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Genomic Analysis of Bacteriophage ΦJL001: Insights into Its Interaction with a Sponge-Associated Alpha-Proteobacterium†

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Bacteriophage ΦJL001 infects a novel marine bacterium in the α subclass of the *Proteobacteria* isolated from the marine sponge *Ircinia strobilina*. ΦJL001 is a siphovirus and forms turbid plaques on its host. The genome sequence of ΦJL001 was determined in order to better understand the interaction between the marine phage and its sponge-associated host bacterium. The complete genome sequence of ΦJL001 comprised 63,469 bp with an overall G+C content of 62%. The genome has 91 predicted open reading frames (ORFs), and 17 ORFs have been assigned putative functions. ΦJL001 appears to be a temperate phage, and the integrase gene was identified in the genome. DNA hybridization analysis showed that the ΦJL001 genome does not integrate into the host chromosome under the conditions tested. DNA hybridization experiments therefore suggested that ΦJL001 has some pseudolysogenic characteristics. The genome of ΦJL001 contains many putative genes involved in phage DNA replication (e.g., helicase, DNA polymerase, and thymidylate synthase genes) and also contains a putative integrase gene associated with the lysogenic cycle. Phylogeny based on DNA polymerase gene sequences indicates that ΦJL001 is related to a group of siphoviruses that infect mycobacteria. Designation of ΦJL001 as a siphovirus is consistent with the morphology of the phage visualized by transmission electron microscopy. The unique marine phage-host system described here provides a model system for studying the role of phages in sponge microbial communities.

Bacteriophages are important components in many natural microbial ecosystems, and they are known to play an important role in maintaining the composition and structure of microbial communities (13). Viruses are highly abundant in the marine environment, reaching concentrations of 10^7 to 10^8 viruses per ml (7, 37). These large numbers of marine viruses can exert significant control in marine bacterial communities with respect to both species composition and genetic transfer (5, 6, 18, 34). To understand the ecological role of viruses in the marine environment, it is necessary to know the infectivity of viruses and the types of interactions that occur between marine viruses and their host bacteria. The potential host bacteria include not only free-living heterotrophic bacteria in the water column but also symbionts of marine invertebrates, including sponges.

Sponges are filter feeders that are capable of filtering thousands of liters of water; for example, a 1-kg sponge can to filter up to 24,000 liters a day (30). The filtering of these enormous amounts of water has the potential for introducing billions of phages into the sponge. Studies of sponge-associated bacterial communities have revealed that several bacterial groups and species are ubiquitous in sponges throughout the world (17). Members of the α subclass of the *Proteobacteria* are a well-represented group in the complex and the highly diverse sponge microbial communities (17, 19). The host bacterium in the host-phage system described here is a member of the α subclass of the *Proteobacteria*. Interestingly, representatives of the α subclass have been shown to be dominant members of the culturable bacterial assemblage in some sponges. A member of this subclass dominated the culturable assemblage associated with the sponge *Halichondria panicea* in the Adriatic Sea, North Sea, and Baltic Sea (2). Analysis of the culturable bacteria present in *Rhopaloeides odorabile* showed that strain NW001, a member of the α subclass, is the dominant culturable bacterium in this Great Barrier Reef sponge (32). The role of members of the α subclass of the *Proteobacteria* in sponges remains unknown, although Althoff et al. (2) and Webster and Hill (32) postulated that strains belonging to the α subclass are true sponge symbionts. In two clearly diseased individuals of *R. odorabile*, strain NW001 could not be isolated, and another member of the α subclass of the *Proteobacteria*, strain NW4437, dominated the culturable bacterial community (32). Strain NW4437 was shown to be pathogenic for the sponge (33). In the absence of certain strains belonging to the α subclass, the health of sponges may be compromised. In other cases, members of the α subclass of the *Proteobacteria* appear to be the cause of necrosis (33).

A phage designed to specifically eliminate a particular member of the α subclass of the *Proteobacteria* could be used as a precise tool for investigating the interaction between these bacteria and sponges. The use of bacteriophages, such as ΦJL001, to specifically target and manipulate microbes in the highly diverse and complex microbial community of sponges should be a invaluable tool for elucidating the roles of sponge symbionts, as well as the roles of phages, in sponge microbial communities. The first description of a complete genome sequence of a marine phage that infects a sponge-associated bacterium is presented here, and the relationship between

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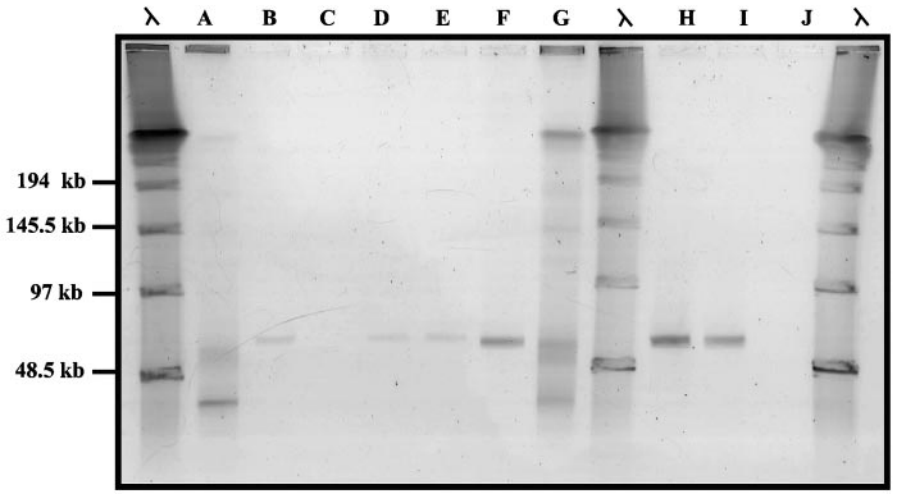

FIG. 1. PFGE analysis of microalgal cultures, showing the appearance of an approximately 60-kb band 8 weeks after addition of viral concentrates. Lanes A and G, viral concentrates; lanes B, D, E, and F, cultures to which concentrate from lane A was added; lanes H and I, cultures to which concentrate from lane G was added; lanes C and J, control cultures to which no viral concentrate was added. Molecular size markers are λ concatemer ladders.

ΦJL001 and its host, strain JL001 isolated from the sponge *Ircinia strobilina*, is described below.

MATERIALS AND METHODS

Sample collection and isolation of sponge-associated bacteria. The sponge *I. strobilina* was collected at Tennessee Reef just off Key Largo during a research cruise of the Harbor Branch Oceanographic Institution on 24 August 1999. *I. strobilina* was collected by SCUBA at a depth ca. 10 m, and a 20-liter water sample was taken from the water column immediately surrounding the sponge. All samples were kept at ambient temperature until they were processed (<3 h). The sponge was surface sterilized by washing it briefly with 70% ethanol, followed by rinsing with sterile artificial seawater. By using aseptic techniques, a 1-cm^3 section of sponge tissue was excised and homogenized in 10 ml of sterilized seawater with a mortar and pestle. Heterotrophic bacteria were isolated from serial dilutions of processed sponge material spread onto marine agar 2216 plates (Difco, Detroit, Mich.), and cyanobacteria and microalgae were isolated by inoculating dilutions of the sponge tissue into $Mn+B_{12}$ liquid medium (31). All organisms were grown at 30°C.

Viral concentration. Prefiltration of the water samples was carried out by two-stage filtration by using no. 3 filters mounted in stainless steel filter holders (Whatman, Clifton, N.J.) and then a 0.2-μm-pore-size polycarbonate filter (Whatman). Viral particles in the water samples were concentrated ca. 200-fold with an S1OY30 Amicon spiral wound cartridge system (Millipore, Bedford, Mass.).

Viral concentrate (1 ml) was added to an algal culture (100 ml) isolated from the *I. strobilina* sponge.

PFGE and Southern hybridization. Viral amplification was monitored by pulsed-field gel electrophoresis (PFGE). Supernatants from an algal culture incubated with viral concentrate were prepared for PFGE by using previously described methods (38). PFGE of samples was performed by using a clamped homogeneous electric field system (CHEF DR-III; Bio-Rad, Richmond, Calif.) under the following conditions: 1% (wt/vol) agarose in 1× Tris-borate-EDTA gel buffer (90 mM Tris-borate, 1 mM EDTA; pH 8.0), 0.5× Tris-borate-EDTA tank buffer, 1- to 15-s pulse ramp, 200-V current at a constant temperature of 14°C, and a run time of 22 h.

DNA plugs containing cells of strain JL001 were prepared for PFGE analysis by a previously described procedure, with slight modifications (24). Lysozyme treatment was performed for 4 h at 37°C, and this was followed by 18 h of incubation at 50°C with 1 mg of proteinase K per ml in a solution containing 100 mM EDTA (pH 8.0), 0.2% (wt/vol) sodium deoxycholate, and 1% (wt/vol) sodium lauryl sarcosine. Plugs were rinsed with 20 mM Tris-HCl–50 mM EDTA (pH 8.0) four times for 1 h at room temperature; Phenylmethylsulfonyl fluoride (1 mM) was included in the second rinse solution. PFGE was performed with a CHEF DR-III apparatus under the following conditions: 0.6% (wt/vol) chromosomal-grade agarose in 1× Tris-acetate-EDTA (TAE) gel buffer and 1× TAE tank buffer. Electrophoresis was performed in two blocks. Block 1 was performed by using a switch time of 20 to 24 min 29 s and a current of 200 V with an angle of 106° for 72 h; block 2 was performed by using a switch time of 5.6 s to 2 min 26 s and a current of 200 V with an angle of 120° for 6 h 28 min. Gels were stained with SYBR Green I (PE Applied Biosystems, Foster City, Calif.) used according to the manufacturer's instructions and were visualized with a FluorImager 573 (Molecular Dynamics, Sunnyvale, Calif.).

Viral and bacterial DNA was transferred from pulsed-field gels to H+ Hybond nylon membranes (Amersham Pharmacia Biotech Ltd., Little Chalfont, Bucks, United Kingdom) (39). Membranes were probed with a ΦJL001-specific ^{32}P-radiolabeled probe at a final concentration of ca. 1×10^6 cpm ml^{-1} (39). The membranes were washed twice for 15 min in 5× SSC–0.5% (wt/vol) sodium dodecyl sulfate (SDS) at 25°C, twice for 15 min in 1× SSC–0.5% (wt/vol) SDS at 37°C, and once for 15 min in 0.1× SSC–1% (wt/vol) SDS) at 37°C (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate).

Isolation of host bacterium and bacteriophages. Heterotrophic bacteria present in the microalgal culture of interest were isolated on marine agar 2216 (Difco). Bacterial cultures were then grown in 50 ml of marine broth in 250-ml flasks with shaking at 30°C. Supernatants used to infect the potential hosts were filtered (pore size, 0.2 μm) to ensure that they were bacterium free but could contain any viral particles present in the original nonaxenic culture. The potential hosts (0.1 ml) were incubated with dilutions of the supernatant from the microalgal culture (0.9 ml), and overlays were made with 0.6% marine agar overlaid on marine agar 2216 plates. The phage was isolated and propagated after incubation at 30°C for 3 days.

Identification of bacterial strain JL001. The DNA of the bacterial isolate was extracted by using a previously described small-scale DNA extraction method (28). PCR amplification of the ca. 1,500-bp 16S rRNA gene fragment from the purified genomic DNA was carried out with primers 8-27F (35) and 1492R (25). Thermal cycling was initiated by denaturation at 94°C for 3 min, and this was followed by denaturation at 92°C for 1.5 min, annealing at 48°C for 1.5 min, and extension at 72°C for 3 min. Thermal cycling was performed in a PTC-200 cycling system (MJ Research, Waltham, Mass.) for 25 cycles of denaturation, followed by a final extension at 72°C for 5 min. The PCR product was subjected to electrophoresis on a 1% agarose gel, stained with ethidium bromide, and visualized by UV excitation. The band of the expected size was excised and purified by using a QIAquick gel extraction kit (QIAGEN, Chatsworth, Calif.). The purified product was sequenced by using an ABI model 373 automated sequencer (Applied Biosystems, Foster City, Calif.). The 16S rRNA gene sequence was aligned manually by using the Phydit software (11), and sequencing data were analyzed by comparison to the sequences of the nearest relatives found by searching the GenBank database with the Basic Local Alignment Search Tool (BLAST) (3). A phylogenetic tree was constructed by using the PHYLIP software and the neighbor-joining method with Jukes-Cantor corrections (27).

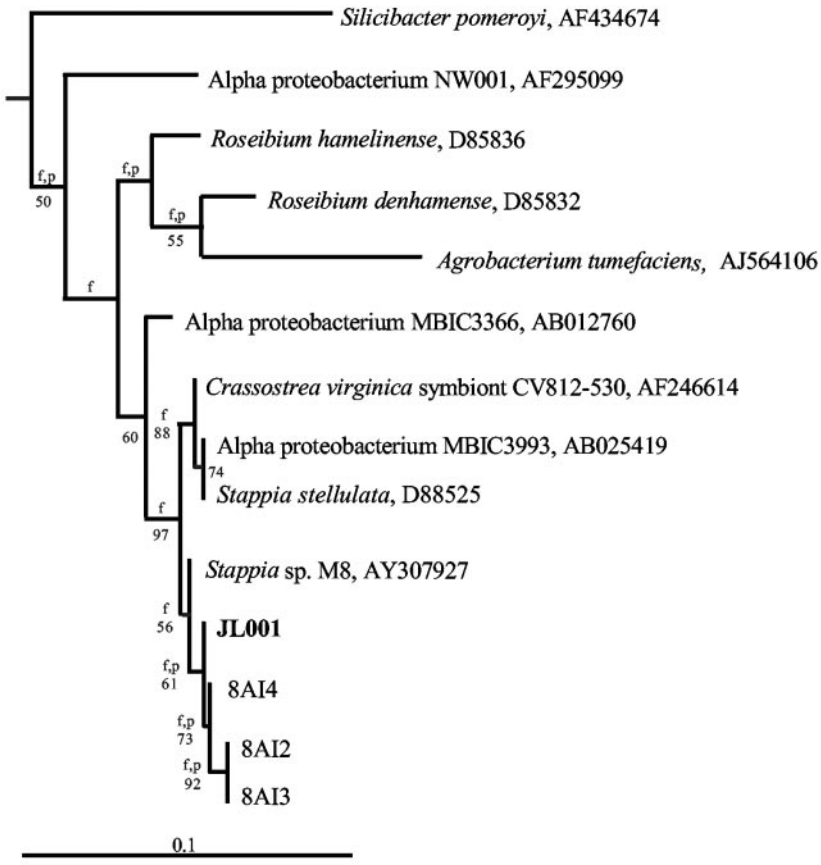

FIG. 2. Neighbor-joining tree based on the 16S rRNA gene sequence, showing the phylogenetic relationship between strain JL001 and its close relatives. Levels of bootstrap support greater than 50% based on a neighbor-joining analysis of 1,000 resampled data sets are indicated at the nodes. Branches that were also found when the Fitch-Margoliash and maximum-parsimony methods were used are indicated by f and p, respectively. Scale bar = 0.1 substitution per nucleotide position.

Induction of putative lysogens. Putative lysogens were inoculated into 100 ml of marine broth to obtain an initial optical density at 600 nm of 0.05. Triplicate cultures were incubated with shaking at 30°C. After overnight incubation, the logarithmic-phase cultures were induced with 0.1, 0.25, and 1.0 μg of mitomycin C per ml or by exposure to a wall-mounted germicidal UV light (Phillips Sterilamp G36T6L 39 W) that was 0.75 m above the cultures for 30, 60 or 90 s. Cultures were maintained in the dark during assays to prevent photorepair effects. Phage titers were determined immediately prior to treatment and at 48 and 96 h posttreatment.

Transmission electron microscopy examination of phage morphology. Lysate was prepared from plaque assays. Plates with confluent lysis were chosen, and phage were collected from the soft agar by shaking the plates gently for 10 min with 10 ml of $Mn+B_{12}$ liquid medium. The supernatant was centrifuged for 10 min at 5,000 × *g* to remove agar and filtered through a 0.2-μm-pore-size filter (Millipore) to remove any of the host bacterium present. This lysate was fixed in 2% (wt/vol) glutaraldehyde. The viral particles were harvested directly onto nickel grids by centrifugation (200,000 × *g*, 30 min) and negatively stained with uranyl acetate (2% [wt/vol] in water).

Phage sequencing. Lysate was harvested and phage DNA was extracted by using methods described previously (28). The viral DNA was randomly sheared by passage through a 26-gauge needle. Passages through the needle were experimentally determined to give good yields of sheared DNA in the 1- to 5-kb size range (results not shown). Sheared DNA was blunt ended by using a DNA terminator end repair kit (Lucigen, Middleton, Wis.). Blunt-ended fragments were gel purified on a 1× TAE–1% (wt/vol) agarose gel. Fragments of the desired size (1 to 5 kb) were excised and extracted with a QIAquick gel extraction kit (QIAGEN). The resulting fragments were ligated into the pSMART vector and electroporated into Ecloni 10G electrocompetent cells (Lucigen). Transformed cells were spread onto Terrific broth plates (Lucigen) and incubated overnight at 37°C. Colonies were subcultured the following day.

Sequencing was performed by Agencourt (Beverly, Mass.) and with an model ABI 373 automated sequencer (Applied Biosystems, Perkin-Elmer) at the Bio-Analytical Services Laboratory of the Center of Marine Biotechnology. Each clone was sequenced with plasmid forward and reverse primers SL1 (5′ CAGT CCAGTTACGCTGGAGTC 3′) and SR1 (5′ CTTTCTGCTGGAGGGGTCA GGTATG 3′). Consensus sequences were assembled by using the AssemblyLIGN (Accelrys, San Diego, Calif.) and PhredPhrap software. For the larger cloned fragments, internal primers were designed from the original sequence by using MacVector (Accelrys) and were synthesized by Sigma-Genosys (The Woodlands, Tex.). The BLAST programs Blastn, Blastx, Blastp, and PSI-BLAST were used to compare continuous sequences (contigs) to nucleotide and amino acid databases (3). Searches for open reading frames (ORFs), were performed online with the WebGeneMark.htm software (4). The predicted genes were compared with sequences in the GenBank database. A hit was considered significant if it had an *E* value of <0.001.

Phylogenetic analysis of a functional gene. The translated amino acid sequence for the putative genes encoding DNA polymerase and integrase was used to construct phylogenetic trees. The amino acid sequence was aligned with the sequences of viruses found in the GenBank database with the program CLUSTAL W by using MacVector 7.1. A Blosum 30 matrix was calculated with a gap penalty of 10.0. Evolutionary distances were calculated by the Jukes-Cantor method, and a distance tree was constructed with the neighbor-joining algorithm. The bootstrap method was employed with 1,000 replicates to estimate the robustness of the tree topologies.

Nucleotide sequence accession numbers. The 16S rRNA gene sequence of strain JL001 has been deposited in the GenBank database under accession no.

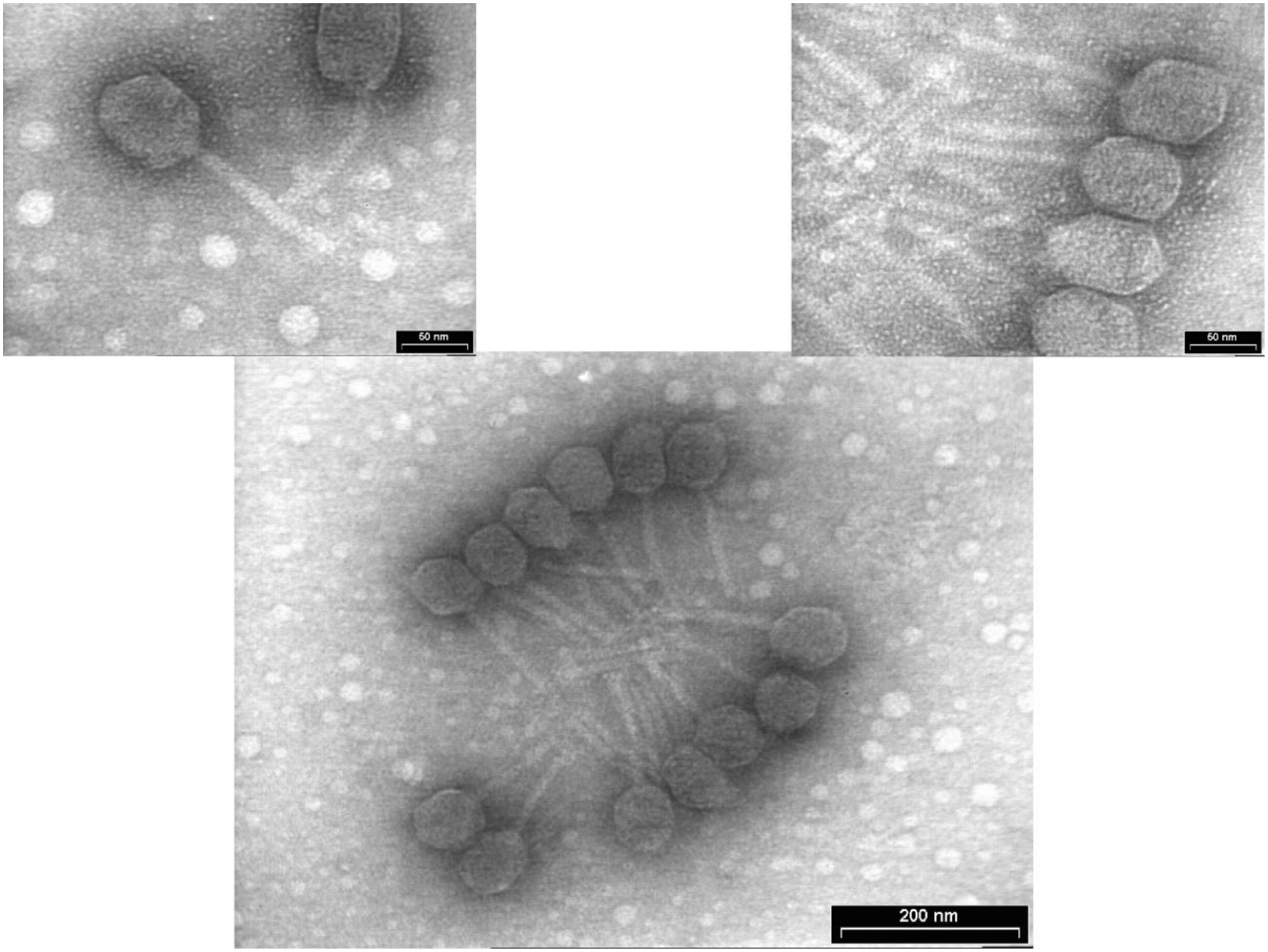

FIG. 3. Morphological characteristics of ΦJL001. The phage head diameter is ca. 75 nm, and the tail length is ca. 125 nm. The top left image shows a phage with a slightly bent tail, indicating that the tail is flexible, which is characteristic of phage in the family *Siphoviridae*.

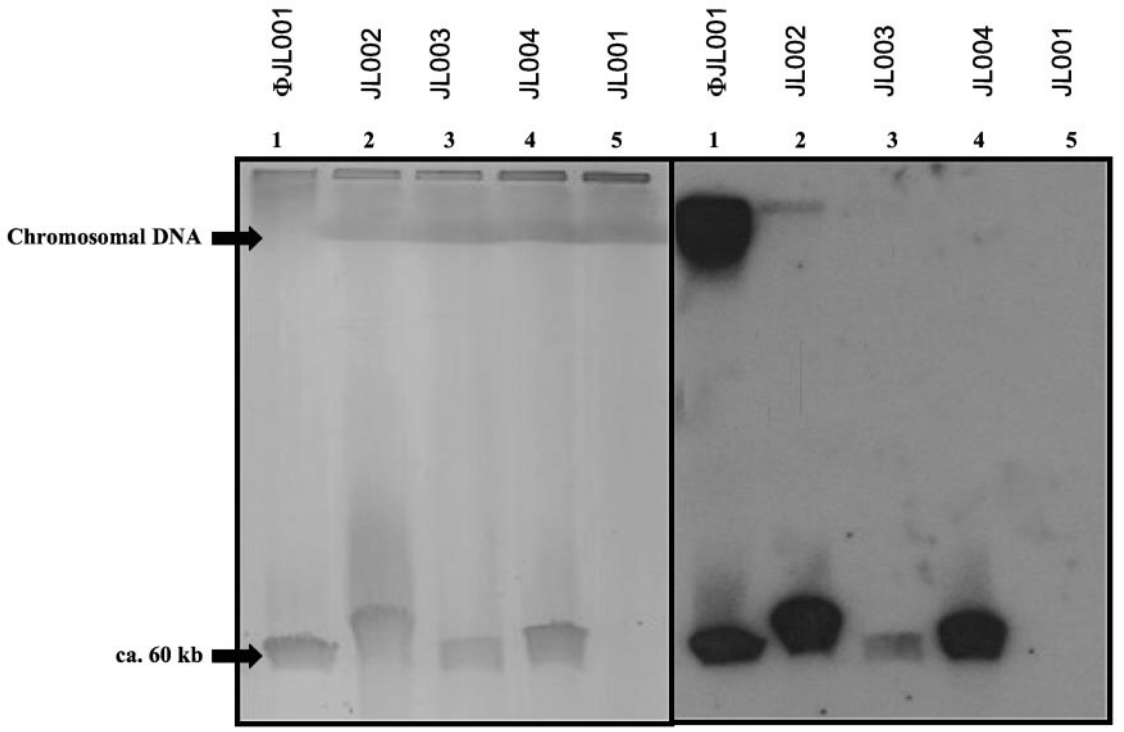

FIG. 4. PFGE (left gel) and Southern hybridization (right gel). Lane 1, ΦJL001; lanes 2, 3, 4, putative lysogen strains JL002, JL003, and JL004, respectively; lane 5, strain JL001.

AY584527, and the complete genome sequence of ΦJL001 has been deposited in the GenBank database under accession no. AY576273.

RESULTS

Isolation of host bacterium. The microalgal culture isolated from the sponge *I. strobilina* infected with the viral concentrate was monitored biweekly by using PFGE to detect viral amplification. A band indicating a possible increase in the level of a specific virus was detected after 8 weeks (Fig. 1). There were no visible signs of lysis of the microalgal culture. Several transfers of the algal culture were made, and a band suggesting viral amplification was consistently observed by PFGE after 8 weeks. Since no lysis of the microalgal culture was observed, we considered the possibility that this band indicated the presence of a bacteriophage infecting a heterotrophic bacterium from the original sample of *I. strobilina* that was still present in the algal culture. The algal culture was plated onto marine agar 2216, and four morphotypes of heterotrophic bacteria were isolated. These bacteria were all closely related α-*Proteobacteria*, and their phylogenic relationship is shown in Fig. 2. Marine agar overlays inoculated with putative phage-containing supernatant from the algal culture were prepared with each of these bacteria. Plaques were observed on the strain designated JL001, indicating that this strain was the host of a phage present in the algal culture. The phage, designated ΦJL001, formed turbid plaques after 3 days of incubation. Strain JL001 had a distinctive light brown colony morphology after 3 days of growth on marine agar 2216 and was estimated to have comprised ca. 8% of the total culturable bacteria originally isolated from the sponge, based on counts of this colony morphology on the initial isolation plates.

Phage biology and lysogenic characteristics. After infection of turbid cultures of the host, strain JL001, with high titers (ca. 10^9 phage particles per ml of culture), lysis of the liquid culture was not observed. It was not possible to make high-titer preparations of ΦJL001 from liquid cultures of the host in the logarithmic growth phase. Instead, high-titer preparations of ΦJL001 were harvested from lawns of confluent lysis in sloppy overlays of strain JL001. These characteristics suggested that ΦJL001 might be a temperate phage rather than a lytic phage. In order to determine if the phage entered into a lysogenic state with its host, strain JL001 was examined for prophage induction by using mitomycin C and UV radiation treatments. The greatest induction occurred 96 h after treatment with mitomycin C at a concentration of 0.1 μg/ml ($2.2 \times 10^{14} \pm 0.4 \times 10^{14}$ PFU/ml, compared with the control containing $3.8 \times 10^{13} \pm 1 \times 10^{13}$ PFU/ml) and UV treatment for 90 s ($5.5 \times 10^{12} \pm 4 \times 10^{12}$ PFU/ml, compared with the control containing $1.0 \times 10^{12} \pm 0.4 \times 10^{12}$ PFU/ml). Induction resulted in increases in phage counts of ca. 0.5 to 1 order of magnitude compared with uninduced controls.

The phage produced several plaque morphologies on its host, including turbid plaques and plaques with haloes, from which three putative lysogens were isolated, which were designated strains JL002, JL003, and JL004. The possibility of a mixed lysate was discounted since phage preparations were prepared from single plaques and each plaque morphology on replating once again gave several different plaque morphologies. 16S rRNA gene sequencing confirmed that these isolates had that same 16S rRNA sequence as the original host bacterium, strain JL001. The colony morphology of the three puta-

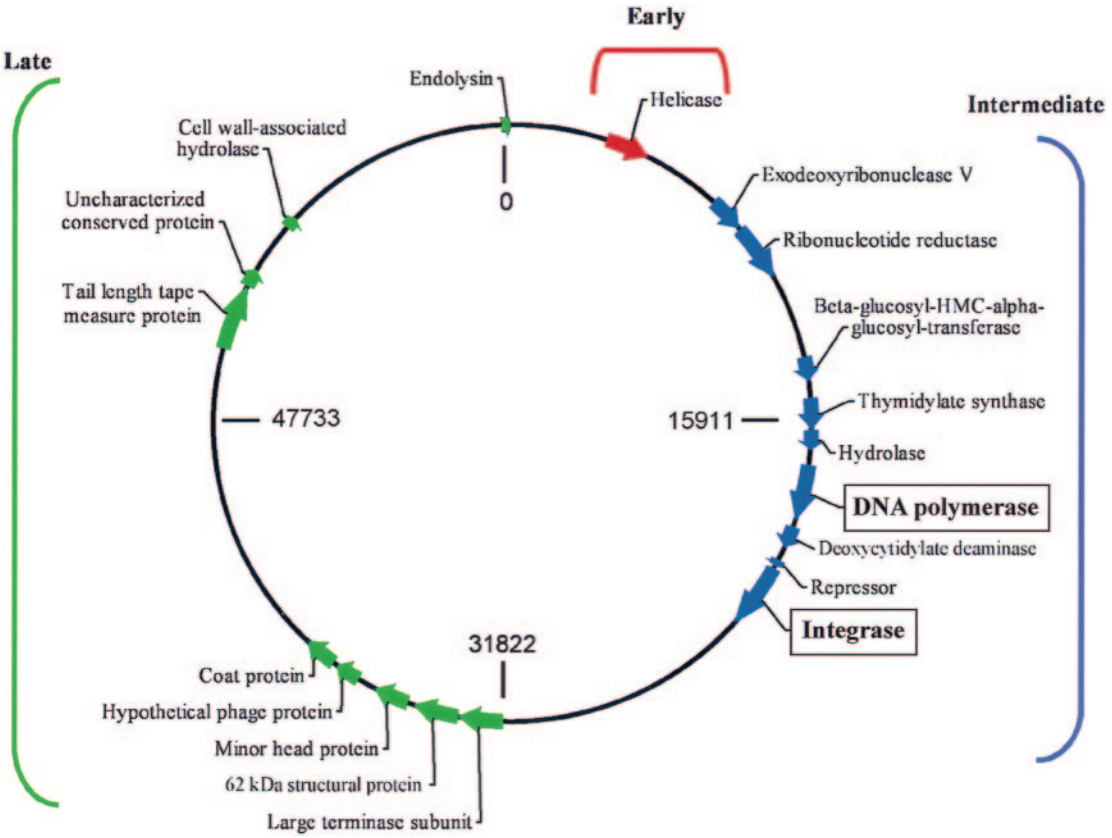

FIG. 5. Circular genomic map of ΦJL001. The predicted genes are clustered into three regions; early genes are indicated by red, intermediate genes are indicated by blue, and late genes are indicated by green.

tive lysogens differed from the colony morphology of strain JL001, and the colonies were more raised colonies and were white rather than light brown. The three putative lysogens had a colony morphology that differed from the colony morphology of the original host strain, which formed smooth and rounded colonies. The putative lysogens formed colonies that were slightly smaller with a rough, crinkled texture and irregular edges. Small white colonies formed on agar overlays but were not isolated.

Homoimmunity of lysogens. When phage ΦJL001 was tested with strains JL002, JL003, and JL004, no plaques or clearing was observed on the bacterial lawns, suggesting homoimmunity of these lysogens to phage ΦJL001, although the possibility that the phenotype was due to cells that were resistant to infection but were not true lysogens cannot be ruled out. Control overlays with strain JL001 showed a high level of plaque formation when they were challenged with ΦJL001.

Transmission electron microscopy examination of phage morphology. Morphological characteristics of ΦJL001 were compiled from multiple micrographs of phage particles in order to minimize size or shape anomalies (Fig. 3). The phage head diameter was ca. 75 nm, while the tail length was ca. 125 nm. The tail morphology is typical of phages in the family *Siphoviridae*.

Integration of ΦJL001 into the genome of strain JL001. The results of Southern hybridization studies of total DNA preparations of strain JL001 and the putative lysogen strains JL002, JL003, and JL004 probed with a radiolabeled fragment of ΦJL001 are shown in Fig. 4. PFGE of preparations of the putative lysogens revealed bands at sizes that corresponded approximately (but not exactly) to the size observed for ΦJL001. The size differences for the band at ca. 60 kb for the putative lysogens and the original phage lysate may have been due to overloaded wells, binding of protein present in the chromosomal preparations, or rearrangements of the phage genome. The ca. 60-kb bands in preparations from the putative lysogens hybridized strongly with the 1,500-bp radiolabeled probe from ΦJL001, while the original host strain did not contain a corresponding band (Fig. 4). No hybridization signal was detected at the position corresponding to the chromosomal DNA bands, indicating that phage ΦJL001 was not integrated into the chromosomes of the putative lysogens.

Phage sequence. The genome of ΦJL001 is comprised of 63,469 bp and is circularly permuted. ΦJL001 has a G+C content of 62%. The overall coverage was 6× to 10×, and a few areas with lower coverage were checked by sequencing of PCR products covering these areas. GeneMark predicted 91 putative ORFs. Putative functions were assigned to 17 of the 91 ORFs, which are indicated in the circular map shown in Fig. 5. On this map, putative genes are transcribed in a clockwise direction, and they appear to be clustered in three regions: early genes, presumably for establishing virus infection (0 to 7,500 bp); intermediate genes, involved in DNA synthesis, modification, and replication (7,500 to 32,000 bp); and late

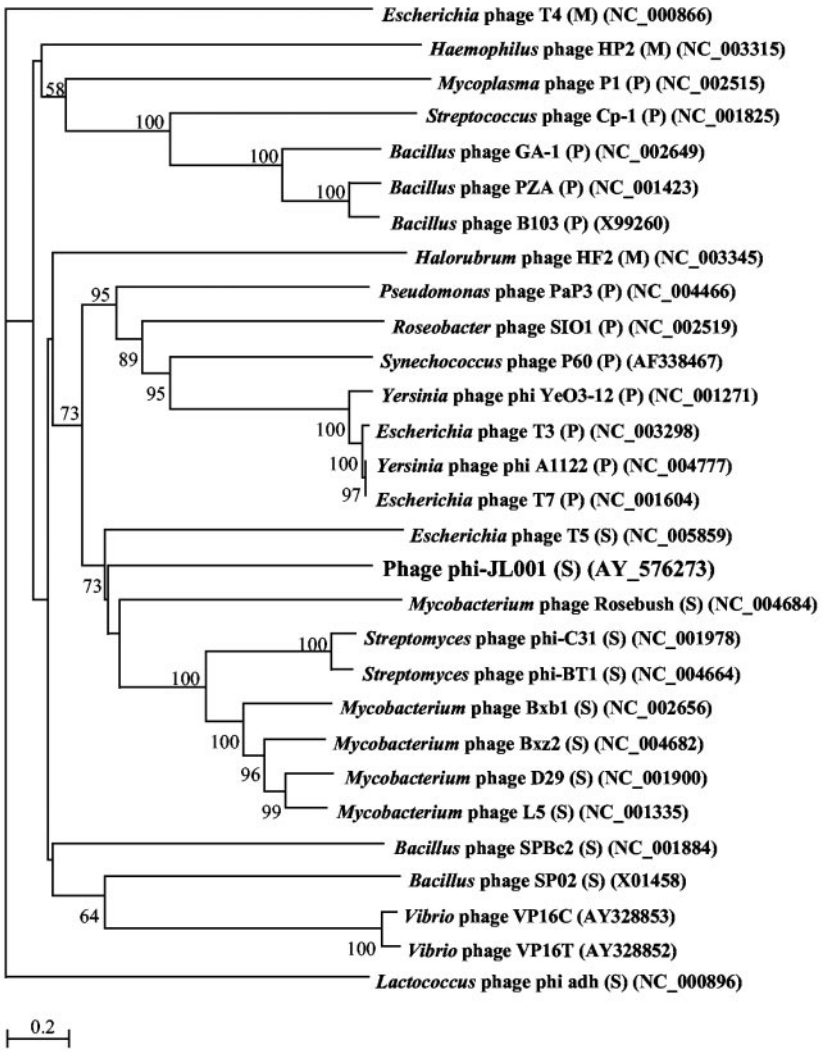

FIG. 6. Unrooted neighbor-joining tree based on the aligned amino acid sequences encoded by the DNA polymerase gene from ΦJL001 and 28 other phages. M, *Myoviridae*; S, *Siphoviridae*; P, *Podoviridae*. A total of 659 residues of the aligned region, including gaps, were used for phylogenetic reconstruction. A Blosum 30 matrix was calculated with a gap penalty of 10.0. The numbers at the nodes are bootstrap values based on 1,000 resamplings. Bootstrap values less than 50 are not shown. Scale bar = 0.2 amino acid substitution per residue.

genes, for the assembly and release of new virus particles (32,000 to 63,000 bp). A putative origin of replication was predicted in the early region by detection of a region with a low G+C content. The designations of the ORFs are shown in Table 1.

Phylogenetic analysis of a functional gene. Comparison of the putative genes encoding DNA polymerase and integrase found in the genomic sequence of ΦJL001 to other phage DNA polymerase and integrase gene sequences deposited in the GenBank database suggested that ΦJL001 is affiliated with the family *Siphoviridae*. Phylogenetic trees based on the DNA polymerase and integrase genes are shown in Fig. 6 and 7, respectively. Both trees show that ΦJL001 is deeply rooted, suggesting that it is quite different from previously described phages. The polymerase gene of ΦJL001 appears to share an origin with a group of siphoviruses that infect mycobacteria.

DISCUSSION

Phage ΦJL001 is the first marine phage that infects a sponge-associated bacterium that has been sequenced. Eleven marine phages have been sequenced previously. These phages are the roseophage SI01 (26), cyanophage P60 (10), the lipid-containing *Pseudoalteromonas espejiana* phage PM2 (20), *Vibrio harveyi* phage VHML (22), three *Vibrio parahaemolyticus* phages (VpV262, VP16T [14], and VP16C [29]), three *Prochlorococcus* phages (P-SSP7, P-SSM2, and P-SSM4 [18a]), and the broad-host-range vibriophage KVP40 (21). Only one of these phages, VHML, exhibits temperate characteristics (22), and none has been described as pseudolysogenic. Genomic analysis of uncultured marine viral communities indicated that the diversity of these communities is extremely high, with the number of viral types ranging from several hundred to several thousand in the two communities that were

TABLE 1. Bacteriophage ΦJL001 ORFs and putative genes

ORF	Positions (bp)	Protein size (amino acids)	Accession no.	*E* value	Significant match	Conserved domain	Function
1	1–375	125	NS[a]				
2	372–854	161	NS				
3	904–1020	39	NS				
4	1017–1277	87	NP_772739	2.00E-06	*Bradyrhizobium japonicum* genome		
5	1294–2931	546	NS				
6	3070–3249	60	NS				
7	32534773	507	ZP_00060797	8.00E-14	Hypothetical protein (*Clostridium thermocellum*)	COG0553.1	HepA, SNF2 family of DNA/RNA helicases
8	4770–5126	119	NS				
9	5243–5479	79	NS				
10	5472–6032	187	NS				
11	6078–6878	267	NS				
12	6974–7471	166	NS				
13	7468–8673	402	NP_539536	3.00E-94	Exodeoxyribonuclease V alpha chain (*Brucella melitensis*)	COG0507.1	RecD, exonuclease V
14	8670–10478	603	ZP_00005311	1.00E-135	Hypothetical protein (*Rhodobacter sphaeroides*)	COG0209.1	NrdA, ribonucleotide reductase
15	10494–10916	141	NS				
16	10929–11507	193	NP_817561	7.00E-08	gp114 (mycobacteriophage CJW1)		
17	11578–11778	67	NS				
18	11832–12287	152	NS				
19	12271–13137	289	NP_821395	6.00E-10	Hypothetical protein (*Streptomyces avermitilis*)		
20	13134–13490	119	NS				
21	13497–13622	42	NS				
22	13637–14455	273	Q06718	1.00E-04	Beta-glucosyl-hydroxymethyl cytosine-alpha-glucosyl-transferase (phage T6)		
23	14472–15071	200	NS				
24	15068–16120	351	P31654	6.00E-32	Deoxyuridylate hydroxymethyltransferase (phage SPO1)	COG0207.1	ThyA, thymidylate synthase
25	16095–16688	198	NS			COG1896.1	Predicted hydrolases of HD superfamily
26	16685–17113	143	NS				
27	17110–17361	84	NS				
28	17361–19256	632	NP_229419	6.00E-33	DNA-directed DNA polymerase 1 (*Thermotoga maritima*)	COG0749.1	PolA, DNA polymerase 1
29	19253–19492	80	NS				
30	19509–20243	245	NP_635521	4.00E-26	Deoxycytidylate deaminase (*Xanthomonas campestris*)	COG2131.1	ComEB, deoxycytidylate deaminase
31	20240–20683	148	NS				
32	20742–20972	77	NS			cd00093.2	Helix-turn-helix XRE family-like proteins
33	21078–23321	748	ZP_00048756	5.00E-42	Hypothetical protein (*Magnetospirillum magnetotacticum*)	pfam05272.1	VirE, virulence-associated protein E
34	23407–23775	123	NS				
35	23861–24127	89	NS				
36	24124–24435	104	NS				
37	24432–24911	160	NS				
38	24908–24997	30	NS				
39	25072–25272	67	NS				
40	25269–25643	125	NS				
41	25654–26037	128	NS				
42	26037–26282	82	NS				
43	26285–26467	61	NS				
44	26552–26797	82	NS				
45	26809–27453	215	NS				
46	27658–27921	88	NS				
47	27965–28141	59	NS				
48	28155–28322	56	NS				
49	28331–28582	84	NS				
50	28579–28941	121	NS				
51	29059–29277	73	NS				
52	29274–29558	95	NS				
53	29555–29785	77	NS				
54	29808–29990	61	NS				
55	30058–30264	69	NS				

Continued on following page

TABLE 1—*Continued*

ORF	Positions (bp)	Protein size (amino acids)	Accession no.	*E* value	Significant match	Conserved domain	Function
56	30261–30464	68	NS				
57	30461–31132	224	NS				
58	31302–31382	27	NS				
59	31498–32127	210	NS				
60	32124–33653	510	ZP_00045650	6.00E-27	Bacteriophage terminase large subunit (*Mesorhizobium loti*)		
61	33653–35212	520	CAB53859	1.00E-15	62-kDa structural protein (bacteriophage MB78)		
62	35202–35438	79	NS				
63	35428–36639	404	ZP_00127008	7.00E-35	Hypothetical protein (*Pseudomonas syringae*)	pfam04233.3	Phage Mu F-like protein, minor head protein
64	36636–36863	76	NS				
65	36860–36976	39	NS				
66	37041–37214	58	NS				
67	37281–38150	290	NP_405657	4.00E-06	Hypothetical phage protein (*Yersinia pestis*)		
68	38296–39420	375	NP_690674	2.00E-41	Coat protein (bacteriophage SPP1)		
69	39514–40329	272	NS				
70	40346–41026	227	NS				
71	41030–42331	434	NS				
72	42328–42693	122	NS				
73	42696–43718	341	NS				
74	43718–45445	576	NS				
75	45445–45633	63	NS				
76	45617–46516	300	NS				
77	46513–46905	131	NS				
78	46902–47312	137	NS				
79	47309–48967	553	NS				
80	48977–49108	44	NS				
81	49171–49584	138	NS				
82	49909–50349	147	NP_791903	7.00E-04	Hypothetical protein (*Pseudomonas syringae*)		
83	50351–52609	753	NP_536371	6.00E-23	Putative tail length tape measure protein	COG5281.1	Mu-like prophage protein
84	52609–53229	207	ZP_00004532	2.00E-31	Hypothetical protein (*Rhodobacter sphaeroides*)	COG5448.1	Uncharacterized conserved protein
85	53231–55000	590	ZP_00004533	2.00E-31	Hypothetical protein (*Rhodobacter sphaeroides*)	COG5449.1	Uncharacterized conserved protein
86	55006–55434	143	NP_421571	9.00E-06	Hypothetical protein (*Caulobacter crescentus* CB15)	COG0791.1	Cell wall-associated hydrolases
87	55435–58893	1153	ZP_00011489	2.00E-35	Hypothetical protein (*Rhodopseudomonas palustris*)		
88	58897–60360	488	NS				
89	60365–62989	875	ZP_00055704	6.00E-05	Hypothetical protein (*Magnetospirillum magnetotacticum*)		
90	63040–63306	89	NS				
91	63312–63632	107	NP_543082	7.00E-26	Putative endolysin (bacteriophage P27)		

[a] NS, not significant.

studied (8). Since it is clear that virioplankton are an active and important component of marine microbial communities (12, 37), it is certainly important to sequence genomes of representative marine viruses. Phage ΦJL001 is of interest in this regard since it shows some pseudolysogenic characteristics; pseudolysogenic phage-host interactions may be shared by many marine bacteria (36).

Morphological characteristics, sequence homology to previously described phages, and phylogenic analysis based on the putative DNA polymerase and integrase genes supported affiliation of ΦJL001 with the family *Siphoviridae*. Comparison of the 91 predicted ORFs with currently available sequences revealed the following relationships. First, there is a high proportion of unique genes (>50%) in the genome of ΦJL001 that are unrelated to genes of other bacteriophages or any other previously sequenced organism. Second, predicted genes with significant hits to genes encoding previously described proteins showed sequence homology to genes of several types of viruses, several groups of bacteria, and even higher eukaryotic organisms. Studies of phage evolution show that double-stranded DNA phages and prophages are mosaics that arose by horizontal transfer of genetic material from a global phage pool. The mosaic nature of ΦJL001 is consistent with findings for previously sequenced phages that indicate that phages are highly mosaic (9, 15, 16, 23).

Basic life histories of marine phages can be elucidated by comparison of their complete genomes to the genomes of other extensively studied phages (26). The genomic sequence

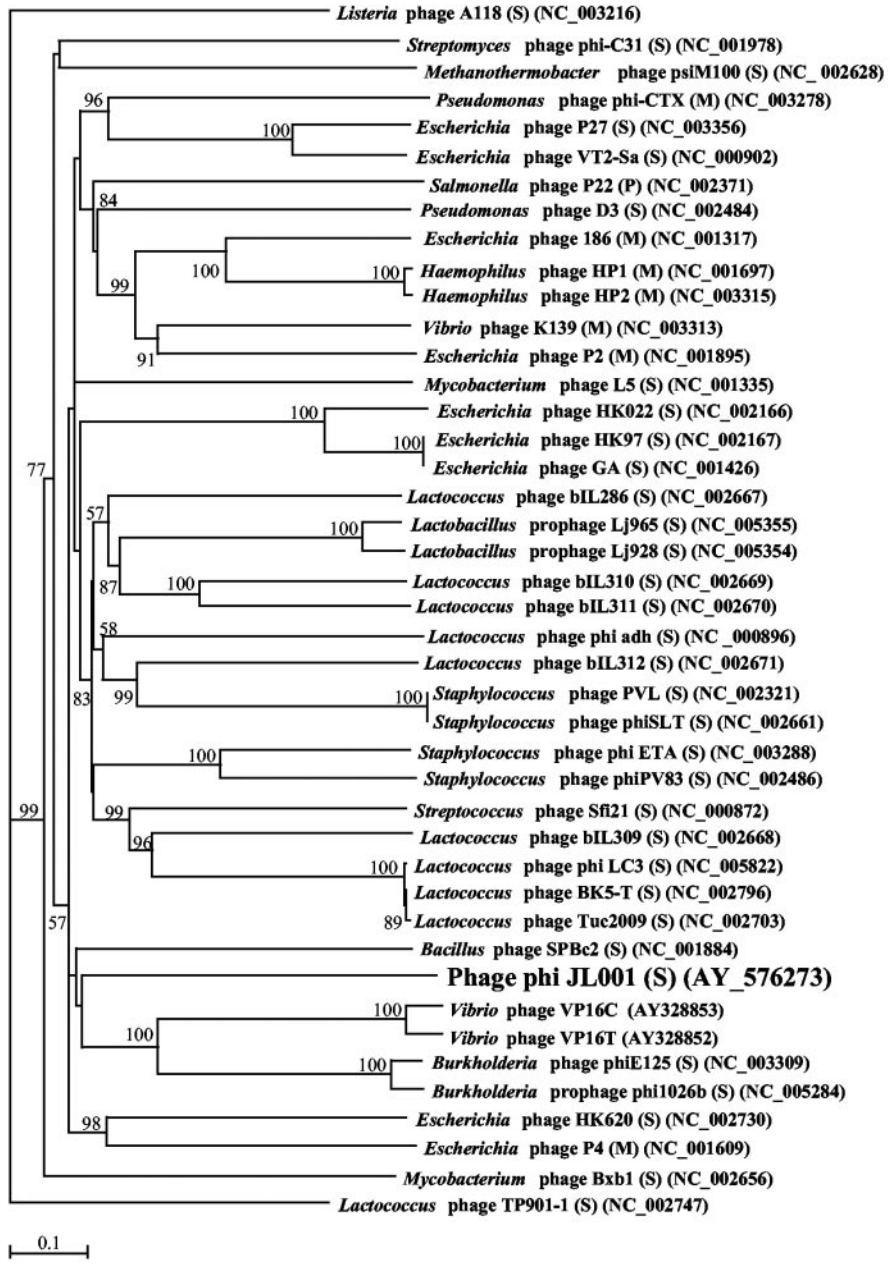

FIG. 7. Unrooted neighbor-joining tree based on the aligned amino acid sequences encoded by the integrase gene from ΦJL001 and 42 other phages. M, *Myoviridae*; S, *Siphoviridae*; P, *Podoviridae*. A total of 405 aligned residues, including gaps, were used for phylogenetic reconstruction. A Blosum 30 matrix was calculated with a gap penalty of 10.0. The numbers at the nodes are bootstrap values based on 1,000 resamplings. Bootstrap values less than 50 are not shown. Scale bar = 0.1 amino acid substitution per residue.

of ΦJL001 is consistent with some known aspects of its biology. Many siphoviruses form lysogenic relationships with their hosts (29). Interestingly, a gene that encodes a putative integrase is found in most lysogenic siphovirus and myovirus genomes, suggesting that the phages are able to integrate their genomes into the host genome and become lysogenized (10). We detected a gene with homology to known integrases in the genome of ΦJL001. Lytic phages typically contain a DNA polymerase gene and some other genes (e.g., primase and helicase genes) associated with DNA replication. Most of the lysogenic phage genomes of members of the *Siphoviridae* and *Myoviridae* do not contain these DNA replication genes. The presence of both a putative DNA polymerase gene and an integrase gene in ΦJL001 is noteworthy considering that ΦJL001 displayed some pseudolysogenic characteristics. Including ΦJL001, only 7 of 84 known siphophage genomes contain both the DNA polymerase and integrase genes.

Lysogeny is characterized by homoimmunity to superinfection, physical and chemical induction, and integration of the phage genome into the host genome (1). Induction of ΦJL001

and the homoimmunity characteristics of ΦJL001 resemble true lysogeny. However, unlike what is observed in true lysogeny, the phage ΦJL001 genome does not integrate into host cellular replicons. This lack of chromosomal integration is consistent with pseudolysogeny (36).

Homoimmunity in phage is often the result of excess repressor molecules that render the lysogenic bacterium immune to superinfection. The continual synthesis of repressor molecules maintains the phage genome as a prophage and prevents the transcription of phage genes, leading to initiation of the lysogenic cycle (1). This interaction may be explained by two factors. First, the lysogen may exhibit certain characteristics of pseudolysogeny due to weak or poor repression of phage DNA transcription. A partially defective repressor protein may cause this effect. Pseudolysogenic characteristics include high host cell abundance along with a high rate of phage production. An unstable repressor would allow a high rate of spontaneous induction along with high host cell abundance. Alternatively, this relationship between phages and bacteria in the marine environment may be the result of a mixture of sensitive and resistant host cells and/or a mixture of virulent and temperate phages.

Phage ΦJL001 is the first phage isolated that infects a sponge-associated host bacterium. The characteristics of ΦJL001 suggest that this virus may have the potential to exert significant control on the population of its host within the sponge microbial community. Further studies of this host-phage system should allow us to better understand the relationship between the host and the phage and, at another level, the role of phages within the very complex sponge microbial community. The use of phages may allow workers to manipulate populations of key microbes in the community of a sponge and could lead to a clearer picture of factors that keep the diversity of microbial communities in balance. This unique host-phage system may provide a model for elucidating interactions that occur within sponge microbial communities.

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