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Abstract

Marinobacter comprises a widespread and ecologically significant genus of marine bacteria with vast metabolic capabilities. However, very few reports on phages infecting this genus exist. In this study, we isolated and characterized a linear dsDNA genome of Marinobacter phage AS1, infecting *Marinobacter sp.* strain, D1S9. The phage is a member of Podoviridae family having a genome size of ~37 kb and a 57 % GC content with no significant overall similarity to any of the available viral whole genome sequences in the database. The genome which encodes for 52 protein coding genes with no tRNA genes, contains 21 protein coding genes with assigned putative functions. High degree of recombination events with other members of the family *Caudovirales* is evident from the mosaic pattern of inheritance of genes such as major capsid protein, terminase large sub unit, portal protein and integrase. Presence of integrase gene, DNA methyl transferases and the occurrence of AS1 sequences within the whole genome sequences of some members of the host taxon were suggestive of its intense association with the host.

Keywords: *Marinobacter*, phage, complete genome sequence, Podovirus, Arabian Sea

1. Introduction

Viruses, primarily bacteriophages are the most prolific and diverse obligate parasites in the marine biosphere where they outnumber bacteria on an average by one order of magnitude (Suttle, 2007, Sime-Ngando 2014). Bacteriophages have indispensable involvement in the ecological and biogeochemical processes by regulating bacterial abundance, bacterial diversity, nutrient fluxes, structuring the microbial food web dynamics and to a great extent in influencing host diversification and speciation through horizontal gene transfer (Middelboe and Lyck, 2002, Bouvier and Del Giorgio 2007, McDaniel et al. 2010, Winget et al. 2011). A considerable fraction of bacterial genetic diversity is acquired through horizontal gene transfer, imparting ecological and pathogenic attributes like antibiotic resistance, resistance to phage infection, toxins and heavy metals (Stern and Sorek, 2010, Xia and Wolz, 2014). Phage genomes are also coevolving concomitantly with their hosts. Compared to other host-parasite systems, bacterial-phage coevolution is faster owing to their large population sizes and short generation times (Buckling and Brockrust 2012). Studies with coevolving populations of hosts and their infective phages revealed two modes of coevolution: arms race dynamics (ARD) and fluctuating selection dynamics (FSD). These two were found to operate at different times in the same population with arms race dynamics giving way to fluctuating selection dynamics in the long run (Hall et al. 2011, Gokhale et al. 2013).

Most of the dsDNA phages share a large pool of genetic elements (Hendrix et al. 1999). Phage genomes exhibit prevalent mosaic architecture as a result of extensive horizontal gene transfer and in fact different genomic segments may have different ancestral origins. So, it is challenging to deduce whole genome similarities and to assign phylogenetic relationships to newly sequenced phages (Hatfull and Hendrix 2011). Isolation and genome characterization of additional phages would help to resolve this problem to some extent.

Despite of the profound knowledge on abundance and role of marine viruses in biogeochemical cycles and host population dynamics, genome level information on phage-host interactions of ecologically significant bacteria are very limited. Sequencing and whole genome characterization of phages infecting these bacteria might provide insights into the phage-host interactions and how they shape the ecological roles of the hosts. *Marinobacter,* an ecologically significant genus of bacteria, and its first representative, *M. hydrocarbanoclasticus,* was proposed by Gauthier in 1992. Since then, new members belonging to this genus have been isolated from diverse marine habitats ranging from oil producing wells to deep-sea benthic sediments with fifty-eight species reported so far (Martin et al. 2003, Yoon et al. 2007, Xu et al. 2008, Zhang et al. 2008, Wang et al. 2009, Wang et al. 2012, Chua et al. 2018). Several species have been associated with marine sponges and microalgae (Green et al. 2006, Kaeppel et al. 2012, Lee et al. 2012, Lupette et al. 2016, Sandhya et al. 2017). Members of this genus are known to have substantial impact on marine snow formation and on various biogeochemical cycles with quite a few strains possessing a repertoire of activities like degradation of various hydrocarbon compounds, denitrification, Fe (II) oxidation, Mn (II) oxidation, Fe (III) reduction and redox cycling of arsenic and fumarate (Kaye et al. 2010, Kaeppel et al 2012, Wang et al. 2012, Handley and Lloyd 2013, Bonis and Gralnick 2015). Although their versatile functional capabilities enable them to survive in diverse environmental conditions and to exploit variable resources (Singer et al. 2011), viruses infecting this genus are reported rarely (Zhu et al. 2018), thus hindering an integrated understanding of the life cycle of these microbes in the marine environments. In this study, we report the isolation of a bacteriophage infecting the genus *Marinobacter* from surface waters of the Arabian Sea (southwest coast of India) and provide first-hand information on the sequencing and whole genome analysis of it.

2. Materials and Methods

2.1. Isolation and purification of Marinobacter phage AS1 and its host

Marinobacter phage AS1 was isolated from the surface water of the tropical Arabian Sea, off the coast of Kochi, southwest coast of India (9.9586º N and 76.0825º E, Fig. 1A) in December 2014 as described by Middelboe et al. (2010). Briefly, 25 mL water sample was amended with 1 mL 10× Zobell marine broth and incubated at room temperature for 2 days in order to propagate potential bacterial hosts and thereby the specific viruses in the sample (Himedia, Mumbai, India). The viral enriched seawater was screened against lawns of bacterial isolates obtained from the same water sample by means of soft agar overlay technique. Culturing of bacteria was done by spread plating water samples on Zobell Marine Agar. The plaques formed on the bacterial lawn after overnight incubation (37 ºC) were picked and transferred to phage buffer (SM buffer: 450 mM NaCl, 50 mM $MgSO_4$, 50 mM Tris, 0.01 % gelatin, pH 8), vortexed well and kept at 4°C. Centrifugation (10,000 ×g, 10 min) was done the next day to remove the soft agar and host cell remnants which was followed by filtration through 0.2µm pore size Acrodisc syringe filters (Sigma-Aldrich, India). The filtrate was then serially diluted and plated by soft agar overlay method. Single plaque from the plate was isolated and the purification was repeated for five consecutive times. The phage stock was stored at 4°C until use.

The bacterial isolate for which the phage was isolated was identified using 16S rRNA gene polymerase chain reaction (Amann et al. 1995). The PCR products were sequenced and identified by BLAST search against non-redundant database of NCBI.

2.2. Transmission Electron microscopy (TEM)

For morphometric characterization of phage by TEM analysis, the purified phage particles were collected onto 400-mesh carbon coated formvar grids by centrifugation (70,000 ×g, 20 minutes at 4ºC) using a SW 40Ti rotor and stained at room temperature for 30 s with uranyl acetate stain (2 % w/v) (Pradeep Ram et al. 2010). Excess stain on grids was removed by rinsing twice with 0.02 µm-filtered distilled water and dried on filter paper. Grids were examined using a JEOL 1200Ex transmission electron microscope (JEOL, Akishima, Japan) operated at 80 kV at a magnification between × 60,000 and × 100,000.

2.3. Phage DNA isolation

Phage particles were precipitated with 8% polyethylene glycol (PEG) 8000 containing 1M NaCl after overnight incubation at 4°C (Lawrence and Steward 2010). The mixture was centrifuged at 10000×*g* for 20 min at 4°C and the pellet was suspended in SM buffer to disaggregate overnight at 4°C. To remove the PEG, KCl was added to the viral suspension to a final concentration of 1M and kept on ice for 30 min. PEG was pelleted by centrifugation at 12000×*g* for 10 min at 4°C and the supernatant containing viral particles were transferred to a fresh tube. The viral suspension was concentrated using Amicon Ultra-15 centrifugal filter unit with a molecular weight cut off of 30 kDa (Millipore, India). The concentrated phage preparation was treated with nucleases (DNase I and RNase A at final concentrations of 1 mg mL^{-1}) to remove any host DNA and RNA. Phage DNA was extracted using the phenol:chloroform (24:1) extraction method as described previously (Yang et al. 2017). Briefly, the nuclease treated phage preparation was treated with proteinase K (100 mg mL^{-1}), 10% (w/v), SDS and 0.5 M EDTA (pH 8) and incubated in water bath at 55 °C for 1 hr. This was followed by two rounds of phenol:chloroform:isoamyl alcohol (25:24:1) extraction at 12,000 rpm at 4°C for 10 min . Again, the aqueous phase was purified by chloroform:isoamyl alcohol (24:1) extraction at 12,000 rpm at 4°C for 10 min. The supernatant was mixed with isoamyl alcohol and

incubated overnight at -20 °C. The pellet was washed with 70% ethanol and air dried. DNA was resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and stored at 4 °C.

2.4. Genome sequencing and bioinformatic analyses

Whole genome sequencing library was prepared with Illumina-compatible NEXTflex rapid DNA sequencing kit (BIOO Scientific, Inc., USA) at Genotypic Technology Pvt. Ltd., Bangalore, India. 100 ng of Qubit quantified DNA was sheared using Covaris S220 sonicator (Covaris, Inc., USA) to generate specific fragments with size range of 300-400 bp. The fragment size distribution was verified on Agilent 2200 TapeStation and subsequently purified using HighPrep magnetic beads (MagBio Genomics, Inc., USA). Purified fragments were end-repaired, adenylated and ligated to Illumina multiplex barcode adapters as per NEXTflex rapid DNA sequencing kit protocol.

Illumina universal adapters used in the study are: 5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3' and Index Adapter: 5'-GATCGGAAGAGCACACGTCTGAACTCCAGTCAC [INDEX] ATCTCGTATGCCGTCTTCTGCTTG-3'.

Adapter-ligated DNA was purified using HighPrep beads. Resultant fragments were amplified for 5 cycles of PCR using Illumina-compatible primers provided in the NEXTflex rapid DNA sequencing kit. Final PCR product (sequencing library) was purified with HighPrep beads, followed by library quality control. Sequencing library was quantified by Qubit fluorometer (Thermo Fisher Scientific, MA, USA) and its fragment size distribution was analyzed on Agilent 2200 TapeStation.

The library was sequenced using Illumina MiSeq (275×2 chemistry) platform. The Illumina paired-end raw reads were quality checked using FastQC (Andrews 2010). The high-quality reads were then assembled using SPAdes assembler (Bankevich et al. 2012). Gene calling and primary

annotation of the assembled genome was done using the RAST server (Aziz et al. 2008). The proteins thus predicted were similarity searched against Uniprot Virus and bacterial protein databases with an E-value cut off of $<10^{-5}$. The conserved domains on proteins were identified using NCBI conserved domain database (Marchler-Bauer et al. 2016). The tRNAs were predicted in the assembled genome using tRNAscan-SE (Lowe and Chan 2016). ARNold12 was used for the identification of rho-independent transcriptional terminators (Naville et al. 2011). The search procedure used two complementary programs Erpin and RNAmotif. Potential promoter sequences and the trans-membrane helices in the proteins were predicted using Martin Reese's Neural Network Promoter Prediction (Reese 2001) and TMHMM2.014 server (http://www.cbs.dtu.dk/services/TMHMM/), respectively. The SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP/) was used to detect presence and location of signal peptide in amino acid sequences. Phylogenetic trees were constructed using MEGA version 7 (Kumar et al. 2016) by maximum likelihood method based on the LG+G+I model with ClustalW aligned amino acid sequences of major capsid protein, phage portal protein, terminase large subunit, major tail protein and integrase. Bootstrap values were based on 100 replications. Genome sequence of Marinobacter phage AS1 was deposited in GenBank database under accession number, MK088078.

3. Results and Discussion

3.1. Growth and morphometric characteristics of Marinobacter phage AS1

The host, *Marinobacer sp.* strain D1S9 is a Gram negative, rod shaped bacterium (Fig 1B) isolated from the surface waters of the Arabian Sea. The host present in the water sample was enriched by the addition of nutrients which in turn amplified the specific viruses for the host. The isolated phages formed clear, round plaques of 1-2 mm diameter with regular edges on lawns of the host after 10-12 h of incubation (Fig 1C). Morphometric characteristics of phage AS1 examined by

transmission electron microscopy analysis indicated it belonged to family Podoviridae of the order Caudovirales. The phage resembled morphotype C1 with an icosahedral head (isometric) having a capsid diameter of ~50nm (Bradley, 1967). With a presence of short, stubby, non-contractile tail, characteristic of this group (Fig. 2). Host range experiments with 9 laboratory isolates of *Marinobacter* did not result any infection, indicating its narrow host range.

3.2. Genome features and annotation

Illumina sequencing and assembly of AS1 genome generated a single contig with a coverage of 92.5%. The assembled genome of Marinobacter phage AS1 was linear with a sequence length of 37 kb with a GC content of 57% (Fig. 3). The general features of AS1 genome are listed in Table 1. An initial whole genome similarity search against the NCBI non-redundant database using basic local alignment search tool (BLAST) showed no close relatives to Marinobacter phage AS1. AS1 genome showed no significant similarity to any of the three already reported Marinobacter phages, PS3 (GenBank accession, MF959999), PS6 (GenBank accession, MF959998) or B23 (Zhu et al. 2018; GenBank accession, KY939598). However, the AS1 phage represents the first Marinobacter phage belonging to the family Podoviridae. When phage AS1 genome was searched against the whole genome shotgun sequences of the taxon Marinobacter (taxid: 2742) using NCBI- BLAST, it showed an identity of 96 % with *Marinobacter manganoxydans isolate UBA5690_contig_21412* (DIHS01000048) and 94 % with *Marinobacter sp.* N4 KEHDKFFH_1 (PSSX01000001) with query coverages of 85% and 73 % respectively. This observation indicates the existence of fragments of AS1 genome within Marinobacter population and its possible interactions with the hosts. A total of 52 protein coding genes were predicted in the genome, of which 21 have assigned putative functions (Table 2). Genes related to phage structure and assembly, DNA modification, transcriptional regulation and host cell lysis were arranged in distinct functional clusters along the genome (Table 3).

The similar sequences of AS1 found within the genomes of *M. manganoxydans* isolate UBA5690_contig_21412 (in reverse orientation) and *Marinobacter* sp. N4 KEHDKFFH_1 are depicted in Fig. 4. They include genes encoding the small and large subunits of terminase, capsid proteins, portal protein, tail proteins, integrase, transcriptional regulators, methyl transferase, endonuclease, lysozyme and hypothetical proteins. Phage sequence was broken up and various functional clusters were shuffled within a particular region of the bacterial genomes.

The structural and assembly module encodes both small and large subunits of terminase, portal protein, serine peptidase, major capsid protein, Gp6, Gp3, major tail protein, tail fiber proteins and tail tape measure protein. The terminase large subunit protein (Gene 2) contains a P-loop having nucleoside triphosphate hydrolase activity and belongs to Terminase_1 family (PF03354). Gene 3 encodes for a protein with a signal peptide (residues 1-21) and a transmembrane helix (residues 29-51) and is homologous to protein Gp3 of *Klebsiella phage* PhiKO2. Besides, a prohead maturation protease (Gene 5) belonging to the MEROPS peptidase family S49 (protease IV family) was also identified in phage AS1. It has a conserved catalytic Serine-Lysine dyad typical of a serine protease domain (cd07022). The proteolytic activity is essential for the phage capsid maturation. The major capsid protein of AS1 (Gene 6) belongs to HK97 family and it forms coiled coil structure at amino acid positions 4-34 and 39-59. The delta domain of HK97 major capsid protein was shown to have coiled coils involved in prohead assembly and maturation and it was also found to be removed by proteases after the assembly (Oh et al. 2014). Gene 8 of the assembled genome codes for a protein containing gp6 domain (cd08054) with 36 conserved oligomerization interface amino acid residues of the domain. Gp6 of bacteriophage HK97 encodes head tail connector protein which forms an oligomeric ring and serves as an interface for head and tail attachment (Cardarelli et al. 2010).

Phage AS1 codes for an integrase gene having 97.2 % homology with that of *Marinobacter sp.* N4. It possess a C-terminal conserved domain similar to Shufflon-specific DNA recombinase Rci and bacteriophage Hp1_like integrase (cd00796) belonging to the superfamily of DNA breaking-rejoining enzymes. These enzymes contain a catalytic domain with six conserved amino acid residues. The presence of integrase gene indicates the potential for a temperate lifestyle which is further strengthened by the absence of any tRNA genes in the genome. It can be assumed that phage AS1 utilizes its host's tRNAs for translating the mRNA. Presence of tRNA genes provide phage with competitive advantage over other phages through more efficient expression of their own genes independent of their host's tRNAs. Whereas, their absence results in a narrow host range, making the phage more particular about selecting the host with similar codon usage bias (Bailly-Bechet et al. 2007).

Two sequence specific DNA methyl transferase genes, DNA adenine methyltransferase (DAM) and DNA (Cytosine-5) methyltransferase (DCM), present in AS1 genome control DNA-protein interactions by methylating adenine and cytosine residues of the DNA sequence. Considering this fact, phage encoded DNA adenine methyl transferase may be interpreted as a coevolutionary adaptation of the phage to protect itself from the host restriction enzymes (Murphy et al. 2014). Normally, specific DNA methyl transferases are part of host restriction-modification system which methylate host DNA at specific sites. This protects the host DNA from cleavage by its own restriction endonucleases which on the other hand destroy the incoming foreign phage DNA. These enzymes were also reported to have functions like controlling the expression of host virulence genes and selective silencing of genes that they methylate (Low et al. 2001, Oakey et al. 2002). In bacteriophage lambda, methylation by DNA adenine methyltransferase is associated with packaging of the phage genome (Sternberg and Coulby 1990), whereas, DNA (Cytosine-5) - methyltransferases are found

rarely in bacteriophage genomes and their functions in the context of viral lifecycle are unknown. Interestingly, a gene coding for the protein HNH endonuclease (gene 52) having homology to 5-methylcytosine specific restriction endonuclease McrA family (COG1403) with two highly conserved histidine and one asparagine residues was identified in AS1 genome nearby the lysozyme gene within the lysis module. McrA is a member of the superfamily HNHc (cI00083) which includes many homing endonucleases, bacterial colicins, pyocins etc. and are rarely reported in Podoviruses. Modified cytosine restriction (Mcr) systems capable of restricting phage λ modified by sequence specific cytosine methylases has been earlier reported in *Escherichia coli* strain K12 (Raleigh and Wilson 1986, Raleigh et al. 1989). The protein Gp74 from lambda-like phage HK97 was reported to possess HNH endonuclease activity and mediate the cleavage of phage DNA (Moodley et al. 2012). Later, Kala et al. (2014) discovered that these endonucleases were associated with the DNA packaging terminase proteins in HK97 and majority of the large terminase subunits linked with HNH endonucleases belonged to the Terminase_1 family (PF03354). The position of AS1 HNH endonuclease, adjacent to terminase and other morphogenetic genes, suggests its potential involvement in DNA packaging.

Genome of phage AS1 encodes pyocin activator protein (gene 36) with a conserved domain belonging to the PrtN family (PF11112) and is involved in the transcriptional activation of the polypeptide endonuclease toxin, pyocin. Other than lysozyme and HNH endonuclease, the lysis module encodes a Cro/CI family transcriptional regulator containing a HTH motif and a phage anti-termination Q type I family protein. The decision of the phage to enter either lytic or lysogenic cycle is determined by Cro/C1 regulatory system which is well characterized and studied in phage λ infecting the bacterium *Escherichia coli* (Ptashne 1967, Eisen et al. 1970, Schubert et al. 2007). The antitermination Q Type I protein positively regulates the phage early and late genes by modifying the

host RNA polymerase and making it to proceed transcription past the terminator sequences. A total of nine trans-membrane helices were predicted in seven proteins. 53 promoter sequences and 20 rho-independent terminator sequences were identified in the assembled genome.

Phylogenetic analysis revealed a mosaic pattern of inheritance of various proteins encoded by phage AS1 (Fig. 5). Major capsid protein, terminase large subunit and portal protein of AS1 had a Siphoviral lineage, whereas, integrase and major tail proteins were closely related to other Podoviral homologs. Recombination driven exchange of genetic material and the resulting genetic mosaicism is wide-spread among *Caudovirales.* Even though prominent morphological differences exist between the three families (specifically tail morphology), the differentiation is not clear cut due to striking sequence level similarities among several members of these different families. A well-known example for this is the genetic relatedness between phages lambda and P22 which are, however, classified under *Siphoviridae* and *Podoviridae*, respectively, based on their tail morphology. No lineage for the tail proteins of AS1 were deduced due to the limited availability of homologs.

Conclusion

Marinobacter phage AS1, isolated in this study, belongs to the family Podoviridae, infect marine bacteria belonging to genus *Marinobacter*, strain D1S9. The presence of integrase gene, occurrence of DNA methyltransferases and the existence of a significant percentage of phage AS1 genes within various Marinobacter genomes are suggestive of their intense association with the host. From the genome analysis, it is evident that phage AS1 relies greatly on its host's replication and translation machinery. Its codon usage bias must be similar to that of the host, since the phage genome does not encode any tRNA genes. Another striking feature of the genome is its mosaicism evident from the inheritance pattern of some of the important proteins with high similarity to their siphoviral counterparts. It also contains most of the morphogenetic, assembly, and lysis genes

involved in the lytic induction. AS1 genome also encodes a gene which positively regulates the expression of the bacterial toxin, pyocin; an interaction which attributes competitive advantage to the host. All the above arguments indicate the possible coevolutionary interactions between the host and the virus. As the members of genus *Marinobacter* are organisms with both phenotypic and metabolic versatility and reported to have many significant ecological functions like their contribution in biogeochemical cycles and marine snow formation, extensive studies on the involvement of these viruses in impacting host metabolism as well as recruitment of this virus to metagenome need to be carried out. The present study is a detailed analysis of the genomic properties of Marinobacter phage AS1, guiding future research on intricate virus-host interactions and the role of phages in the ecological functioning of the genus *Marinobacter.*

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Table 1. Genome features of Marinobacter phage AS1

Feature	**Marinobacter phage AS1**
Genome size	36,994 bp
GC content	57 %
Total no. of proteins	52
No. of proteins with putative function	21
tRNA genes	None
Proteins with trans-membrane helices	7

Table 2. Genome annotation of Marinobacter phage AS1

Gene	Start (bp)	Stop (bp)	Length (bp)	Strand	Putative function	E-value
1	70	531	462	+	Phage terminase small subunit P27 family	3.2E-111
2	531	2216	1686	+	Terminase large subunit	0.0
3	2210	2404	195	+	Gp3	2.8E-19
4	2404	3669	1266	+	Putative portal protein	0.0
5	3657	4589	933	+	Serine peptidase	0.0
6	4663	5946	1284	+	Major capsid head protein	0.0
7	6000	6224	225	+	Hypothetical protein	1.8E-48
8	6262	6795	534	+	Gp6	6.4E-116
9	6797	7348	552	+	Hypothetical protein	4.5E-128
10	7348	7902	555	+	Hypothetical protein	5.6E-127
11	7899	8348	450	+	Hypothetical protein	6.2E-98
12	8352	9107	756	+	Major tail protein	8.3E-8
13	9172	9321	150	+	Hypothetical protein	-
14	9385	9600	216	+	Hypothetical protein	1.3E-30
15	9665	12883	3219	+	Tail tape measure protein	2.0E-28
16	12880	13278	399	+	Hypothetical protein	8.3E-88
17	13275	14765	1491	+	Tail fiber	3.1E-81

18	14779	16893	2115	+	Tail fiber	1.4E-125
19	16893	18011	1119	+	Hypothetical protein	1.1E-82
20	18016	19926	1911	+	Hypothetical protein	0.0
21	19913	20146	234	+	Hypothetical protein	5.2E-56
22	20955	20143	813	-	Putative lipoprotein	9.2E-76
23	21434	20979	456	-	Hypothetical protein	2.4E-96
24	21810	22895	1086	+	Integrase	0
25	23007	23207	201	+	Hypothetical protein	4.0E-07
26	24260	23265	996	-	DNA methyl transferase	9.7E-131
27	25730	24270	1461	-	Hypothetical protein	-

Table 2. Genome annotation of Marinobacter phage AS1 (continued)

28	26213	25878	336	-	Hypothetical protein	2.8E-46
29	26407	26210	198	-	Hypothetical protein	1.3E-43
30	26661	26404	258	-	Hypothetical protein	6.5E-57
31	28046	26658	1389	-	DNA (Cytosine-5)- methyl transferase 1	0.0
32	28417	28043	375	-	Hypothetical protein	2.7E-27
33	28711	28421	291	-	Hypothetical protein	2.2E-37
34	29094	28720	375	-	Hypothetical protein	3.3E-811
35	29299	29105	195	-	Hypothetical protein	3.5E-48
36	29617	29354	264	-	Pyocin activator protein	3.0E-49
37	29892	29614	279	-	Protein containing phage anti repressor protein domain	3.2E-15
38	30317	29889	429	-	Hypothetical protein	5.6E-81
39	30981	30502	480	-	Hypothetical protein	1.5E-69
40	31316	30978	339	-	Hypothetical protein	6.5E-53
41	32428	31313	1116	-	Hypothetical protein	5.2E-82
42	32880	32473	408	-	Hypothetical protein	5.1E-61
43	32981	33217	237	+	Cro/cl family transcriptional regulator	2.0E-41
44	33282	34235	954	+	Hypothetical protein	0.0
45	34285	34629	345	+	Hypothetical protein	1.3E-70

46	34619	34969	351	+	Phage anti termination Q type I family- like protein	1.5E-15
47	35096	35527	432	+	Lysozyme	3.6E-91
48	35524	35802	279	+	Hypothetical protein	5.5E-53
49	35786	36016	231	+	Hypothetical protein	6.0E-57
50	36013	36462	450	+	Hypothetical protein	1.6E-43
51	36464	36592	129	+	Hypothetical protein	2.8E-13
52	36592	36975	384	+	HNH endonuclease	2.3E-87

Table 3. Functional categorization of phage AS1 proteins

Function	**No. of proteins**
Phage structure and assembly	13
Phage defense/DNA modification	2
Transcriptional regulation	4
Host lysis	1
Phage integration	1
Hypothetical proteins	31

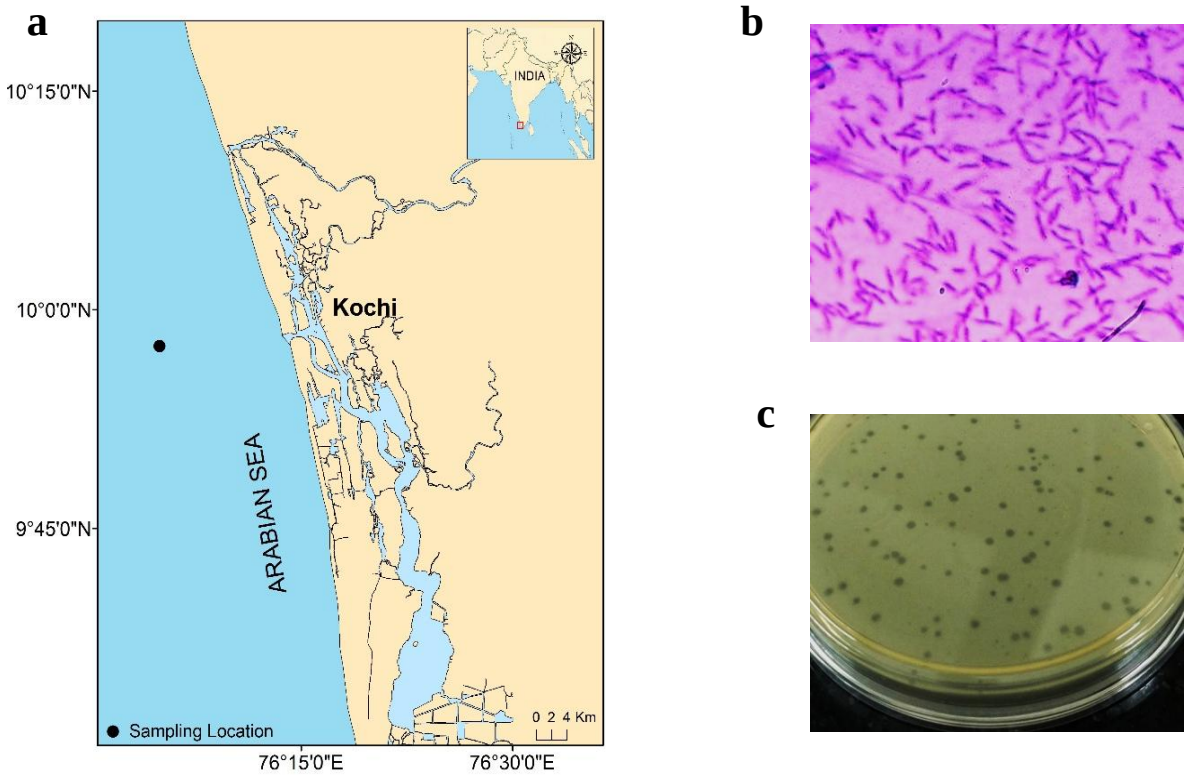

Fig. 1 Isolation of phage AS1. (**a**) Sampling location in the Arabian Sea, (**b**) rod shaped Marinobacter host cells after staining with crystal violet, (**c**) clear round plaques of phage AS1 formed on a lawn of host

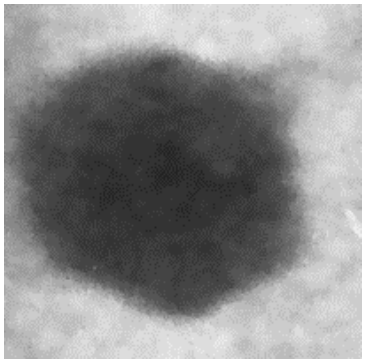

Fig. 2 Morphology of the isolated phage. Electron micrograph of phage AS1. Scale bar equals 50 nm.

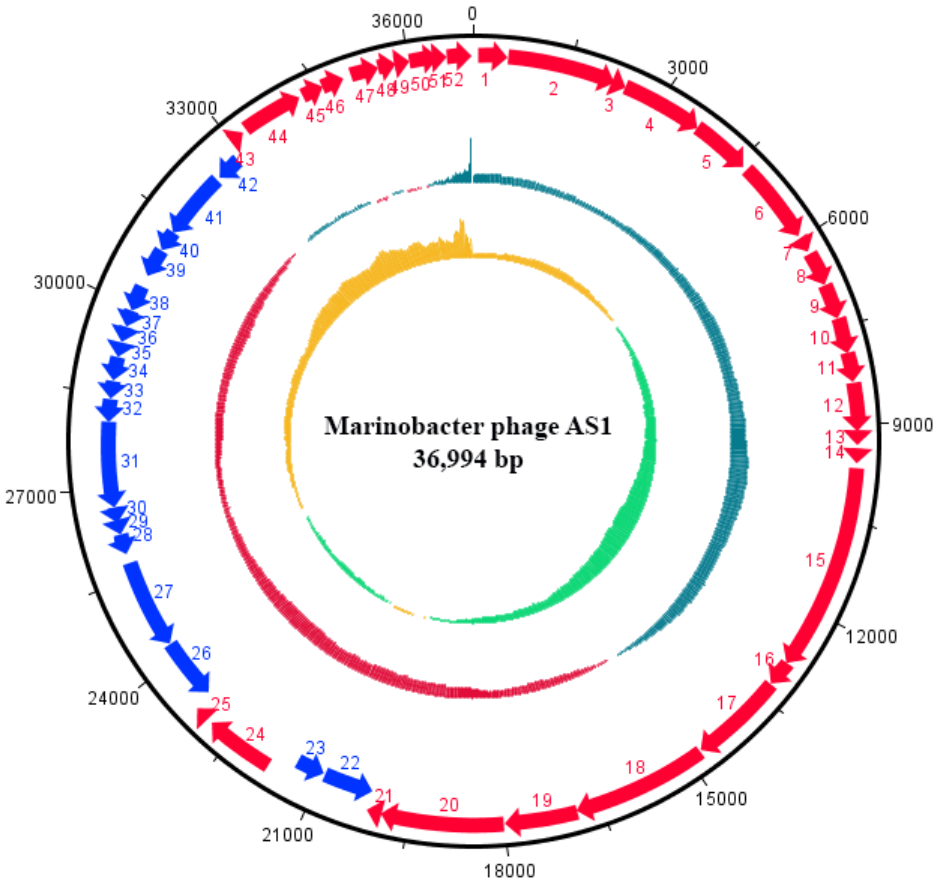

Fig. 3 Circular representation of the double stranded linear genome of Marinobacter phage AS1 featuring (from outside to inside) coding DNA sequences with predicted functions in the forward strand (red), reverse strand (neon blue), GC content (maroon & blue) and GC skew (green & yellow).

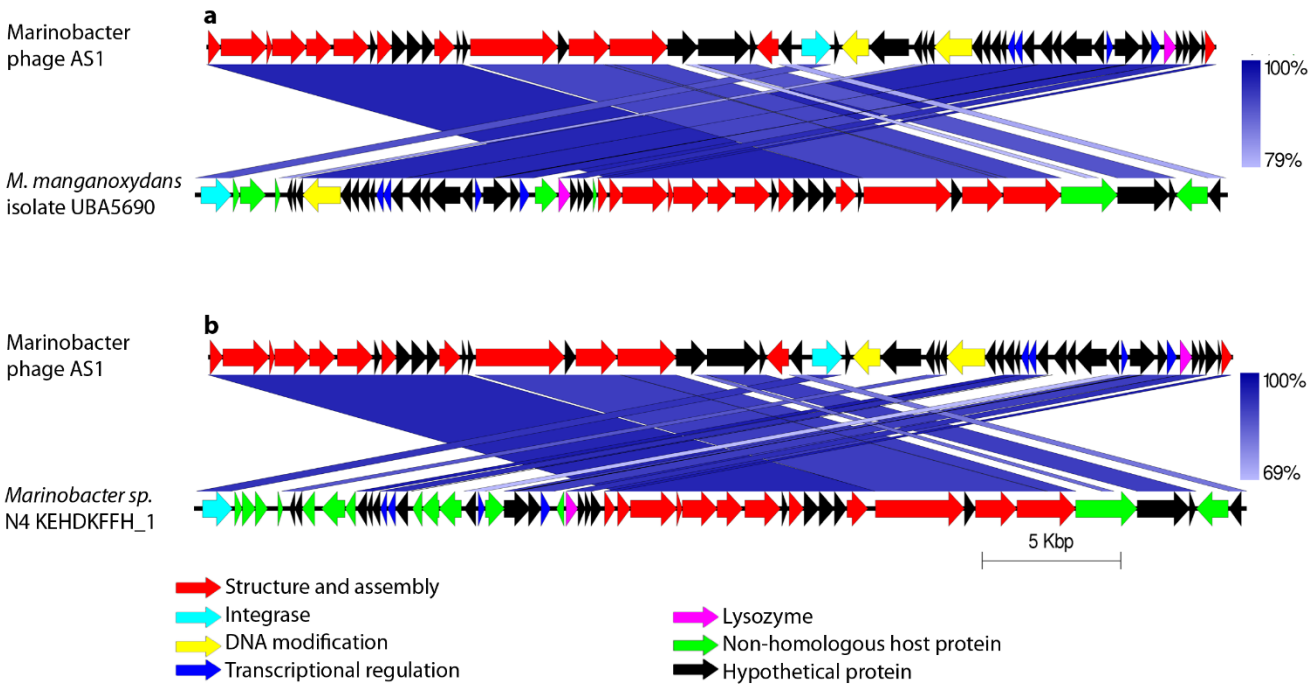

Fig. 4 Comparison of similar sequences found within the sequences of host genus. Comparison with (**a**) *M. manganoxydans* isolate UBA5690 and (**b**) *Marinobacter sp.* N4 KEHDKFFH_1. The intensity of blue colour indicates the percentage of similarity between the two sequences.

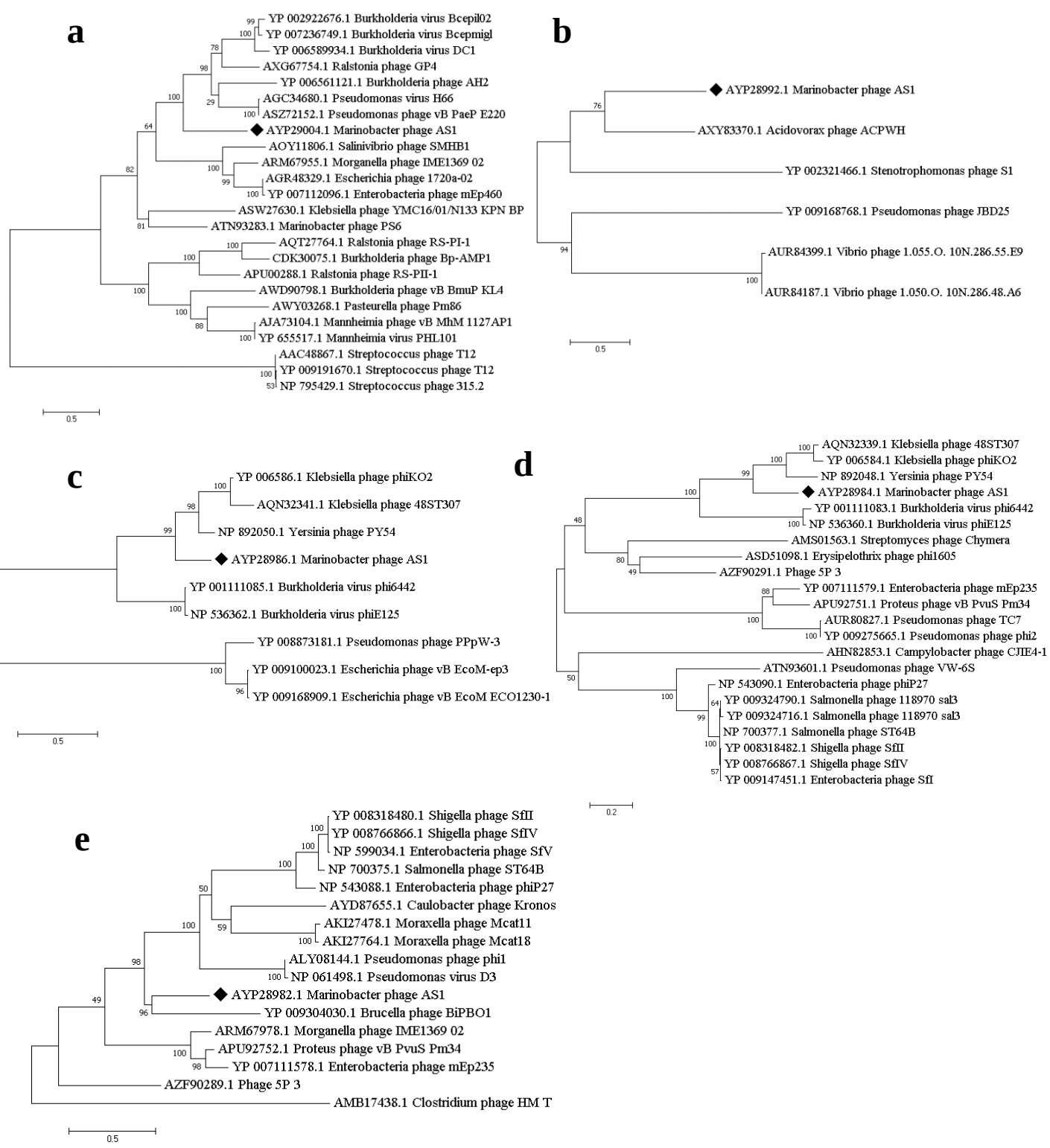

Fig. 5 Phylogenetic trees of AS1 proteins. (**a**) Integrase, (**b**) major tail protein, (**c**) major capsid protein, (**d**) portal protein and (**e**) terminase, large subunit.