Characterization and complete genome sequence of bacteriophage vB_Vc_SrVc2, a marine phage that infects Vibrio campbellii

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Abstract

*Vibrio campbellii* is widely distributed in the marine environment and is an important pathogen of aquatic organisms such as shrimp, fish, and mollusks. The emergence of multi-drug resistance among these bacteria resulted in a worldwide public health problem, which requires alternative treatment approaches such as phage therapy. In the present study, we isolated a phage vB_Vc_SrVc2 from white shrimp hepatopancreas with symptoms of AHPND. Phage vB_Vc_SrVc2 is a member of the genus *Maculivirus* and the family *Autographiviridae*, with high lytic ability against *Vibrio* isolates. Phage has a high resistance to a broad range of temperatures, salinity, UV radiation and chloroform. The genome size was 43,157 bp, with a GC content of 49.2% that encodes 49 putative ORFs, no tRNAs, showed three single nucleotide polymorphisms, two small deletions and one nucleotide insertion compared to SrVc9, showing slightly different infectivity profiles.. No lysogeny related genes were detected in vB_Vc_SrVc2 genome. Overall phage vB_Vc_SrVc2 has a good potential for therapeutic use in the aquaculture industry against *V. campbellii* infections.

Introduction

*Vibrio* genera are a widespread bacteria in marine environments and various species inside this genera are responsible for disease outbreaks in wild and reared aquatic organisms; most notably penaeid shrimp, several fish species, and mollusc [1]. Luminous *Vibrios* related to *V. harveyi* and *V. campbellii* have been implicated in disease outbreaks in shrimp larviculture facilities and in grow-out ponds worldwide [2], and more recently have been associated with infections in corals [3, 4]. In the past decade, shrimp production in SE Asia and Mexico has dropped sharply with significant economic losses due to acute hepatopancreatic necrosis disease (AHPND) caused by *Vibrio* spp. [5]. Initially, *V. parahaemolyticus*, which becomes virulent by acquiring a unique extrachromosomal AHPND-associated plasmid carrying *pirAB vp* (VP<sub>AHPND</sub>), was the only pathogen known to cause AHPND. Later, non-*V. parahaemolyticus* AHPND-causing *Vibrio* started emerging, and *V. harveyi*-like, *V. owensii* and *V. campbellii* strains have been reported [4, 6, 7]. *In vivo* model with crustaceans like *Artemia franciscana*, and *Litopenaeus vannamei*, has been used to study the pathogenicity of *Vibrio* species and potential treatments against *Vibrio* infections [8, 9].

One potential treatment is the use of bacteriophages (phages) that infect and kill bacteria during their replication cycle. Phages are made of a protein shell measuring between 24 and 200 nm, containing proteins and nucleic acids [10]. Those are in general small as their length vary between 17 and 700 kb [11]. Phages can be roughly divided into two groups: temperate phages and lytic phages. These differ by their infection cycle which consists in the integration of phage genetic information into the host's DNA and eventual killing of the host for temperate phages and immediate killing for lytic phages. Temperate phages are generally avoided for phage therapy as they may promote horizontal gene transfer and can thereby spread antimicrobial resistance genes or virulence genes, which would be counterproductive [12, 13]. Considering these disadvantages during phage selection, we describe genomic and physical characteristics of a lytic phage against *Vibrio campbellii*. 


Materials And Methods

Bacterial strains

*Vibrio campbellii* strain (CIBGEN-002) was used for phage isolation [14], and other *Vibrio* strains *V. parahaemolyticus* (CIBGEN-001), *V. campbellii* (CIBGEN-003), and *V. alginolyticus* (CIBGEN-004) were used for host range assay, efficiency of plating, and killing curves only. All strains are available at Genomic Laboratory’s collection of CIBNOR. For experimental assays, strains were reactivated in 5 mL of TSB medium supplemented with 2.5 % NaCl (TSB) at 35 ºC during 24 h. Before their use, the optical density of strains were adjusted in sterile saline solution at 2.5% NaCl to an absorbance (600 nm), corresponding to $\sim2.1\times10^8$ CFU mL$^{-1}$ (estimated with serial dilution in 2.5% NaCl and plating on TSA).

Bacteriophages isolation and propagation

Using hepatopancreas of shrimps with AHPDN signs, the isolation of phage vB_Vc_SrVc2 was performed as previously described in [14] using the standardized procedure described by [15] with slight modifications. Using a positive lytic zone, the sample was diluted using serial dilutions to obtain individual phage plaques according to [15]. A unique phage plaque corresponding to phage vB_Vc_SrVc2 was re-isolated three times to ensure the purity of the phage.

For phage vB_Vc_SrVc2 high production [16], 500 mL of TSB medium was inoculated with bacteria CIBGEN-002 and phage vB_Vc_SrVc2; and incubated at 25°C during 24 h. All volume was centrifuged at 5000 g for 30 min to remove bacterial debris, and finally filtered using a 0.2 µm filter. New phage stock titter was determined using the double-agar overlay plate assay [15], and correspond to $4.6\times10^9$ PFU mL$^{-1}$. Phage stock was maintained at 4°C until its use.

Phage host range

A spot lysis test was used to assess the host range of phage vB_Vc_SrVc2. Briefly, 20 µL of phage suspension was spotted on lawns of each of the four *Vibrio* strains (Table 1) using the agar overlay method [15] on TSA plates. Plates were incubated at 30 ºC during 24 h. Zones with no bacteria growth were considered as lytic activity (positive interaction between phage-host).

Efficiency of plating

Considering that every phage has different production of progeny on different hosts, we evaluated the efficiency of plating of vB_Vc_SrVc2 phage with each susceptible host (Table 1). Previously, phage stock was diluted to $10^{-8}$ using serial dilutions, and bacterial strains were grown to a concentration of $10^9$ CFU mL$^{-1}$. For phage quantification 100 µL of bacterial was mixed with 100 µL of corresponding phage lysate dilution, and added to a double-layer agar plate. The process was repeated with each strain and phage dilution. Plates were incubated at 30 ºC during 24 h. EOP was determined using the number of phages obtained in a new host divided by number of phages obtained in original host. These values were classified as ‘High,’ ‘Moderate,’ and ‘Low’ based on the productive infection on the target bacterium [17].
Killing curves

Killing curve assay was performed to evaluate the growth inhibition due to vB_Vc_SrVc2 phage presence over every bacterial strain (Table 1), using the method previously described by [14] but using a multiplicity of infection of 0.01 (MOI = 0.01) for this time.

Effect of temperature, salinity, UV, and chloroform on phage stability

Phage stability was tested in different values of temperature, salinity, UV radiation, and chloroform exposition. Exposition time and values of every variable was performed using the method of [14]. In all cases, serial dilutions were prepare to determinate final phage concentrations with double-agar plate assay [15].

Phage DNA purification and sequencing

A 1 mL volume of a vB_Vc_SrVc2 stock (1·10^9 PFU mL^-1) was treated with DNase I and RNase A (Sigma-Aldrich, Germany) to remove any exogenous host genomic DNA and RNA, respectively [18]. Total DNA was then isolated using, 500 µL of lysis buffer (NaCl 100 mM, Tris 50 mM, EDTA 100 mM, SDS 1%), was added and vigorously vortexed for 10 s. Finally, 20 µg mL^-1 of proteinase K was added to the phage sample and incubated at 55 ºC for 60 min. Phage DNA was purified by phenol-chloroform protocol and subsequent ethanol precipitation [73]. After phage nucleic purification, we ran on gel and observed a single band, indicative of DNA. Sequencing libraries were prepared using NexteraXT library preparation kit. Sequencing was carried out on a NextSeq550 (2 x 150 bp).

Bioinformatics analysis

Reads were trimmed with trim_galore v0.6.6 with default settings. Trimmed reads were assembled with SPAdes v3.12.0 using the –only-assembler option [19]. The resultant phage contig was checked for assembly errors with Pilon v1.24 with default settings [20]. A manual reordering of the genome was required to set the possible starting point of vB_Vc_SrVc2 (SrVc2) and therefore the orientation of genes. Phage vB_Vc_SrVc9 (SrVc9) was taken as reference (accession number LR794124.1) using Geneious Prime v2021.0.3 with the ‘map to reference’ option and Minimap2 v.2.20 as an aligner with default settings [21]. The resulting sequence was used alongside the original paired-end reads to map and index the genome using Minimap2 v.2.17 [22] and Samtools v1.7 [23] respectively. Finally, the genome was reviewed again with Pilon to correct any possible errors.

Open reading frames (ORFs) were predicted with Prodigal V2.6.3 [24] and the RAST server [25] with default settings. All predicted proteins and their functions were manually assigned based on the BLASTp [26] and HHpred [27] results, the former against the NCBI non-redundant database and the latter against four downloaded databases (PDB_mmCIF70, SCOPe v70.2, Pfam-A v.331 and NCBI Conserved Domains v3.18). Putative transmembrane helices in proteins were screened using SOSUI [28] and TMHMM [29] servers. ARAGORN v.2.36 [30] and tRNAscan-SE v.2.0 [31] were employed to detect tRNA coding
sequences. Possible virulence and antibiotic resistance genes were performed using the Virulence Factor [32] and Comprehensive Antibiotic Resistance Databases [33]. The viral taxonomic position of vB_Vc_SrVc2 was determined using the whole genome sequence and two phylogenetic markers from the predicted proteins (DNA Polymerase and major capsid protein). The species, genus, and family level were estimated with the OPTSIL program [34], the recommended clustering thresholds [35], and an F value (fraction of links required for cluster fusion) of 0.5 [36] were calculated through VICTOR [35]. All trees were visualized in the Interactive Tree Of Life (iTOL) [37]. The final graphics, including the comparative map and trees were edited with the Inkscape software [38]. Finally, the genome sequence of phage vB_Vc_SrVc2 was deposited in GenBank under the accession number MW331544.1.

Results

Isolation and phage characterization

Phage vB_Vc_SrVc2 was isolated from shrimps with AHPND, that was considered a good option due to previously Vibrio AHPND presence in commercial production (Data not show). Phage vB_Vc_SrVc2 was named according to [39] naming convention. This phage generate small plaques (∼ 1 mm) over CIBGEN-002 at 30ºC after 48 h.

Phage characteristics

During spot test, three of four Vibrio strains were susceptible to phage vB_Vc_SrVc2 forming complete lysis zones, however all strains correspond to different species suggesting that phage vB_Vc_SrVc2 may have a broad host range. During EOP test, only CIBGEN-004 has a high value, meanwhile CIBGEN-001 have a low value (Table 1).

Bacterial reduction assay

The phage vB_Vc_SrVc2 showed a good ability to inhibit the growth of three hosts during 12 h (Fig. 1), with final inhibition percentages of 61.6 % for CIBGEN-001, 52.5 % for CIBGEN-002, and 25.7 % for CIBGEN-004. Phage vB_Vc_SrVc2 showed the best inhibition activity against CIBGEN-001, reducing bacterial growth since 6 h; at difference with CIBGEN-002 and CIBGEN-004. However, non-lytic effect was recorded over CIBGEN-003 (D) strain during all the experiment time.

Effect of temperature, salinity, UV, and chloroform on phage stability

Phage vB_Vc_SrVc2 remained stable until 20 s of UV exposure (Fig. 2), but after 25 s of exposition a significant loss was found (p < 0.05), corresponding to 4.6·10^8 PFU·mL^{-1}. However, phage vB_Vc_SrVc2
did not disappear even after 60 s of UV exposure, with a final concentration of $5.3 \times 10^8$ PFU·mL$^{-1}$.

Figure 2 vB_Vc_SrVc2 phage survival exposed to different times of UV radiation. Data are shown as the means and standard deviation of three independent assays. Capital letters show significant difference between treatments.

Temperature exposure affect the stability of vB_Vc_SrVc2 phage between 30 to 60 ºC (Fig. 3) with a significant loss of $3.5 \times 10^8$ PFU·mL$^{-1}$ ($p < 0.05$), corresponding to 36 % at 60 ºC; however vB_Vc_SrVc2 phage did not disappear even at 80 and 100 ºC, where the final concentration of viable virions is $1.4 \times 10^5$ and $4.9 \times 10^3$ PFU·mL$^{-1}$ respectively.

Figure 3 vB_Vc_SrVc2 phage survival at different temperatures during one hour of incubation. Data are shown as the means and standard deviation of three independent assays. Capital letters show significant difference between treatments.

Salinity did not cause a significant loss of phage vB_Vc_SrVc2 even at 35 ppt concentration ($p > 0.05$), but the increase to 40 ppt caused the loss of 100 % of phage viable virions (Fig. 4).

With respect to chloroform effect, we did not find a significantly loss of phage vB_Vc_SrVc2 with 2 % chloroform concentration ($p > 0.15$), causing only a loss of 15 % during one hour of exposure.

Figure 4 Effect of different salinities of TSB in the survival of phage vB_Vc_SrVc2 during one hour of incubation. Data are shown as the means and standard deviation of three independent assays. Capital letters show significant difference between treatments.

**Genome analysis**

After de novo assembly, a linear DNA genome of 43,157 bp with a GC content of 49.2% was obtained. All genes were found to be on the same strand as has been seen in other phages of the same genus.

Phage vB_Vc_SrVc2 shares a 99.99% identity and 100% query coverage with phage vB_Vc_SrVc9, the genome alignment of the two phages made with MAFFT [40] and visualized with Jalview [41] showed three single nucleotide polymorphisms, two small deletions and one nucleotide insertion (Online Resource 1). In the case of the putative primase [(ORF 10QQM14900.1)], the polymorphisms observed were not found in other phages related to phage vB_Vc_SrVc2 when they were compared in BLASTp against VP93, MGD1, FE11 and others. Therefore these changes were classified as missense mutation since the changes shown were sufficient to produce two amino acid substitutions in the conformation of the protein. For ORF 43 [QQM14933.1], the nucleotide change resulted in a synonymous substitution, since the triplet "ACA" in phage vB_Vc_SrVc2 and "ACG" in SrVc9 produce the same amino acid (threonine) for the protein. Additionally, phage vB_Vc_SrVc2 maintains an identity and query cover above 90% with other vibriophages reported in the National Center for Biotechnology Information database (March 2021) (Online Resource 2). No tRNA genes were found in phage vB_Vc_SrVc2 genome, yet none of the other
related phages seem to use tRNAs of their own, suggesting that SrVc2 is dependent on the host translation machinery [42].

Genome analysis showed 50 putative ORFs (Online Resource 3) predicted by Prodigal and RAST that were crosschecked manually by BLASTp (E-value less than 10 − 4) and HHpred databases. Most proteins with known functions were associated with DNA replication. A total of 26 proteins (46%) were annotated as hypothetical and the remaining 34 proteins (54%) were assigned a function. Within these functions, some proteins involved in replication (helicase), lysis (peptidase), nucleotide metabolism (endonuclease) and DNA packaging (ATP-binding protein), as well as structural (major capsid protein, tail fiber) were readily identified. Only three proteins showed the presence of transmembrane helicases (THMs) including the protein 21 and 27 with 1 single TMH and the protein 49 with 3 TMHs. Although none of these three proteins have a known function, they provide protein conformational insights that might be helpful in further analysis. DNA polymerase (ORF 13) and major capsid protein (ORF 33) were used for phylogenetic tree constructions due to their relative conservation [43]. Phage vB_Vc_SrVc2 was classified according to the guidelines of the International Committee on Taxonomy of Viruses [44].

The machine-learning based lifestyle prediction tool PhageAI [45] suggested a virulent lifestyle for phage vB_Vc_SrVc2 and no genes that could provide antibiotic resistance were found according to the Virulence Factor [32] and Comprehensive Antibiotic Resistance databases [33].

**Phylogenetics analysis and comparative genomics**

Phage vB_Vc_SrVc2 is a vibriophage isolated using *V. campbellii* as host. Based on the complete genome sequence, the taxonomic position indicated that phage vB_Vc_SrVc2 is more closely related with nine bacteriophages (Fig. 5), where has a closer identity with vibriophages VP93 and MGD1 besides phage vB_Vc_SrVc9. The resulting phylogenetic trees obtained from phylogenetic markers such as DNA polymerase shows a clear differentiation of phage vB_Vc_SrVc2 and vB_Vc_SrVc9 from other phages within the same group (Fig. 6), while major capsid protein analysis encompasses phage vB_Vc_SrVc2 within a broader subgroup, including phage vB_Vc_SrVc9, VP93, and MGD1 (Fig. 7). These phages have been classified within the genus *Maculvirus* and the family *Autographiviridae*.

Figure 5 Phylogenetic tree constructed from VICTOR analyses compared to 5 phage genus and an additional unclassified group. Phage vB_Vc_SrVc2 is marked in black to highlight its position within the genus Maculvirus. Each phage was sorted according to its classification based on the NCBI taxonomy database. The bootstrap consensus was set to 1000 replicates

Figure 6 Phylogenetic tree constructed from the conservation of DNA polymerase (ORF 13) above 90% with 811 amino acids compared to phages of the same genus. Phage vB_Vc_SrVc2 is marked in black to show its location within the same group. A clear differentiation of phage vB_Vc_SrVc2 and vB_Vc_SrVc9 from the rest of the phage is shown. The bootstrap consensus was set to 1000 replicates
Figure 7 Phylogenetic tree constructed from the conservation of major capsid protein (ORF 33) above 90% with 332 amino acids compared to phages of the same genus. Phage vB_Vc_SrVc2 is marked in black to show its location within the same group. Within a larger group, vB_Vc_SrVc2, vB_Vc_SrVc9, VP93 and MGD1 remain more closely related. The bootstrap consensus was set to 1000 replicates.

Phage genomes visualized with Easyfig [46] showed a set of conserved genes in phages VP93 and OWB (Fig. 8). Phage VP93 maintained a gene organization most similar to phage vB_Vc_SrVc2, with small differences like a non-coding section at the end of the genome present in both SrVc9 and OWB, and in particular genes associated with structural proteins remain largely conserved among the three phages. It is important to mention that, although hypothetical, many genes coding for proteins mainly involved in DNA replication and more frequently in structural proteins in SrVc2 and SrVc9, are not found in VP93 but are found in OWB (ORF 29, 30, 34, 46). In addition, a peptidase in VP93 (ORF 8) reported by [Hu et al, 2020] is absent in OWB, SrVc2 and SrVc9.

Figure 8 Comparative genomic map made with Easyfig between the phages most related to phage vB_Vc_SrVc2. Genes involved in DNA replication as well as structural genes are shown to be highly conserved. The figure was finally adapted using Inkscape.

Discussion

Aquaculture industry needs new options to prevent and control bacterial infections in its different areas, and perhaps many treatments were explored, the use of phages is still and attractive option to substitute or complement other therapies [47]. One of the main advantage of phages is their quantity and diversity found in nature, where is a limitless source of new phages [48]. Considering this, the isolation of new phages is necessary to access a new phage strains with the potential for phage therapy and provide new genomic information inside them. Here, we isolated a new lytic phage vB_Vc_SrVc2 against *Vibrio parahaemolyticus* AHPND and described its potential for phage therapy in aquaculture.

According to Fernandez et al, (2019) phage particles consist only of acid nucleic with a proteinaceous envelope, making them a more complex and labile compounds than other antimicrobials, so a previous analysis of their stability to different environmental conditions (marine water, fresh water, digestive tract, soil, etc.) is necessary for phage selection [48]. Also, the isolation of phages from same sites where we found the pathogenic bacteria, it is a good option to increase the proportion of adequate phages; because a high probability to found specific phages well adapted to site conditions is expected, considering a posterior re-introduction in similar sites during phage therapy application [48, 49]. Based on this point, we isolated vB_Vc_SrVc2 phage from samples of white shrimp ponds previously reported with AHPND problems, who which was stable at different values of temperature and salinity as expected, with at least a 40 % survival of phage virions. Also, the similar phage survival results were obtained at different UV radiation and chloroform values.

vB_Vc_SrVc2 phage was able to recognize and replicate using three vibrio species, with EOP values from 0.04 and 0.5, however a reduced host availability limited a deeper characterization. Also, vB_Vc_SrVc2
has a lower EOP with CIBGEN-001 and CIBGEN-004 host compared to EOP values of phage vB_Vc_SrVc9 using the same host. The smaller number of genetic differences between the two phages, narrows down the possible genes involved in this. However, further work is; however a new test is needed to determine the exact mechanism that causes the difference in EOP if phages differences are due to recognitions sites or other characteristics. Recognition of strains or even species inside a genera is a desirable result because bacteriophages could use similar receptors on membrane surface of related bacteria, like Harveyi clade [50, 51]; but this characteristic is not common in all phages [17]. Also, phage vB_Vc_SrVc2 was able to inhibit bacterial growth during 12 h with a unique dose of phage (MOI 0.1 for each strain). All these characteristic, showed that vB_Vc_SrVc2 phage is a good candidate for phage therapy against Vibrio infections and also could be used with other phages (phage cocktail) [52, 53] or other agents like probiotics [54, 55] and even antibiotics [56, 57].

vB_Vc_SrVc2 is incorporated into the recently created genus Maculvirus with a genomic organization and GC content similar to other vibriophages previously reported in GenBank (March 2021). The variation between the genomes of vB_Vc_SrVc2, VP93 and OWB is primarily concentrated in regions containing protein-coding genes that include transcriptional regulators, bacterial surface binding receptors and proteins with unknown function, while the main differences between vB_Vc_SrVc2 and vB_Vc_SrVc9 are in nucleotide metabolism, such as amino acid substitutions in the primase in vB_Vc_SrVc2, however, these changes were not found in the active or metal-binding site of the protein [58] and appear unlikely to have marked effects on function, suggesting alternative events, like competitive interaction between phages [59], host-specific changes [60] or genomic variations or small intraspecies genomic variations [61] that may favor slightly different infection profiles between vB_Vc_SrVc2 and vB_Vc_SrVc9 despite their high genomic identity. Further evaluation would be necessary to determine whether minor changes in the genome were solely responsible for the shift in infectivity range.

Interestingly, although several host recognition proteins in vB_Vc_SrVc2 maintain a percentage of identity above 90% compared to phages of the same genus, major differences in the amino acid sequence of the tail fiber protein between vB_Vc_SrVc2, VP93 and OWB may indicate distinct cell-surface targets for each of these phages since the changes observed between vB_Vc_SrVc2 and VP93 are conserved with little change, while greater divergence exists between vB_Vc_SrVc2 and OWB. These changes in host recognition patterns have been previously described in genetically identical phages infecting Vibrio anguillarum, suggesting that non-genomic mechanisms affect the functional properties of phages, expanding diversity beyond genomic variations [60] However, it has also been documented, that small intraspecies genomic variations can have phenotypic consequences due to minor changes in the genome, which can cause a significant change in the range of infectivity [61], for example, small point mutations can help in the evasion of host restriction-modification systems [62] or specific nucleotide variations, over generations, can change the phage's host range [63].

It is important to note, that the phages most closely related to SrVc2 like vB_VpaS_OWB isolated from the Atlantic Ocean water [64], Vibriophage VP93 isolated from the Pacific Ocean of Chile [65], and vB_VpaP_KF1 (KF1) & B_VpaP_KF2 (KF2) isolated from Western and Southern coastal areas of Korea
are also capable to infect *Vibrio parahaemolyticus* strains. Previous studies on protein expression of KF1 and KF2 phages confirmed the role of two homologous proteins found in vB_Vc_SrVc2, both ORF 8 coding for a peptidase and ORF 41 coding for a glycosyl hydrolase, demonstrated to be lytic proteins, being the peptidase, the one that showed the highest lytic activity [67]. Genomic information on MGD1, DE17, and FE11 phages are available in GenBank but there are no references on the biology or host of these phages yet. To date, vB_VcaS_HC (Accession number: MK559459.1) comprises the second reference (besides vB_Vc_SrVc9) to a vibriophage capable of infecting *Vibrio campbellii* [68] however vB_VcaS_HC contains a potential lysogeny genepotential lysogeny related gene (RecA) in its genome. BLASTnp analysis showed no significant similarity between vB_Vc_SrVc2 and vB_VcaS_HC.

On the other hand, using genes encoding for individual proteins (major capsid protein and DNA polymerase) we concluded that phage vB_Vc_SrVc2 belonged to the *Autographiviridae* family. These genes are widely used to assess viral diversity [69], because containas they several conserved regions [70] that allow a better resolution for phage relationships. Additionally, the high identity with other vibriophages also classified within this family as well as the systematic classification resulting from using VICTOR Victor's server, were indicative of phage vB_Vc_SrVc2 taxonomic position. In addition, the phylogenetic classification obtained from the DNA polymerase allowed a much better differentiation among members of the *Maculvirus* genus, even with the high genetic identity presented by phage vB_Vc_SrVc2 and phage vB_Vc_SrVc9. The aforementioned could indicate a useful phylogenetic marker for this specific genus. Although it is recommended that it be used with additional phylogenetic markers, known for their ubiquity among phages and high level of sequence conservation such as the terminase large subunit (TerL) to avoid false phylogenetic signals [71].

Phage therapy represents an opportunity against the spread of multi-resistant bacterial pathogens. Phage vB_Vc_SrVc2 offers an alternative against infections caused by *Vibrio campbellii* due to its genetic composition characterized for therapeutic applications [72]. The absence of genes that promote lysogeny or multi-resistance, the use of lytic lifestyle, and antibacterial efficacy evaluated by experimental techniques make phage vB_Vc_SrVc2 a suitable candidate for therapeutic use in the aquaculture industry.

**Declarations**

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**Tables**

Table 1. Host range of phage vB_Vc_SrVc2 against four bacterial strains from different Vibrio species: *V. parahaemolyticus, V. campbellii, V. alginolyticus*. EOP is expressed as: the fraction of phages’ infectivity vs. tested strains to phages’ infectivity vs. host bacteria. High (EOP≥ 0.5), Moderate (EOP≥0.1<0.5) and Low (EOP≤ 0.1).
| Bacterial host       | Host range | EOP  | Growth inhibition (%) |
|---------------------|------------|------|------------------------|
| CIBGEN-001 (V. parahaemolyticus) | Yes        | 0.04 | 58.6 ± 3.9             |
| CIBGEN-002 (V. campbellii)          | Yes        | 1.0  | 54.0 ± 0.9             |
| CIBGEN-003 (V. campbellii)          | No         | N/A  | N/A                    |
| CIBGEN-004 (V. alginolyticus)       | Yes        | 0.5  | 23.7 ± 2.2             |

* Not applicable

**Figures**
Figure 1

Killing curves (A) V. parahaemolyticus CIBGEN-001; (B) V. campbellii CIBGEN-002; (C) V. campbellii CIBGEN-003 and (D) V. alginolyticus CIBGEN-004 (○) control curve and (▲) strain infected with SrVc2; (MOI = 0.01). Data are the average of three repetition OD600 (n = 3)
Figure 2

vB_Vc_SrVc2 phage survival exposed to different times of UV radiation. Data are shown as the means and standard deviation of three independent assays. Capital letters show significant difference between treatments.
**Figure 3**

vB_Vc_SrVc2 phage survival at different temperatures during one hour of incubation. Data are shown as the means and standard deviation of three independent assays. Capital letters show significant difference between treatments.

**Figure 4**

Effect of different salinities of TSB in the survival of phage vB_Vc_SrVc2 during one hour of incubation. Data are shown as the means and standard deviation of three independent assays. Capital letters show significant difference between treatments.
Phylogenetic tree constructed from VICTOR analyses compared to 5 phage genus and an additional unclassified group. Phage vB_Vc_SrVc2 is marked in black to highlight its position within the genus Maculivirus. Each phage was sorted according to its classification based on the NCBI taxonomy database. The bootstrap consensus was set to 1000 replicates.
Phylogenetic tree constructed from the conservation of DNA polymerase (ORF 13) above 90% with 811 amino acids compared to phages of the same genus. Phage vB_Vc_SrVc2 is marked in black to show its location within the same group. A clear differentiation of phage vB_Vc_SrVc2 and vB_Vc_SrVc9 from the rest of the phage is shown. The bootstrap consensus was set to 1000 replicates.

Figure 7

Phylogenetic tree constructed from the conservation of major capsid protein (ORF 33) above 90% with 332 amino acids compared to phages of the same genus. Phage vB_Vc_SrVc2 is marked in black to show its location within the same group. Within a larger group, vB_Vc_SrVc2, vB_Vc_SrVc9, VP93 and MGD1 remain more closely related. The bootstrap consensus was set to 1000 replicates.

Figure 8

Comparative genomic map made with Easyfig between the phages most related to phage vB_Vc_SrVc2. Genes involved in DNA replication as well as structural genes are shown to be highly conserved. The figure was finally adapted using Inkscape.