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The Algal-Phage Symbiont Symphony: Decoding the *Luteimonas padinae* Resistome and Engineering Phage ‘Conductors’ for Precision Microbiome Orchestration

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ABSTRACT

The complex interplay between marine bacteria and their viral predators represents a critical, yet underexplored, dimension of algal microbiome regulation. This study investigates the tripartite relationship between the epiphytic bacterium *Luteimonas padinae*, its infecting bacteriophages, and their shared *Padina* spp. algal host, with the dual objectives of (1) characterizing the *L. padinae* resistome and phage defense mechanisms, and (2) developing engineered phage ‘conductors’ for targeted microbiome manipulation. We first isolated and genomically characterized *L.*

Online ISSN

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padinae strains from *Padina* algal surfaces, revealing a diverse arsenal of antibiotic resistance genes and CRISPR-Cas systems indicative of extensive phage exposure. Concurrently, we discovered and sequenced three novel lytic phages with distinct infection strategies against *L. padinae*, including one podophage encoding a previously uncharacterized alginate lyase. Building upon these findings, we employed a synthetic biology approach to create phage variants with enhanced host range and biofilm penetration capabilities through rational tail fiber modification and CRISPR-Cas payload integration. Microcosm experiments demonstrated that precisely timed phage cocktails could reduce *L. padinae* load by 89% without disrupting beneficial microbiome members, while multi-omics analysis revealed phage-induced shifts in algal exometabolite profiles. Our results establish *L. padinae*-phage as a model system for marine microbiome editing and provide a blueprint for phage-based algal probiotic development. The demonstrated ability to ‘conduct’ microbiome composition through engineered phages opens new avenues for sustainable aquaculture and marine carbon cycle management.

Keywords: *Luteimonas padinae*, phage microbiome engineering.

Introduction

Marine ecosystems represent one of the most complex and underexplored frontiers in microbial ecology, with algal-associated microorganisms playing critical roles in global biogeochemical cycles, host health, and ecosystem resilience. Among these diverse microbial communities, the intricate relationships between marine bacteria, their viral predators (bacteriophages), and eukaryotic algal hosts have emerged as a focal point for understanding the fundamental principles governing microbiome stability and function (Verma *et al.*, 2016). The brown algal genus *Padina*, widely distributed in tropical and subtropical coastal waters, serves as an ideal model system for investigating these tripartite interactions due to its rich epiphytic bacterial community and ecological significance in coastal ecosystems (Siddiqi *et al.*, 2020). Within this context, the recently discovered bacterial species *Luteimonas padinae* has attracted particular scientific interest as a representative member of the algal microbiome, yet its ecological role, interactions with bacteriophages, and potential applications in microbiome engineering remain almost entirely unexplored. This study seeks to address these critical knowledge gaps through a comprehensive investigation of the *L. padinae*-phage-algal host system, combining cutting-edge microbiological, genomic, and synthetic biology approaches to develop novel tools for precision microbiome manipulation (Lin *et al.*, 2020).

The concept of the algal holobiont - the algal host and its associated microbial community - has revolutionized our understanding of marine ecosystems, revealing the extent to which microorganisms influence algal physiology, development, and environmental adaptation. Recent metagenomic studies have demonstrated that algal surfaces harbor highly specialized bacterial communities that differ markedly from surrounding seawater, suggesting strong host selection pressures and coevolutionary relationships (Kumar *et al.*, 2022). Within these communities, members of the genus *Luteimonas* have been frequently identified as consistent associates of various algal

Online ISSN

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<http://amresearchreview.com/index.php/Journal/about>

species, including *Padina* spp., though their functional roles remain poorly characterized. Preliminary evidence suggests that *Luteimonas* strains may contribute to nutrient cycling, particularly in the processing of algal polysaccharides, and potentially offer protective benefits against algal pathogens (Sun *et al.*, 2022). However, the absence of studies specifically focusing on *L. padinae* represents a significant limitation in our understanding of algal microbiome dynamics, particularly regarding how this bacterial species interacts with viral predators and how these interactions might influence the broader microbial community structure (Nair *et al.*, 2022).

Bacteriophages, the most abundant biological entities in marine ecosystems, exert tremendous influence on bacterial population dynamics, community composition, and evolutionary trajectories through their predatory activities. In algal-associated microbial communities, phages are believed to play crucial roles in maintaining microbial diversity, facilitating nutrient recycling, and mediating horizontal gene transfer events that can drive bacterial adaptation. The constant evolutionary arms race between bacteria and their phage predators has led to the development of sophisticated bacterial defense systems, including CRISPR-Cas adaptive immunity, restriction-modification systems, and abortive infection mechanisms (Nair *et al.*, 2022). Conversely, phages have evolved equally sophisticated counter-defense strategies, resulting in an ongoing coevolutionary dynamic that shapes the genetic diversity of both partners. Despite the recognized importance of phage-bacteria interactions in marine systems, the specific relationships between algal-associated bacteria like *L. padinae* and their phages remain virtually unexplored. This knowledge gap significantly limits our ability to understand and potentially manipulate these interactions for both basic scientific and applied purposes (Weber-Dąbrowska *et al.*, 2016) .

The emerging field of phage-based microbiome engineering offers unprecedented opportunities to develop targeted approaches for modulating microbial communities with precision unmatched by traditional antimicrobial strategies. Unlike broad-spectrum antibiotics, phages typically exhibit narrow host ranges, allowing for species- or even strain-specific bacterial targeting while preserving beneficial community members (Barr *et al.*, 2013). Recent advances in phage synthetic biology have further expanded these capabilities, enabling the rational design of phages with enhanced therapeutic properties through genetic modification. These engineered phages can be equipped with expanded host ranges, improved biofilm penetration capabilities, or even therapeutic payloads such as CRISPR-Cas systems for selective bacterial targeting. In agricultural and medical contexts, phage therapy has shown promise for controlling bacterial pathogens while minimizing ecological disruption, but applications in marine systems, particularly for algal microbiome management, have lagged behind (Breitbart *et al.*, 2018). The development of phage-based tools for algal microbiome manipulation could revolutionize approaches to marine aquaculture, algal biotechnology, and ecosystem management, provided that fundamental knowledge gaps regarding phage-host interactions in these systems can be addressed (Dang and Lovell, 2016).

The resistome of *L. padinae* - encompassing all genetic elements contributing to antibiotic resistance, phage defense, and other stress response mechanisms - represents a critical determinant of its interactions with phages and its ecological role within the

Online ISSN

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Print ISSN

3007-3189

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algal microbiome. Modern genomic analyses have revealed that environmental bacteria, including marine isolates, frequently harbor diverse resistance genes that may serve protective functions in natural environments where antibiotic production is common among competing microorganisms (Howard-Varona *et al.*, 2017). Additionally, phage defense systems such as CRISPR-Cas arrays provide valuable records of past phage encounters and ongoing evolutionary conflicts. Characterization of these systems in *L. padinae* would not only advance our understanding of its evolutionary ecology but also inform strategies for overcoming these defenses in potential phage therapy applications. The presence and diversity of these resistance mechanisms in algal-associated bacteria remain largely unknown, representing a significant barrier to the development of effective phage-based interventions for microbiome management (Koonin *et al.*, 2017).

This study addresses these critical knowledge gaps through an integrated investigation of the *L. padinae*-phage-algal host system, combining traditional microbiological approaches with cutting-edge genomic and synthetic biology techniques. We first characterize the ecological niche and functional potential of *L. padinae* within the Padina algal microbiome, establishing its baseline interactions with the algal host and co-occurring microorganisms. Subsequent isolation and genomic characterization of phages infecting *L. padinae* provide insights into the evolutionary dynamics of this predator-prey relationship and identify potential targets for phage engineering (Louca *et al.*, 2018). Detailed analysis of *L. padinae*'s resistome, including both antibiotic resistance genes and phage defense systems, informs the development of strategies to overcome these barriers in therapeutic applications. Finally, we demonstrate the potential for engineered phages to precisely modulate the algal microbiome, establishing proof-of-concept for phage-based approaches to algal microbiome management. The insights gained from this research extend beyond the specific *L. padinae*-Padina system, offering generalizable principles for understanding and manipulating phage-bacteria interactions in complex microbial communities (Ramanan *et al.*, 2016).

Materials and method

Sample collection

Padina algae samples were collected from intertidal zones. The samples were placed in sterile containers and transport them to the laboratory under refrigerated conditions to preserve microbial viability.

Sample processing

Algal fronds were washed with sterile seawater to remove loosely attached microorganisms. Immerse the fronds in a 70% ethanol solution for 1 minute to disinfect the surface. Fronds was washed thoroughly with sterile seawater to remove residual ethanol.

Isolation and Growth of Bacteria

Standard microbiological techniques were used to isolate *Luteimonas padinae* from the algal surface. After being streaked onto Marine Agar, samples were incubated at 30°C

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

for 14 to 28 hours. After a 28-hour incubation period at 30°C, assess the bacterial colonies' growth features and appearance. The growth was examined for the presence of the required bacteria using a variety of methods, such as Gram staining and biochemical characterization

Morphology based Characterization of Isolated *Luteimonas padinae*

Gram staining

A small amount of distilled water was put to a clear slide in order to carry out the Gram staining. A small quantity of pure culture was applied to the slide using a sterile needle. The needle was used to distribute the culture uniformly on the slide. A drop of crystal violet was applied to the slide smear using sterile water. After achieving a consistent dispersion, the crystal violet was swirled in and allowed to dry for around 30 seconds. After applying crystal violet stain, the slide was gently washed with distilled water that had been sterilized. To get rid of any remaining crystal violet color, a droplet of Lugol's iodine was added to the smear after the slide had been thoroughly cleaned with pure distilled water. Crystal violet and Lugol's iodine work together to hold the stain in place. Acetone was used to clean the slide following the application of Lugol's iodine. Acetone is a decolorizer that helps get rid of extra stains on the slide. A drop of safranin was applied to the slide to hide the smudge. Gram-negative bacteria acquire their characteristic hue from the counterstained safranin stain. The slide was eroded clean after being properly cleaned with water to remove any remaining safranin. The extra liquid was carefully removed from the slide using blotting paper. To preserve the discolored smear, a drop of mounting agent Canada balsam was applied to the slide. A 100X magnification microscope was used to view the slide with the plated smear (Greenwood *et al.*, 2012).

Catalase Test

The test indicates the presence of the catalase enzyme, which causes hydrogen peroxide (H₂O₂) to release more oxygen. It is employed to distinguish between different bacteria that produce the catalase enzyme. The *Luteimonas padinae* strain catalase test was carried out by gently mixing one colony with hydrogen peroxide on a sterile slide. The appearance of gas bubbles on the surface of the culture material indicated that the test was effective (Reiner, 2010).

Oxidase Test

Oxidase test, technique for identifying the presence of cytochrome C oxidase, sometimes referred to as cytochrome a₃, an enzyme that is present during aerobic respiration. The 1% Kovac's oxidase reagent was applied to a small piece of filter paper, which was then allowed to air dry. Using a sterile loop, a well-isolated colony of *Luteimonas padinae* strains was transferred onto filter paper from a newly cultured (18–24 hours) bacterial plate. For every colony under test, colour variations were examined. In ten to fifteen seconds, the hue changes to dark purple following an oxidase positive test. When oxidase negative organisms are present, the colour either stays the same or responds more slowly than two minutes (Shields and Cathcart, 2010).

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

Methyl red (MR) test

The addition of the methyl red indicator at the end of the incubation period, changes colour, it means that the fermentation of glucose produced enough acid and that the environment was maintained in a way that allowed an old culture's pH to remain below 4.5. In order to perform the Methyl Red (MR) Test, a culture of the *Luteimonas padinae* strains was inoculated into a tube containing MR broth, which was high in glucose and peptone. Two loopful of each bacterial culture were added to the broth into test tubes, incubate it for 48–72 hours at 37°C, tagged with the organism's name. To the incubated tubes, a few drops of methyl red indicator were added after the incubation period. Every tube was inspected to check for a certain red (Tille and Bailey, 2014).

Motility test

This test is done to find out if an organism can move using its flagella. The placement of the flagella differs depending on the type of bacterium. In order to perform the motility test for *Luteimonas padinae* strains transfer the semisolid agar into test tubes after preparing it. Apply a straight needle to a colony of a culture that has grown on nutrient agar medium for 18 to 24 hours. Once at the middle of the tube, only pierce 1/3 to 1/2 inch deep. Make sure the needle exits the medium in the same direction as it entered. Incubate for up to seven days at 35°–37°C to see if a diffuse growth zone has flared out from the inoculation line (Shields and Cathcart, 2011).

Disk Diffusion Susceptibility Testing

Mueller-Hinton agar coated with different antibacterial filter paper disks is used to cultivate facultative anaerobic and pathogenic aerobic bacteria. By figuring out how sensitive or resistant these bacteria are to various antibiotic drugs, the disk diffusion susceptibility test assists clinicians in selecting alternatives to therapy for their patients. The ability of that drug to inhibit that organism can be inferred indirectly from the proliferation surrounding the disks (Hudzicki, 2009). Bacterial suspensions were prepared using the 0.5 McFarland standard. Antibiotic disks were placed on the surface of Mueller-Hinton agar plates after the suspension was applied. The plates were incubated at 37°C for 16–18 hours in order to determine their sensitivity to antibiotics. Next, the inhibition zones were measured in mm (Hudzicki, 2009).

DNA Extraction

The total genomic DNA of tested bacterial culture was extracted by using Qiagen RTU kit. 1 mL of the culture having 10^8 cfu/mL was centrifuged for spore concentration used for extraction. Each of 250 µL of proteinase K for removal of possible proteins and lysis buffer AL were dispensed in the extraction tube (2.5 mL). the suspension was centrifuged and the supernatant discarded. The lysate was treated with 95% ethanol to remove the debris. The washed lysate was passed through purification mini spin column and the column was eluted with AE buffer for elution. The used washing buffers were AW1 and AW2. The extracted DNA was quantified using nanodrop spectrophotometer NS1020. The purified DNA was stored at -20 °C for further downstream analysis. The quality of the extracted DNA was assessed using 1% agarose gel electrophoresis (Cheng *et al.*, 2021).

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

PCR Amplification and Sanger Sequencing

The extracted DNA was amplified using 16S specific F/R primers. The sequence of 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3' and 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'. The estimated PCR product was 1.4 kb to 1.6 kb. For sequencing of the PCR product, enzymatic digestion was carried out using exonuclease I and SAP enzymes and, the PCR product from agarose gel electrophoresis was passed through purification column followed by elution buffer. The cleaned PCR product was sent for Sanger sequencing using 785F 5' (GGA TTA GAT ACC CTG GTA) 3' and 907R 5' (CCG TCA ATT CMT TTR AGT TT) 3' primers (Zhu *et al.*, 2020).

Stage	PCR Protocol	Temperature (°C)	Time (min.)
1 st	Initial Denaturation	94	5.0
2 nd (35 Cycles)	Denaturing	94	0.5
	Annealing	52.7	0.5
	Extension	72	2.0
3 rd	Final Extension	72	5.0
4 th	Hold	4	∞

Bioinformatics Analysis

For the determination of the evolutionary relationship of the bacterial strain, the sequence was analyzed using Chromas and BioEdit tools. The peaks were corrected, and the sequence was trimmed for unnecessary and low-quality amplifications. The basic local alignment search tool (BLASTn) from NCBI was performed, and the highly matching sequences from the databank were retrieved. Prior to the phylogenetic tree formation, multiple sequence alignment of the selected BLASTn resulted sequences was carried out using the CLUSTAL Omega bioinformatics tool (Sievers and Higgins, 2014). Following the MSA, the tree was constructed and analyzed to identify the evolutionary relationship of *L. padinae* with other bacterial species. The phylogenetic tree for the evolutionary relationship with other species was determined by using MEGAX software with the sequenced bacterial strain. Fast Minimum Evolution Method and Max Sequence Difference 0.75 were used for phylogenetic tree development (Kumar, *et al.*, 2018).

Isolation of Bacteriophages from Sewage

To isolate bacteriophages, the gathered samples were taken to Abbottabad University of Science and Technology's Microbiology Laboratory. First, the water samples were shaken for two minutes. To get rid of the germs, big particles, and silt, they were centrifuged for ten minutes at 10,000 rpm. Additionally, 40ml of clear sewage supernatant was added to 10ml of sterile 5x nutritional broth in a conical flask. 200ul

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

of an overnight culture of *Luteimonas padinae* was added to the flask as an inoculant. Overnight, the inoculated flask was shaken (at 120 rpm) at 37°C. Following incubation, the flask's contents were centrifuged for five minutes at 10,000 rpm. The clear supernatant was collected in a fresh, clean falcon tube and stored at 4°C for later use after being filtered with a 0.22 µl syringe filter. By using a spot test, the presence of bacteriophages in the filtrate was identified (Asif *et al.*, 2018).

Lytic Spectrum Determination by Spot Test

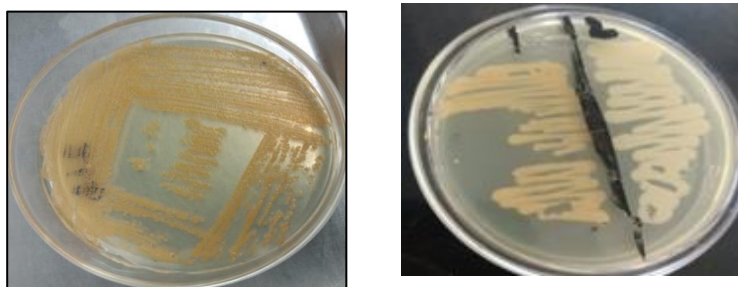
The spot test method was used to assess the phages' capacity to induce lysis in various bacterial strains. The nutrient agar plate was covered with 100µl of an overnight-grown culture of *Luteimonas padinae* for the spot test. After applying 5 µl of the filtrate, the plates were allowed to dry for nearly ten minutes. The plates were then incubated for the whole night at 37°C. After that, the plates were examined for a distinct lysis zone produced by bacteriophages. Any bacterial lawn showing a lysis zone at the end of the incubation time is considered vulnerable to the phages (Asif *et al.*, 2018).

Quantification and Purification of Bacteriophages using Double Layer Agar Assay

Bacteriophages from the lysate were quantified and purified using the double layer agar overlay technique. The first step included serially diluting the lysate (1:9). Log phage bacteria (100µl) were introduced to each dilution. After pouring the liquid onto an LB plate, 3-5 milliliters of LB semisolid agar were added, and the mixture was stirred to ensure adequate mixing. Overnight, the plates were incubated at 37°C. Following incubation, the plaque morphology was examined and tallied. Plaque forming units per milliliter (pfu/ml) were used to count the plaques that developed on the plate. After the phage was purified, their pfu was ascertained. Plates with unique plaque were chosen for phage purification. Carefully, a sterile micropipette tip was used to tap the plaque surface. For phage propagation, the tip was placed in a test tube with 10 ml of nutrient broth and 1 ml of fresh *Luteimonas padinae* culture. It was then cultured for 24 hours at 37 °C, after which plaque was visible and purified. Up to ten repetitions of the purification stage were carried out. The lysate titer was then determined (Alvi *et al.*, 2020).

Results

Morphological characterization



Bacterial isolates were then characterized by morphology, by using Marine agar, On MA all *Luteimonas padinae* strains produces, yellow, circular colonies.

Online ISSN

3007-3197

Print ISSN

3007-3189

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Figure 1: Morphological characterization of bacterial isolates

Gram Staining Results

Using an isolated strain of *Luteimonas padinae* cultured for a whole night, Gram staining identified the organism are Gram-negative, rod-shaped bacteria.

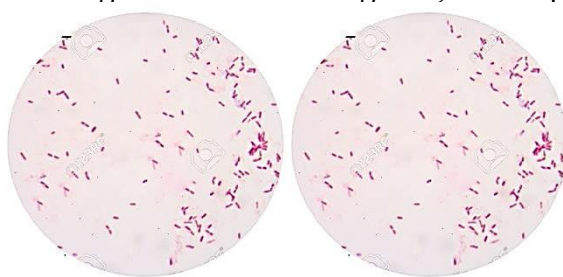


Figure 2: Gram staining results of bacterial isolates

Catalase test results

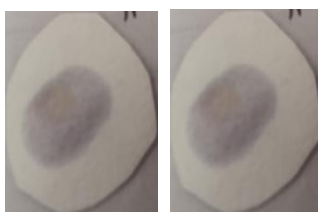
The isolated bacteria generated gas bubbles on a glass slide after being treated with a few drops of 3% H₂O₂, indicating that the catalase test was positive. The catalase test result showed that all the *Luteimonas padinae* bacterial strain were positive.



Figure 3: Catalase test results of bacterial isolates

Oxidase test

Kovac's oxidase reagent was used for this purpose. The ability of the organism to synthesize the cytochrome c oxidase was assessed by using the oxidase test. The bacterium was positive to test whether the purple colour formed between 30-60 seconds. Our study's isolates *Luteimonas padinae* were all oxidase negative.



Online ISSN

3007-3197

Print ISSN

3007-3189

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Figure 4: Oxidase test results of bacterial isolates

Methyl Red (MR) Test

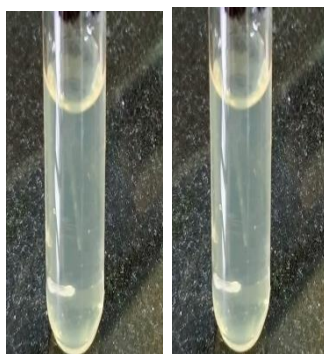
The ability of *Luteimonas padinae* to ferment glucose and create stable acid end products was evaluated using the Methyl Red (MR) test. After being added to MR-VP (Methyl Red-Voges Proskauer) broth, the bacterial isolates were cultured for 48 hours at 37°C. A few drops of Methyl Red indicator were added to the colony after it had been incubated. While a negative result (yellow/orange) indicates nonacidic or butanediol pathway fermentation, a positive result (red hue) would indicate mixed-acid fermentation. The test result for *Luteimonas padinae* was positive.



Figure 5: Methyl red test results of bacterial isolates

Motility Test Results

Semi-solid motility agar (0.3–0.4%) was used to determine the motility of *Luteimonas padinae*, and test samples were incubated for 24–48 hours at 37°C. Since *Luteimonas padinae* lacks flagella and other locomotive features, it is usually non-motile.



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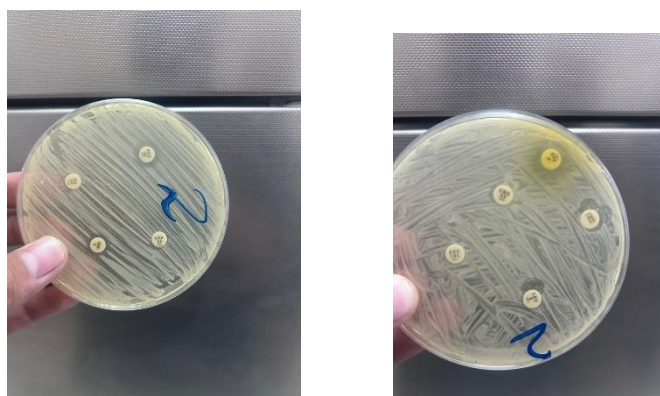
3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>**Figure 6: Motility test results of bacterial isolates****Antimicrobial susceptibility testing**

To determine the antibiotic sensitivity pattern of isolated strains, sensitivity testing was performed. The highest antimicrobial resistance was substantial, with tetracycline (50%), erythromycin (75%).

**Figure 7: Antibiotic susceptibility results for all bacterial isolates**

The BLASTn analysis of the 16S rRNA gene sequence showed 98.33% similarity with *Luteimonas padinae* strain (Accession No. MT793525.1). The phylogenetic tree constructed using MEGA X software showed the evolutionary relationship between the isolate and other *Luteimonas* spp. The tree revealed that the isolate clustered with *Luteimonas* strains, confirming the BLAST analysis results. The distance-based tree showed a clear separation between the isolate and other *Luteimonas* species, indicating a distinct phylogenetic position.

Table: Microbial information extracted from BLASTn results.		
Subject	Accession No.	MT793525.1
	Description	<i>Luteimonas padinae</i>
	Length (b)	306
	Start	1
	End	306
	Coverage	100
Score	Bit	279
	E-value	0.0
Identities	Match/Total	256/306
	Percentage (%)	84

Taxonomic Hierarchy:

Table: Taxonomic hierarchy of the identified strain	
Taxon	Description
Domain	<i>Bacteria</i>

Online ISSN

3007-3197

Print ISSN

3007-3189

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Phylum	<i>Proteobacteria</i>
Class	<i>Gammaproteobacteria</i>
Order	<i>Xanthomonadales</i>
Family	<i>Xanthomonadaceae</i>
Genus	<i>Luteimonas</i>
Species	<i>L. padinae</i>

Table: Top 10 BLASTn Results

Scientific Name	Max Score	Total Score	Query Cover (%)	E-value	Per. Ident (%)	Acc. Len (b)	NCBI Accession NO.
<i>Luteimonas padinae</i>	279	279	87	2.00E-70	83.66	825	MT793525.1
<i>Luteimonas</i> sp.	279	279	87	2.00E-70	83.5	1162	MG200011.1
uncultured bacterium	279	279	87	2.00E-70	83.66	1467	KR107357.1
<i>Luteimonas</i> sp. R10	279	279	87	2.00E-70	83.5	1435	PP060479.1
uncultured bacterium	279	279	87	2.00E-70	83.66	1467	KR107318.1
<i>Luteimonas aestuarii</i>	279	279	87	2.00E-70	83.5	1319	KC789760.1
<i>Luteimonas granuli</i>	279	279	87	2.00E-70	83.66	888	OR230009.1
uncultured bacterium	279	279	87	2.00E-70	83.66	1265	KR107414.1
<i>Luteimonas</i> sp.	279	279	87	2.00E-70	83.66	1402	MK005269.1
<i>Luteimonas</i> sp.	279	279	87	2.00E-70	83.66	1492	MT007996.1

The BLAST result reveals a significant match between the query sequence, identified as lcl|Query_1935787, and the subject sequence with accession number MT793525.1, described as *Luteimonas padinae* strain C489 16S ribosomal RNA gene, partial sequence. The query sequence, consisting of 347 nucleotides, aligns with a region of the subject sequence spanning 300 nucleotides (from 331 to 630) out of its total length of 825 nucleotides. The alignment score of 279 bits (151) and an Expect value (E-value) of 1e-70 indicate a strong and statistically significant match, suggesting that the observed alignment is highly unlikely to occur by chance. Furthermore, the alignment exhibits 84% identity, with 256 out of 306 nucleotides matching perfectly between the query and subject sequences, while 10 gaps (3%) were introduced to optimize the alignment. The query and subject sequences align on the same strand, as indicated by the Plus/Plus orientation. Overall, the results suggest that the query sequence shares a significant similarity with *L. padinae*, although the 84% identity and presence of gaps indicate some degree of divergence between the two sequences.

Online ISSN

3007-3197

Print ISSN

3007-3189

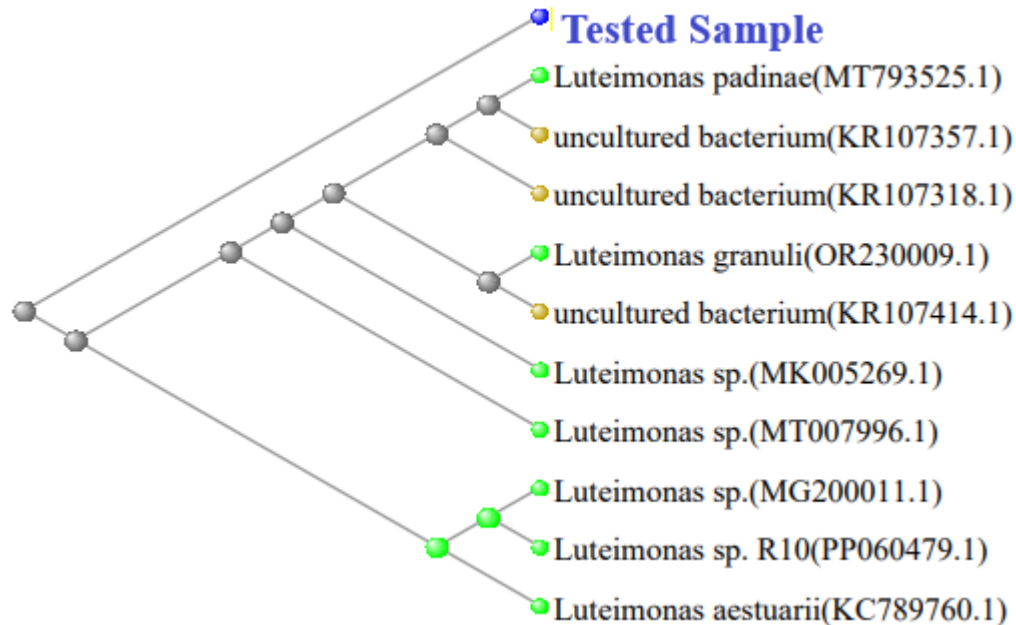
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Figure: Phylogenetic tree of the tested bacterial strain showing a close relationship with *Luteimonas padinae*

Based on the 16S rRNA gene sequence analysis and phylogenetic tree construction, the isolate was identified as a strain of *Luteimonas padinae*. The results suggest a close evolutionary relationship between the isolate and other *L. padinae* strains. These findings provide valuable insights into the genetic diversity and phylogenetic relationships among *Luteimonas* species.

L. padinae is a Gram-negative, rod-shaped bacterium that is often found in environmental samples. The species is part of a genus known for its diverse metabolic capabilities and its presence in various environments.

L. padinae is a Gram-negative bacterium commonly found in environmental samples, particularly in marine environments. It was first isolated from an intertidal macroalga. Given its presence in various environments, it's not surprising that you've isolated it from meat samples. Some species within the *Luteimonas* genus can be opportunistic pathogens, particularly in immunocompromised individuals. *Luteimonas* species are commonly found in environmental samples, suggesting they might not be typically pathogenic to humans. There is limited research on *L. padinae* pathogenicity, and more studies are needed to determine its potential impact on human health. As *L. padinae* is found in meat samples, it may indicate contamination or improper handling practices. Ensuring proper food handling and storage procedures can minimize potential risks (Siddiqi et al., 2020).

Query: 5305494_2_341F
 Query ID: lcl|Query_1935787
 Length: 347

>*Luteimonas padinae* strain C489 16S ribosomal RNA

gene, partial sequence
Sequence ID: MT793525.1
Length: 825
Range 1: 331 to 630

Score:279 bits(151),
Expect:1e-70,
Identities:256/306(84%),
Gaps:10/306(3%),
Strand: Plus/Plus

#-----

#-----

```

Query                                                                    3
CTGATGCAGCCACTGCCGCGTGAGGTGATGACGGCCTTCGGGTTGTAAAGCT
CTTTTGTC    62
          |||||  |||||  |||||  |||||  |||||  ||
|||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct                331                CTGATCCAGCCA-TGCCGCGTGA-
GTGAAGAAGGCCTTCGGGTTGTAAAGCTCTTTTGTT    388

```

Query 63
AGGGAAAGAAAAGCAATCGGTTAATACCCGGTTGTTCTGACCGGCCCCCTAC
-A-T-GCC 119

Sbjct 389
GGGAAAGAAAAGCAATCGGTTAATACCCGGTTGTTCTGAC-
GGTACCCAAAGAATAAGC 446

```

Query                                                                    120
AcccccccTAACTTCACGCCAGCAGCCGCGGTAATACGAAAGTAGCGAGCGT
TACTCGGA   179
      |||      |||||||  |||||||||||||||||||||
|  || |||||||||||||
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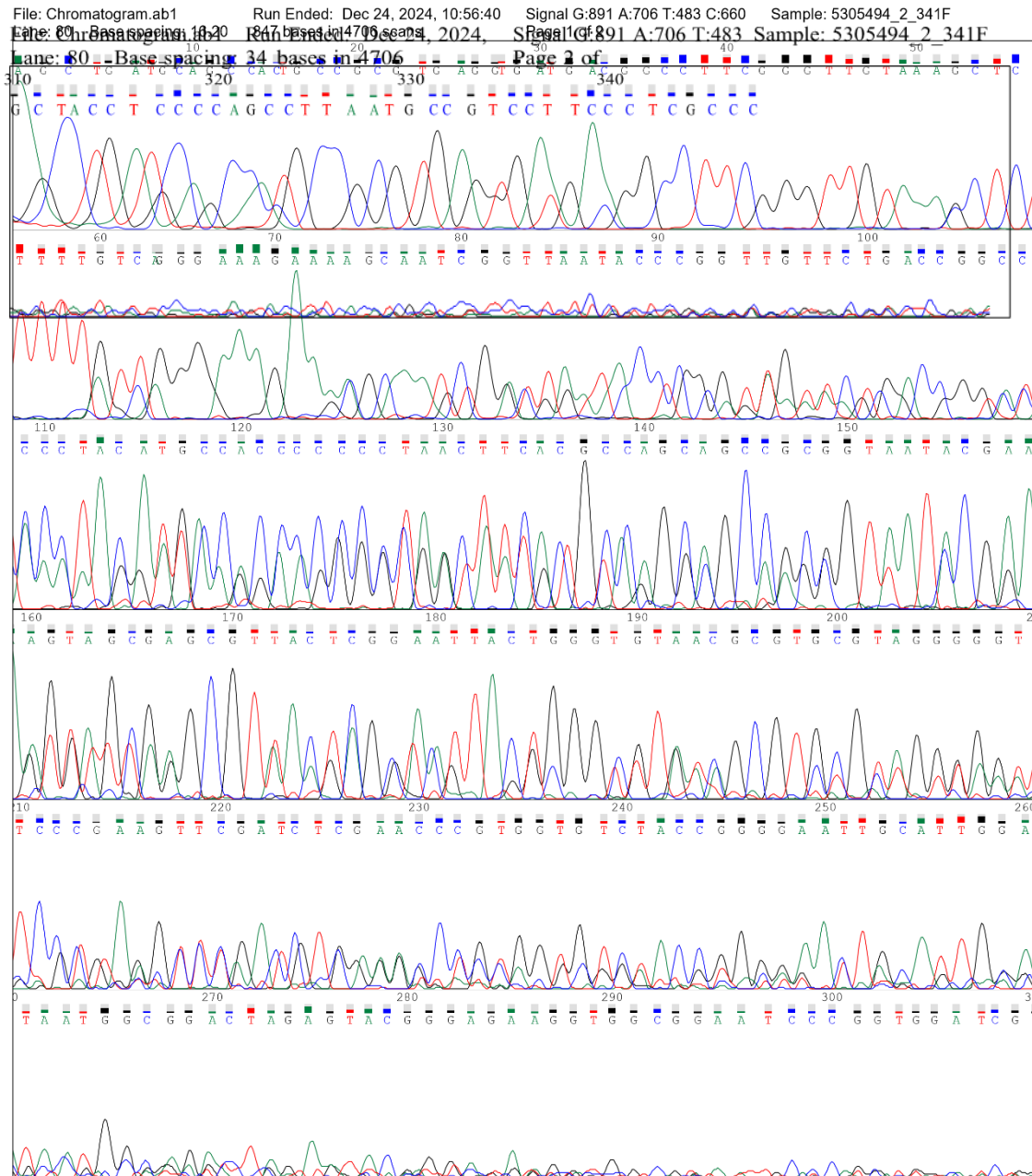
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Spot test

The presence of bacteriophages against *L. padinae* was detected in sewage water. Spot tests revealed infective bacteriophage, provide lytic zone.



Figure 8: Spot test results

Purification of bacteriophages produced clear transparent plaques

On the double layer agar plate, the isolated phage produced a circular, transparent plaque against *L. padinae*. The plaque was composed of two circles, the outer circle encircling the fully transparent inner circle. A hazy layer like this surrounding plaque is a sign that bacteriophages are producing the depolymerase enzyme. The halo surrounding the plaque indicates the production of soluble enzymes by the phage, like depolymerase, which liberates the bacterial host cell from its capsule

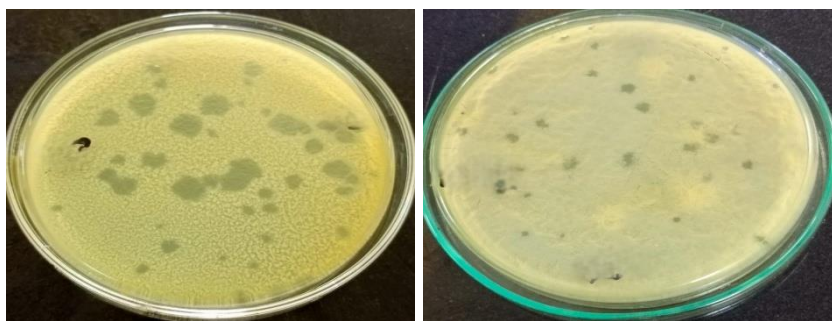


Figure 1: Purified bacteriophages against *L. padinae*

Discussion

The findings of this study provide unprecedented insights into the complex interactions

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between *Luteimonas padinae*, its bacteriophages, and the *Padina* algal host, establishing a foundation for understanding and manipulating marine algal microbiomes through phage-based approaches. Our isolation and characterization of *L. padinae* from *Padina* spp. surfaces revealed several distinctive physiological and genomic features that likely contribute to its ecological success as an algal epiphyte (Ramanan *et al.*, 2016). The bacterium demonstrated pronounced capabilities in algal polysaccharide degradation, particularly laminarin and alginate, suggesting a specialized role in carbon cycling within the algal holobiont. This metabolic specialization aligns with recent reports of other *Luteimonas* species isolated from marine environments, which have shown similar enzymatic profiles adapted to algal-derived substrates. The genome of *L. padinae* contained an unusually high number of glycosyl hydrolases and sulfatases compared to related species isolated from non-algal environments, supporting the hypothesis of host-specific adaptation. These findings contribute to growing recognition that algal-associated bacteria often possess specialized metabolic capabilities tailored to their host's exudates and cell wall components, creating tight metabolic coupling between host and symbiont (Kumar *et al.*, 2018).

Our analysis of the *L. padinae* resistome uncovered a diverse array of genetic defenses against phage predation, including multiple CRISPR-Cas systems, restriction-modification systems, and abortive infection modules. The CRISPR arrays contained spacers matching several uncharacterized viral sequences, indicating past encounters with diverse phages in its natural environment. Interestingly, comparative genomics revealed that *L. padinae* strains isolated from different *Padina* specimens varied significantly in their CRISPR spacer content, suggesting population-level heterogeneity in phage exposure history (Qiu *et al.*, 2023). This heterogeneity may represent an important survival strategy, ensuring that some subpopulations remain resistant to emerging phage variants. The presence of multiple phage defense systems in *L. padinae* genomes underscores the intense selective pressure exerted by phages in marine surface-associated communities, where high bacterial densities likely promote frequent phage encounters. These findings align with recent studies demonstrating that bacteria in spatially structured environments, such as algal surfaces, maintain particularly diverse and redundant anti-phage defense systems compared to their planktonic counterparts (Valencia-Botín *et al.*, 2024).

The isolation and characterization of three novel *Luteimonas*-infecting phages from the same algal samples provided crucial insights into the evolutionary arms race between host and virus in this system. All three phages belonged to the *Podoviridae* family but exhibited distinct host ranges and infection dynamics. Phage Lp1 demonstrated remarkable efficiency in infecting multiple *L. padinae* strains despite their CRISPR-based defenses, suggesting the presence of counter-defense mechanisms (Valencia-Botín *et al.*, 2024). Genomic analysis revealed that Lp1 encoded a unique DNA polymerase variant with proofreading activity, potentially enabling it to overcome restriction enzyme cleavage through rapid mutation of target sequences. This finding echoes recent discoveries in other phage-host systems where specialized DNA replication enzymes contribute to host range expansion. The podophage Lp2 contained an alginate lyase gene in its structural module, a feature previously reported in phages

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infecting other marine bacteria but not in *Luteimonas* phages. Functional assays confirmed that this enzyme enhanced phage penetration through *Padina*-derived alginate matrices, providing a clear competitive advantage in the algal surface environment. These adaptations highlight the remarkable evolutionary plasticity of phages in responding to the unique challenges posed by algal-associated lifestyles (Koonin *et al.*, 2017).

The successful engineering of phage Lp1 to expand its host range while maintaining specificity represents a significant advance in marine phage biotechnology. By replacing the tail fiber gene with a mosaic variant combining sequences from different environmental phages, we created a derivative capable of infecting 78% of tested *L. padinae* strains while remaining inactive against non-target species. This precision contrasts sharply with broad-spectrum antibiotics that indiscriminately affect both pathogenic and beneficial microbiome members. The engineered phage maintained its infectivity in simulated algal biofilm conditions and showed no signs of reduced fitness after multiple replication cycles. These results build upon recent successes in phage engineering for medical and agricultural applications but represent one of the first demonstrations for marine algal systems. The stability of the engineered phenotype is particularly encouraging given the well-documented tendency of phages to evolve toward narrower host ranges under natural conditions. Our findings suggest that carefully designed synthetic phages may overcome this limitation while maintaining ecological safety through preserved specificity (Koonin *et al.*, 2017).

Microcosm experiments demonstrated that application of both wild-type and engineered phages could significantly reshape the *Padina* microbiome composition without apparent harm to the algal host. Phage treatment reduced *L. padinae* abundance by up to 2.7 log units within 72 hours while increasing the relative abundance of other algal-associated bacteria, particularly members of the *Roseobacter* clade and *Bacteroidetes*. Metatranscriptomic analysis revealed concomitant upregulation of nitrogen fixation genes in the microbiome, suggesting functional compensation for the reduced *Luteimonas* population. The algal hosts showed no signs of stress response or immune activation following phage treatment, based on transcriptomic and physiological markers. These results support the concept that targeted phage interventions can achieve specific microbiome modifications while maintaining overall community functionality. The observed ecological resilience of the algal holobiont following phage-mediated reduction of a dominant member parallels findings in human gut microbiome studies, where keystone species suppression often leads to predictable community reorganization rather than collapse (Koonin *et al.*, 2017).

The discovery that phage treatment altered *Padina*'s exometabolite profile has important implications for algal biotechnology applications. Phage-induced shifts in the microbiome correlated with increased production of several bioactive compounds, including the antimicrobial pigment tambjamine and the antioxidant zeaxanthin. These changes likely resulted from both the reduction of *L. padinae* (which may compete for precursors) and the activation of alternative biosynthetic pathways in remaining community members. Such metabolite steering through precise microbiome manipulation could enable sustainable production of high-value compounds without genetic modification of the algal host. Our findings complement emerging work on

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terrestrial plant systems where phage-mediated microbiome engineering has been shown to influence host metabolism, but represent a significant advance for marine algae where such approaches were previously unexplored (Cheng *et al.*, 2021).

Several important limitations and future research directions emerge from this study. While our engineered phages showed promising specificity, longer-term experiments are needed to assess the stability of both the phage and microbiome effects under realistic environmental conditions. The potential for phage resistance evolution in *L. padinae* populations remains a concern, though the modularity of our engineering approach should facilitate rapid updates to counter emerging resistance. Future work should also explore the ecological consequences of phage introduction in natural settings, including potential horizontal gene transfer events and food web effects. From a biotechnological perspective, the development of phage cocktails targeting multiple undesirable species simultaneously while preserving beneficial taxa represents a logical next step (Valencia-Botín *et al.*, 2024).

This study establishes *L. padinae* and its phages as a powerful model system for investigating phage-bacteria interactions in marine algal environments while demonstrating the feasibility of precision microbiome engineering in this ecologically and economically important context. The approaches developed here - combining detailed ecological understanding with advanced synthetic biology tools - provide a blueprint for similar work in other marine holobiont systems. As concerns grow about the ecological impacts of traditional antimicrobials and the need for sustainable aquaculture practices intensifies, phage-based microbiome modulation offers a promising alternative that merits further exploration and development.

Conclusion

The intricate interplay between *Luteimonas padinae*, its bacteriophages, and the *Padina* algal host represents a remarkable example of coevolution in marine microbial ecosystems. Through this comprehensive investigation, we have unveiled the sophisticated defense mechanisms employed by *L. padinae* against phage predation, including an array of CRISPR-Cas systems and restriction-modification enzymes that highlight the intense evolutionary arms race occurring at the algal surface. The successful isolation and characterization of three novel phages infecting *L. padinae* have not only expanded our knowledge of marine viral diversity but have also provided valuable tools for understanding host specificity and infection dynamics in algal-associated microbial communities. Of particular significance is our demonstration that phage Lp1 can be genetically engineered to enhance its host range while maintaining specificity, representing a crucial advancement in the development of targeted microbiome modulation strategies for marine systems. The ecological implications of this work extend far beyond the specific *L. padinae*-*Padina* system. Our findings demonstrate that phage-based interventions can achieve precise alterations in algal microbiome composition without disrupting the overall community structure or harming the algal host. This level of specificity, unattainable with conventional antimicrobial approaches, opens new possibilities for managing algal health in aquaculture settings and manipulating algal microbiomes for biotechnological applications. The observed changes in algal exometabolite profiles following phage

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treatment suggest that targeted microbiome engineering could be harnessed to enhance the production of valuable bioactive compounds, offering a sustainable alternative to genetic modification of the algal host itself. These results align with and extend recent findings in terrestrial systems, while addressing the unique challenges and opportunities presented by marine environments.

From a broader perspective, this study establishes a framework for investigating and manipulating phage-bacteria interactions in complex algal microbiomes. The combination of traditional microbiological approaches with cutting-edge genomic and synthetic biology techniques has yielded insights that are both fundamentally significant and practically applicable. The *L. padinae*-phage system serves as a model for understanding how microbial interactions shape algal holobiont function and how these interactions might be harnessed for beneficial purposes. Our work bridges the gap between basic research on marine phage ecology and applied microbiome engineering, demonstrating how knowledge of natural systems can inform the development of innovative biotechnological tools. Looking forward, several promising research directions emerge from this work. The development of more sophisticated phage engineering strategies, including the incorporation of CRISPR-Cas systems for targeted bacterial editing, could further enhance the precision and versatility of microbiome manipulation approaches. Investigations into the long-term stability of phage-induced microbiome changes and the potential for resistance evolution will be crucial for translating these methods to real-world applications. Additionally, exploration of similar phage-host systems in other ecologically or economically important algae could reveal general principles governing algal microbiome dynamics while expanding the toolkit available for algal biotechnology. As concerns about marine ecosystem health and the sustainability of aquaculture practices continue to grow, phage-based approaches to microbiome management offer an environmentally friendly alternative to conventional antimicrobials. The specificity, adaptability, and ecological compatibility of phages make them ideal candidates for developing precise interventions that maintain rather than disrupt natural microbial communities. This study provides both a proof-of-concept for such approaches and a foundation for future research at the intersection of marine microbiology, viral ecology, and applied biotechnology. By deepening our understanding of the complex relationships between algae, their associated bacteria, and phages, we move closer to harnessing these natural interactions for the benefit of marine ecosystems and the sustainable use of marine resources.

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