IS5
189 transposase-flanked *pirAB*^{Vp} genes being integrated into the chromosomal region[41, 42].

190 ***Structural variations in the MVP1 genome***

191 Chromosome 2 of Isolate MPV1 consists of a 160 kb genomic region that is mostly absent in five out of
192 six pVa plasmid-harboring *V. parahaemolyticus* isolates included in this comparative genomic analysis.
193 Functional annotation of the genes located in this region revealed two gene clusters that may further
194 contribute to host pathogenicity in addition to the binary Pir-like toxins on the pVa plasmid. The first
195 gene cluster located from 1,822,499 to 1,836,895 bp in chromosome 2 consists of three relatively large
196 genes (Gene Locus Tag: BSR23_26145, BSR23_26150 and BSR23_26155) coding for another type of
197 putative insecticidal toxins. Functional annotation using InterProScan revealed the presence of protein
198 domains commonly associated with the toxin-complex (Tc) toxins (Figure 3). In addition, Phyre2 protein
199 modeling also indicated their high structural homology to their respective homologous toxin
200 components (*Extended data:* Supplemental Files 2-4). In *Xenorhabdus nematophilus*, the three toxin
201 components formed a native toxin complex and the ingestion of this complex led to growth inhibition of
202 two different insect larvae, *Helicoverpa zea* and *Heliothis virescens* [43]. In addition, further tests showed
203 that the native toxin complex is capable of binding to solubilized gut membranes of *H. zea* larvae in
204 addition to inducing pore formation in black lipid membranes [43]. Given the functional resemblance of
205 such toxins to the Pir-like binary toxins, the presence of all three genes coding for the complete set of
206 insecticidal Tc toxins in *V. parahaemolyticus* may contribute to mortality without AHPND lesions in
207 shrimps, as previously reported [44].

208 Interestingly, isolate MVP1 also harbor a *fuc* operon that is not commonly present in members of this
209 species (*Extended data:* Supplemental Table 1). The presence of this operon in MVP1 translates into the
210 genomic potential for the internalization of fucose for incorporation into the capsular polysaccharides or

211 bacterial glycoproteins. While it is also possible that the *fuc* operon may contribute to improved
212 utilization of fucose as an energy source, Williams *et al* did not observe a greater capacity to utilize
213 fucose in an EMS-causing *V. parahaemolyticus* isolate 13-028/A3 [45].

214 ***High prevalence and divergence of the LuxM/OpaM/AinS family autoinducer synthesis proteins***
215 ***among Vibrio spp.***

216 NCBI annotation of MVP1 proteome revealed the presence of only the LuxM/OpaM-type AHL synthase.
217 An in-house HMMsearch for the LuxI autoinducer synthase domain (PFAM signature: PF00765) in the
218 MVP1 proteome also did not reveal significant hits, indicating that MVP1 employs a single system for the
219 biosynthesis of AHL signal (*Extended Data* : Supplemental File 5). Additional UniProt query search of
220 "PF00765 AND Vibrio parahaemolyticus" on 14th November 2019, showed only one positive hit across all
221 currently available *V. parahaemolyticus* proteomes. However, further investigation of the positive hit,
222 AAY51_09590, revealed that this belonged to a *Citrobacter* spp. previously misclassified as *V.*
223 *parahaemolyticus* [46]. In contrast, *V. harveyi* was shown to utilize at least two quorum-sensing systems
224 e.g. LuxI that synthesizes 3-oxo-C6-HSL and the AinS that synthesizes C8-HSL [47]. Despite being classified
225 as members of the same family for sharing the similar protein domain signature, AinS exhibited a
226 substantially lower pairwise amino acid divergence (~ 30%) in comparison to LuxM and OpaM. By
227 rooting the LuxM/OpaM phylogenetic tree with the AinS from *Aliivibrio fischeri* as the outgroup, we
228 observed a strongly supported monophyletic cluster consisting of the functionally validated LuxM and
229 OpaM in addition to their homologs from other *Vibrio* spp (Figure 4A). The pairwise amino acid identity
230 among members in this LuxM/OpaM clade averages around 50% (Figure 4B), consistent with the
231 relatively short branch length among members of the clade.

232 In Indian white shrimps (*Fenneropenaeus indicus*), co-injection of a pathogenic *V. parahaemolyticus*
233 strain DHAP1 and purified recombinant AHL lactonase, AiiA, reduced *Vibrio* viable counts and biofilm
234 development in the shrimp intestine, suggesting the role of AHL-mediated quorum-sensing system in
235 host colonization [48]. While the presence of intact *opaM* in several sequenced *V. parahaemolyticus*
236 isolates supports their ability to accumulate AHL and engage in quorum-sensing activity, this is best
237 validated using a simple biosensor or LC/MS approach that can not only verify the AHL producing
238 phenotype but also provide insight into the structural diversity of AHL signals [49-51]. Furthermore,
239 elucidating the role of *opaM* in mediating host colonization and pathogenesis among AHPND-causing *V.*
240 *parahaemolyticus* via transposon mutagenesis will be instructive.

241

Conclusions

Using approximately 40× genome coverage of Nanopore long reads, we produced the first chromosome-scale genome assembly of a Malaysian *pirAB*^{Vp}-bearing *V. parahaemolyticus* isolate. Although the genome completeness of the initial Flye assembly was relatively poor, polishing of the genome assembly with Illumina reads improved its completeness to a level that is comparable to a high-quality microbial genome assembly. Structural variations identified from genomic comparisons provide new insights into the genomic features of *V. parahaemolyticus* MVP1 that may be associated with host colonization and pathogenicity.

Data availability

Underlying data

Vibrio parahaemolyticus strain MVP1 chromosome 1, complete sequence, Accession number CP043421: https://www.ncbi.nlm.nih.gov/nuccore/CP043421

Vibrio parahaemolyticus strain MVP1 chromosome 2, complete sequence, Accession number CP043422: https://www.ncbi.nlm.nih.gov/nuccore/CP043422

Vibrio parahaemolyticus strain MVP1 plasmid pVa, complete sequence, Accession number CP043423: https://www.ncbi.nlm.nih.gov/nuccore/CP043423

Raw Illumina reads and basecalled Nanopore Reads have also been deposited in NCBI Sequence Read Archive under the BioProject PRJNA355061.

Extended data

Zenodo: Nanopore long reads enable the first complete genome assembly of a Malaysian Vibrio parahaemolyticus isolate bearing the pVa plasmid associated with acute hepatopancreatic necrosis disease,**10.5281/zenodo.3563171.** Creative Commons Attribution 4.0 International.

This project contains the following extended data:

Supplemental File 1: Main genome assemblies (Unpolished Flye assembly, Polished Flye assembly, Unicycler Hybrid Assembly and Unicycler Illumina-only assembly) generated in this study for comparison and their BUSCO output.

Supplemental File 2: Phyre2 protein modeling output of the putative MVP1 TcdA toxin

Supplemental File 3: Phyre2 protein modeling output of the putative MVP1 TcdB toxin

Supplemental File 4: Phyre2 protein modeling output of the putative MVP1 TccC toxin

Supplemental File 5: InterProScan output of the NCBI-predicted MVP1 proteome.

Supplemental Table 1: NCBI BlastN output using the *fuc* genes of *Vibrio parahaemolyticus* MVP1 as the query to search against the *Vibrio* reference WGS database as of 21 Oct 2019

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Produces neither Pir (Vp) Toxins nor Acute Hepatopancreatic Necrosis Disease Lesions. Applied and environmental microbiology. 2017 Aug 15;83(16). PubMed PMID: 28576761. Pubmed Central PMCID: PMC5541212. Epub 2017/06/04. eng.

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Figure Legends

Figure 1. Genomic comparisons of pVa plasmid-harboring *Vibrio parahaemolyticus*. Left, *V. parahaemolyticus* MVP1 chromosome 1 compared against five other *V. parahaemolyticus* chromosome 1. Right, *V. parahaemolyticus* MVP1 chromosome 2 compared against five other *V. parahaemolyticus* chromosome 2. The innermost rings show GC content while the second innermost rings show Illumina sequencing depth along the genome (blue). The remaining rings indicate BLAST comparisons of other V. parahaemolyticus genomes against MVP genome assembly. Arrows indicate genes of interest and were labeled with the predicted protein names.

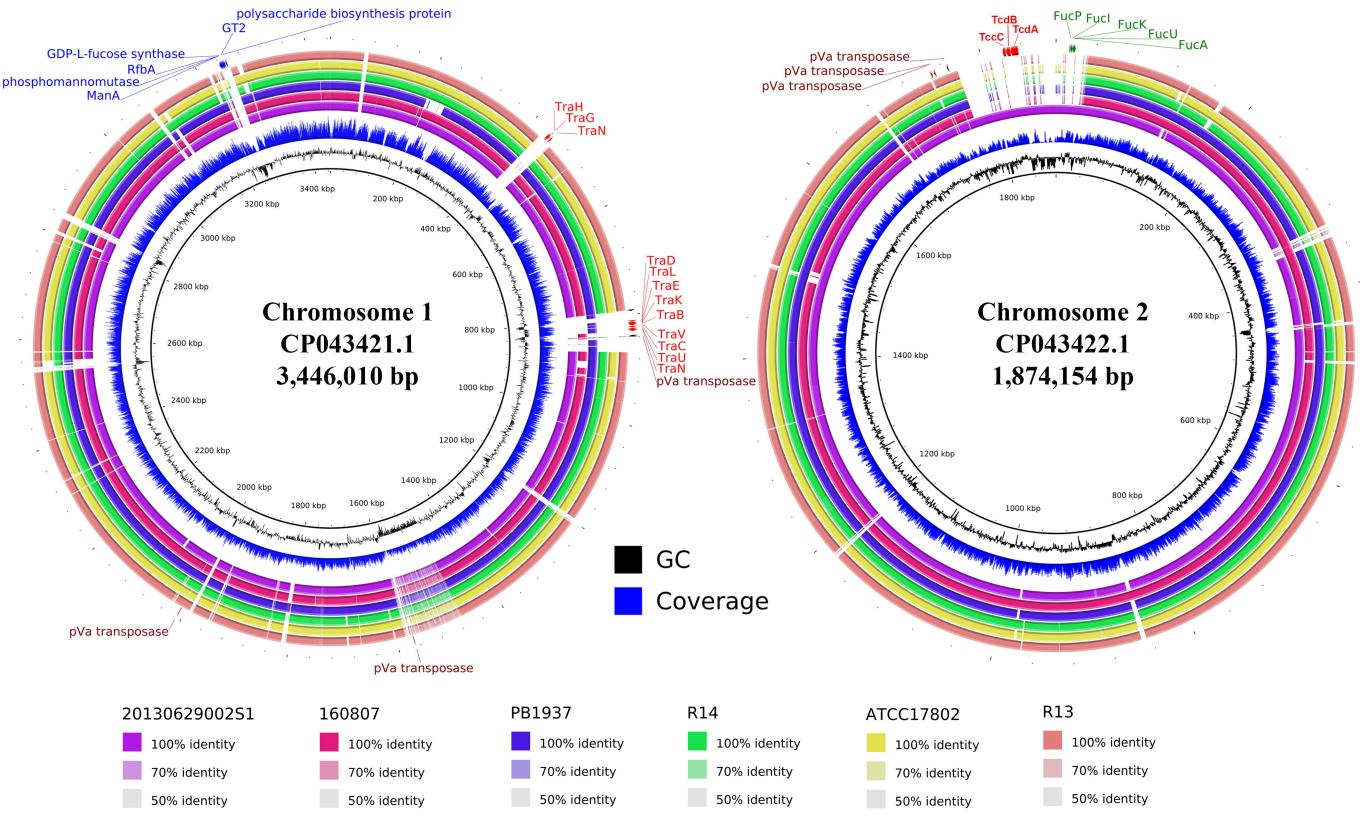
Figure 2. Alignment of Illumina-assembled contigs, Illumina and Nanopore reads to the annotated *Vibrio parahaemolyticus* MVP1 pVa plasmid sub-region containing the *pirAB*^{Vp} genes. Direction of arrow in the annotation indicates transcription orientation. Blue and red arrows in the Nanopore read alignment indicate forward and reverse strands, respectively.

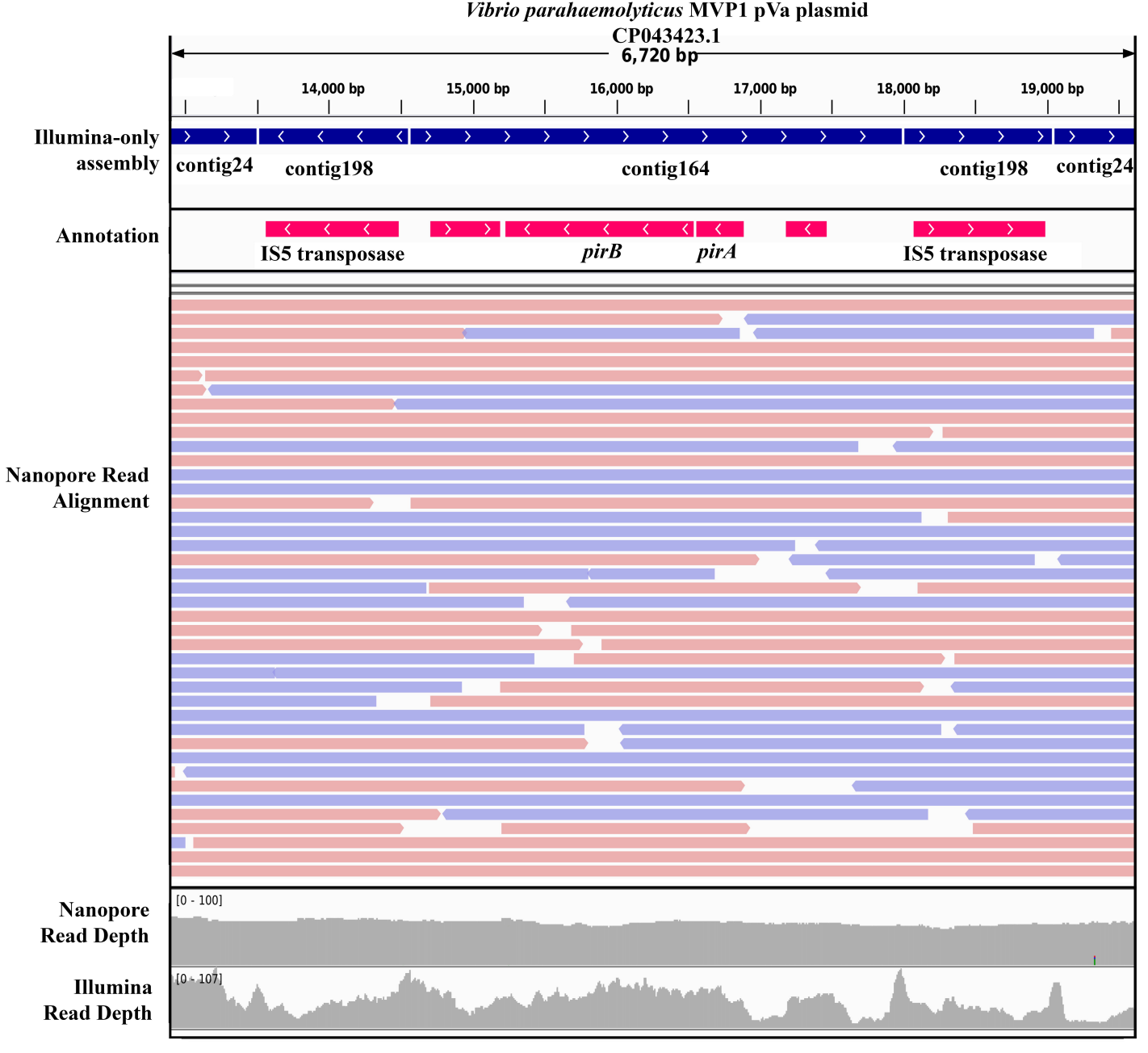
448 **Figure 3. InterProScan identification of conserved protein domains in the putative toxin complex**
449 **family proteins coded by three linked genes located on Chromosome 2 of *Vibrio parahaemolyticus***
450 **MVP1.**

451 **Figure 4. Phylogenetic analysis and sequence divergence of the AinS-like family proteins in *Vibrio* spp.**
452 (A) Maximum likelihood tree depicting the evolutionary relationships of AinS-like family protein clusters
453 among Vibrio spp. Number after the "=" sign in each tip label indicates the number of proteins
454 represented by the cluster. Node were colored according to the SH-like local support values and branch
455 lengths indicate number of substitutions per site. (B) Pairwise identity matrix of the identified AinS-like
456 family proteins in *Vibrio* spp.

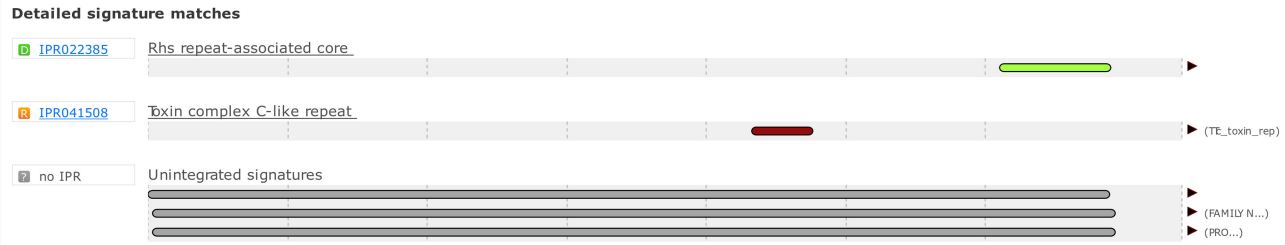
Table 1. Statistics and BUSCO completeness assessment of the *Vibrio parahaemolyticus* MVP1 assembly

Assembler (input data)	Genome Length (bp)	GC%	# contigs	N50 (bp)	BUSCO (gammaproteobacteria_odb9, n=452)			
					Single-copy	Duplicated	Fragmented	Missing
Unicycler (Illumina)	5,167,326	45.47%	198	54,109	98.9	0.9	0.2	0
Unicycler (Hybrid)	5,378,063	45.34%	9	1,738,848	98.9	0.9	0.2	0
Flye (Nanopore)	5,405,284	45.23%	3	3,456,517	37.4	0	45.1	17.5
Flye+Pilon (Nanopore+Illumina)	5,389,211	45.30%	3	3,446,010	99.1	0.7	0.2	0

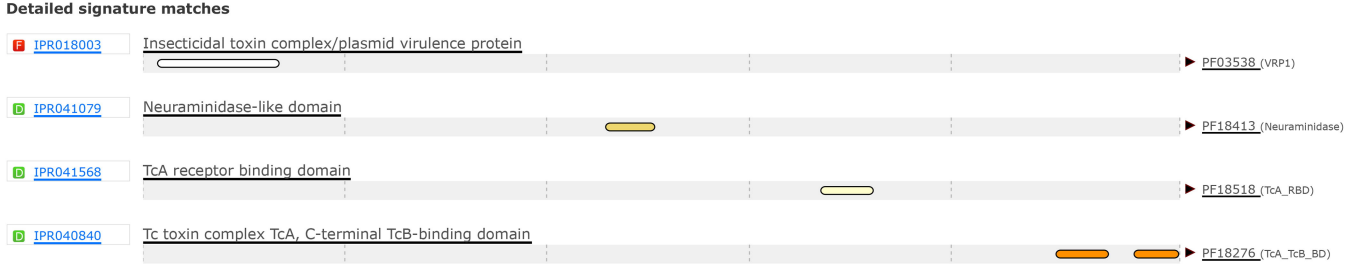

polysaccharide biosynthesis protein
GT2
GDP-L-fucose synthase
RfbA
phosphomannomutase
ManA
TraH
TraG
TraN
TraD
TraL
TraE
TraK
TraB
TraV
TraC
TraU
TraN
pVa transposase
pVa transposase
pVa transposase
Chromosome 1
CP043421.1
3,446,010 bp
200 kbp
400 kbp
600 kbp
800 kbp
1000 kbp
1200 kbp
1400 kbp
1600 kbp
1800 kbp
2000 kbp
2200 kbp
2400 kbp
2600 kbp
2800 kbp
3000 kbp
3200 kbp
3400 kbp
TcdB
TccC
TcdA
FucP
FucI
FucK
FucU
FucA
pVa transposase
pVa transposase
pVa transposase
Chromosome 2
CP043422.1
1,874,154 bp
200 kbp
400 kbp
600 kbp
800 kbp
1000 kbp
1200 kbp
1400 kbp
1600 kbp
1800 kbp
GC
Coverage
2013062900 2S1
100% identity
70% identity
50% identity
160807
100% identity
70% identity
50% identity
PB1937
100% identity
70% identity
50% identity
R14
100% identity
70% identity
50% identity
ATCC17802
100% identity
70% identity
50% identity
R13
100% identity
70% identity
50% identity


Vibrio parahaemolyticus MVP1 pVa plasmid
CP043423.1
6,720 bp
14,000 bp
15,000 bp
16,000 bp
17,000 bp
18,000 bp
19,000 bp
Illumina-only assembly
contig24
contig198
contig164
contig198
contig24
Annotation
IS5 transposase
pirB
pirA
IS5 transposase
Nanopore Read Alignment
Nanopore Read Depth
[0 - 100]
Illumina Read Depth
[0 - 107]

BSR23_26145 Chromosome 2: 1,821,846-1,822,496



BSR23_26150 Chromosome 2: 1,824,874-1,829,145



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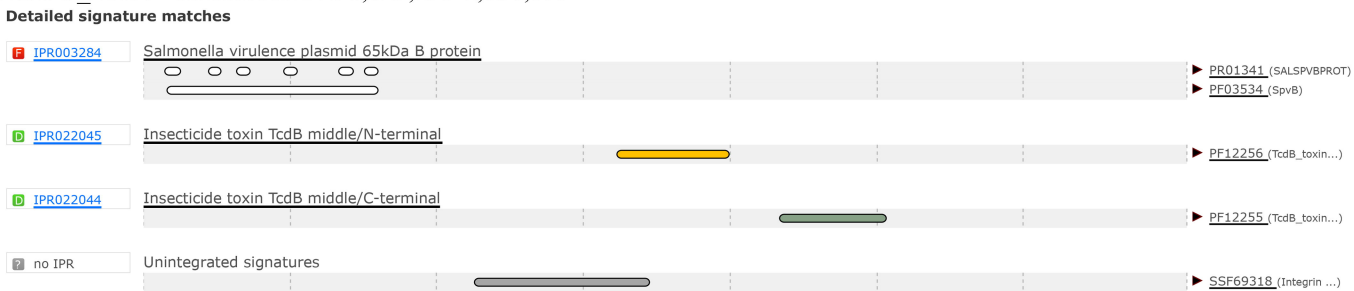

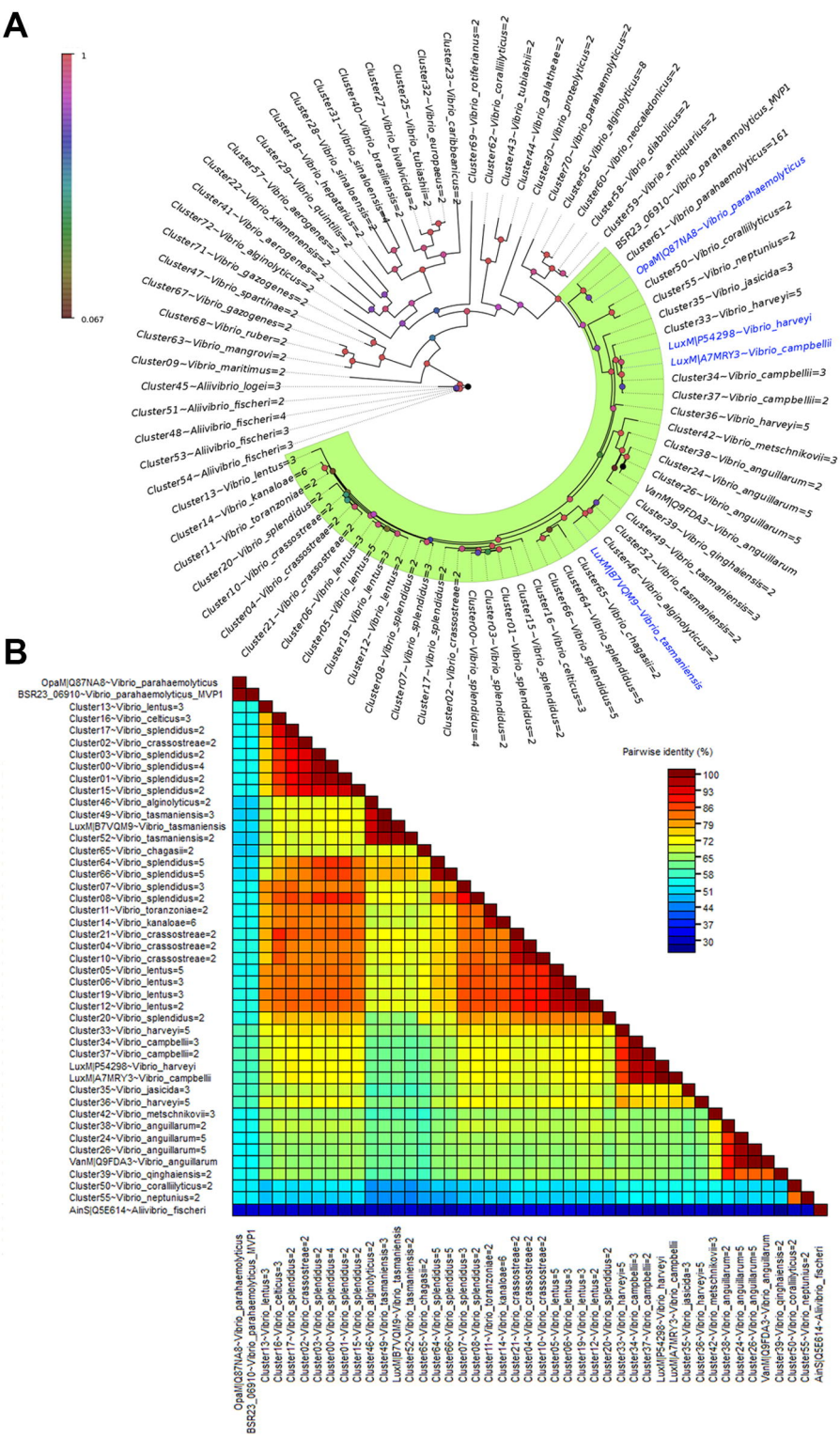
A
1
0.067
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Cluster32~Vibrio_europaeus=2
Cluster25~Vibrio_tubashii=2
Cluster27~Vibrio_brasiliensis=2
Cluster40~Vibrio_sinaloensis=2
Cluster31~Vibrio_quintilis=2
Cluster28~Vibrio_hepatarius=2
Cluster18~Vibrio_sinaloensis=2
Cluster29~Vibrio_aerogenes=2
Cluster57~Vibrio_aerogenes=2
Cluster22~Vibrio_xiamenensis=2
Cluster41~Vibrio_aerogenes=2
Cluster72~Vibrio_alginolyticus=2
Cluster71~Vibrio_alginolyticus=2
Cluster47~Vibrio_gazogenes=2
Cluster67~Vibrio_spartinae=2
Cluster68~Vibrio_gazogenes=2
Cluster63~Vibrio_ruber=2
Cluster09~Vibrio_mangrovi=2
Cluster45~Aliivibrio_logei=3
Cluster51~Aliivibrio_fischeri=2
Cluster48~Aliivibrio_fischeri=4
Cluster53~Aliivibrio_fischeri=3
Cluster54~Aliivibrio_fischeri=3
Cluster13~Vibrio_lentus=3
Cluster14~Vibrio_kanaloae=6
Cluster11~Vibrio_toranzoniae=2
Cluster20~Vibrio_splendidus=2
Cluster10~Vibrio_crassostreae=2
Cluster04~Vibrio_crassostreae=2
Cluster21~Vibrio_crassostreae=2
Cluster06~Vibrio_lentus=3
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Cluster19~Vibrio_lentus=3
Cluster12~Vibrio_lentus=2
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Cluster16~Vibrio_celticus=3
Cluster66~Vibrio_splendidus=5
Cluster64~Vibrio_splendidus=5
Cluster65~Vibrio_chagasii=2
LuxM|B7VQM9~Vibrio_tasmaniensis
Cluster69~Vibrio_rotiferianus=2
Cluster62~Vibrio_coralliilyticus=2
Cluster43~Vibrio_tubiashii=2
Cluster44~Vibrio_galatheae=2
Cluster30~Vibrio_proteolyticus=2
Cluster70~Vibrio_parahaemolyticus=2
Cluster56~Vibrio_alginolyticus=8
Cluster60~Vibrio_neocelonicus=2
Cluster58~Vibrio_diabolicus=2
Cluster59~Vibrio_antiquarius=2
BSR23_06910~Vibrio_parahaemolyticus_MVP1
Cluster61~Vibrio_parahaemolyticus=161
OpaM|Q87NA8~Vibrio_parahaemolyticus
Cluster50~Vibrio_coralliilyticus=2
Cluster55~Vibrio_neptunius=2
Cluster35~Vibrio_jasicida=3
Cluster33~Vibrio_harveyi=5
LuxM|P54298~Vibrio_harveyi
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Cluster37~Vibrio_campbellii=2
Cluster36~Vibrio_harveyi=5
Cluster42~Vibrio_metschnikovii=3
Cluster38~Vibrio_anguillarum=2
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Cluster26~Vibrio_anguillarum=5
VanM|Q9FDA3~Vibrio_anguillarum
Cluster39~Vibrio_qinghaiensis=2
Cluster52~Vibrio_tasmaniensis=3
Cluster46~Vibrio_alginolyticus=2
LuxM|B7VQN9~Vibrio_tasmaniensis
B
Pairwise identity (%)
100
93
86
79
72
65
58
51
44
37
30
OpaM|Q87NA8~Vibrio_parahaemolyticus
BSR23_06910~Vibrio_parahaemolyticus_MVP1
Cluster13~Vibrio_lentus=3
Cluster16~Vibrio_celticus=3
Cluster17~Vibrio_splendidus=2
Cluster02~Vibrio_crassostreae=2
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Cluster00~Vibrio_splendidus=4
Cluster01~Vibrio_splendidus=2
Cluster15~Vibrio_splendidus=2
Cluster46~Vibrio_alginolyticus=2
Cluster49~Vibrio_tasmaniensis=3
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Cluster52~Vibrio_tasmaniensis=2
Cluster65~Vibrio_chagasii=2
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Cluster37~Vibrio_campbellii=2
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LuxM|A7MRY3~Vibrio_campbellii
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Cluster36~Vibrio_harveyi=5
Cluster42~Vibrio_metschnikovii=3
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