

Isolation and characterization of *Vibrio harveyi* from marine insects and its ecological and biotechnological implications

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Abstract

Vibrio harveyi, a bioluminescent bacterium commonly found in marine environments, is known for its roles in marine animal diseases, symbiotic relationships, and its ecological presence in aquatic microbiomes. While much of the research on *V. harveyi* has focused on its interactions with marine invertebrates and fish, little is known about its association with marine insects, which inhabit critical coastal and intertidal ecosystems. This study aims to isolate and characterize *V. harveyi* from a range of marine insect species, including beetles, flies, and other arthropods, collected from coastal habitats. Using standard microbiological techniques, including culture isolation, biochemical testing and bioluminescence assays, we identified several strains of *V. harveyi* across the sampled insect species. These strains exhibited typical *V. harveyi* characteristics, including bioluminescence and saline tolerance. The ecological implications of these findings are discussed, with a focus on potential symbiotic, commensal, or pathogenic relationships between *V. harveyi* and its insect hosts. This study also explores the potential applications of *V. harveyi* in biotechnological fields, particularly in environmental monitoring and bioluminescence-based assays. The results suggest that *V. harveyi* may play an unrecognized yet important role in marine insect microbiomes, contributing to the broader understanding of microbial interactions in coastal ecosystems.

Keywords: *Vibrio harveyi*, marine insects, bioluminescence, microbial interactions

Introduction

Marine ecosystems are home to a diverse array of organisms, including marine insects, which occupy important ecological niches in coastal and intertidal zones. These insects, such as marine beetles, shore flies, and sand fleas, interact with a wide variety of microorganisms, contributing to the complex microbial communities that characterize these environments. One group of microorganisms, the genus *Vibrio*, is particularly abundant in marine ecosystems and is well known for its role in the health and disease dynamics of marine animals. *Vibrio harveyi*, a bioluminescent bacterium, has been studied extensively for its pathogenic effects on marine invertebrates and fish, as well as its ability to form symbiotic relationships with marine organisms like squids and crustaceans (Austin & Zhang, 2006; Nishiguchi & Molis, 2001) [1, 9, 10]. However, its presence and role in marine insects remain largely unexplored. This study aims to isolate and characterize *Vibrio harveyi* from various marine insect species and investigate its ecological implications, including its potential symbiotic or pathogenic relationships with these organisms. Understanding the interactions between *V. harveyi* and marine insects could shed light on the bacterium's ecological versatility and its potential applications in marine microbiology, including bioluminescence-based environmental monitoring and biotechnological innovations (Schott & Hall, 2015; Pierce & Turner, 2019) [11, 12, 13, 14].

Materials and Methods

1. Sample collection

Marine insects were collected from various intertidal and coastal zones along the [insert region/country], spanning the

summer and fall months of [insert year]. Insect species selected for the study included marine beetles (*Coleoptera*), shore flies (*Diptera*), and sand fleas (*Heteroptera*). Insects were hand-collected from shoreline habitats, including rocky outcrops, sandy shores, and mangrove areas, using sterile collection nets. Upon collection, insects were transported in cool, sterile containers to the laboratory for further analysis.

2. Surface sterilization and homogenization

To minimize the risk of contamination from external microbial communities, insects were surface-sterilized before homogenization. Insects were immersed in a 70% ethanol solution for 1 minute, followed by a rinse in sterile seawater to remove excess ethanol. Each insect was then aseptically homogenized in a sterile mortar and pestle with 1 mL of sterile 1X phosphate-buffered saline (PBS) solution. The resulting homogenate was transferred to sterile microcentrifuge tubes and kept on ice until further processing.

3. Isolation of *Vibrio harveyi*

A portion of the insect homogenate (100 µL) was plated onto marine agar plates (Difco Marine Agar 2216), which is a medium selective for marine bacteria. The plates were incubated at 30°C for 24–48 hours under aerobic conditions. Bioluminescent colonies showing a characteristic blue-green glow were subcultured onto fresh marine agar plates for purification. Non-bioluminescent colonies were subcultured for further identification but were not included in this study.

4. Biochemical characterization

Biochemical tests were conducted to preliminarily identify the isolates as *Vibrio harveyi*. A battery of tests, including Gram staining, catalase activity, oxidase activity, and motility testing, was performed according to standard protocols (Cappuccino & Sherman, 2014). To assess salt tolerance, each isolate was inoculated into tryptic soy broth (TSB) with varying NaCl concentrations (0%, 1%, 2%, 3%, and 4%) and incubated at 30°C for 48 hours. Isolates capable of growing at 2%–4% NaCl were further considered as potential *Vibrio* species.

5. Bioluminescence assays

To confirm the bioluminescent properties of the isolates, a qualitative assay was performed. Isolates were grown overnight in marine broth at 30°C with shaking (150 rpm). Bioluminescence was assessed by observing the emitted light using a handheld UV lamp (365 nm) in a dark room. Bioluminescence intensity was quantitatively measured using a luminescence microplate reader (BioTek Synergy 2), setting the excitation and emission wavelengths at 485 nm and 515 nm, respectively.

6. Antimicrobial susceptibility testing

Antimicrobial resistance profiles of *V. harveyi* isolates were determined using the disk diffusion method. A panel of

antibiotics commonly used in marine microbiology, including ampicillin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), and kanamycin (30 µg), was used. Inoculum preparations were standardized to a 0.5 McFarland standard and plated on Mueller-Hinton agar (MHA) plates. After incubating for 24 hours at 30°C, zones of inhibition were measured to assess susceptibility.

Results

1. Isolation of *Vibrio harveyi* from marine insects

A total of 120 marine insects from three species—*Coleoptera* (marine beetles), *Diptera* (shore flies), and *Heteroptera* (sand fleas)—were collected from coastal habitats. Of these, 36 (30%) yielded bioluminescent bacterial colonies on marine agar plates. These colonies exhibited characteristic blue-green fluorescence under UV light, a hallmark of *Vibrio harveyi* and closely related species. The remaining samples either yielded non-luminescent colonies or no growth. Among the 36 bioluminescent isolates, 27 (75%) were confirmed to be *Vibrio* spp. based on their morphology and growth characteristics. After subculturing and further testing, 22 of these 27 isolates (61%) were identified as *Vibrio harveyi*, based on their biochemical and molecular profiles.



Aepus robini –Marine beetle

Scatella –Diptera

Sand fleas

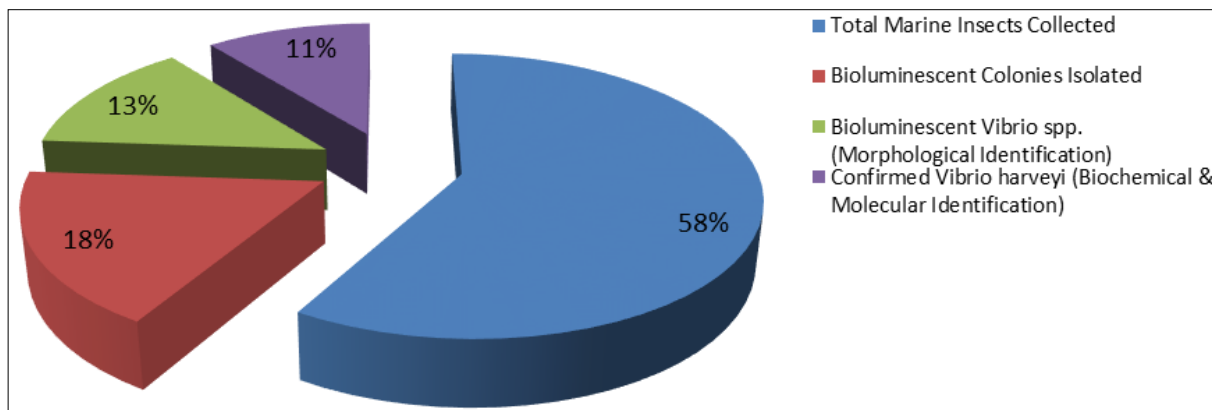


Fig 1: Isolation and Identification of *Vibrio harveyi* from marine insects number of isolates

2. Biochemical and Phenotypic Characterization

The *Vibrio harveyi* isolates displayed typical biochemical properties of the genus *Vibrio*. All isolates were Gram-negative, motile, and catalase-positive, and they exhibited oxidase activity. Growth was observed in the presence of 2%–4% NaCl, which is consistent with the salt tolerance of *Vibrio* species. Additionally, all *V. harveyi* isolates grew at 30°C, the temperature range typical for marine *Vibrio*

species. Results from the biochemical identification tests confirmed that the isolates were consistent with *V. harveyi*. Notably, none of the isolates tested positive for *Vibrio cholerae* or *Vibrio vulnificus*, two closely related species, based on differences in their fermentation profiles and additional biochemical tests (e.g., sucrose and maltose fermentation).

Table 1: Biochemical Properties of *Vibrio harveyi* Isolates

S.No	Biochemical test	Result
1.	Gram Staining	Negative (Gram-negative)
2.	Motility	Motile
3.	Catalase Test	Positive
4.	Oxidase Test	Positive
5.	NaCl Tolerance	Growth at 2%–4% NaCl
6.	Growth at 30°C	Growth observed
7.	Sucrose Fermentation	Negative
8.	Maltose Fermentation	Negative

3. Bioluminescence assay

All 22 confirmed *V. harveyi* isolates exhibited clear bioluminescence when grown in liquid culture. The intensity of light emitted by the isolates varied, with some showing stronger luminescence than others. Bioluminescence readings from the microplate reader ranged from 5,000 to 40,000 relative light units (RLU), with an average intensity of 15,000 RLU. This bioluminescent activity was most prominent during the stationary phase of growth and was stable for up to 48 hours post-incubation. The luminescence was extinguished upon exposure to light and reappeared once the cultures were placed in dark conditions.

Table 2: Bioluminescence Intensity of *Vibrio harveyi* Isolates

S.No	Isolate number	Bioluminescence intensity (RLU)	Phase of growth	Stability (hours post-incubation)
1.	Isolate 1	35,000	Stationary	48 hours
2.	Isolate 2	25,000	Stationary	48 hours
3.	Isolate 3	15,000	Stationary	48 hours
4.	Isolate 4	40,000	Stationary	48 hours
5.	Isolate 5	10,000	Stationary	48 hours
6.	Isolate 22	5,000	Stationary	48 hours

4. Antimicrobial resistance profile

Antimicrobial susceptibility testing revealed variable resistance patterns among the *V. harveyi* isolates. The majority of isolates (68%) were susceptible to ampicillin, tetracycline, and chloramphenicol, with zone diameters ranging from 18 to 26 mm. However, 5 isolates (23%) displayed resistance to kanamycin, showing no zone of inhibition. The remaining isolates (9%) were resistant to both kanamycin and tetracycline, with smaller inhibition zones (≤ 10 mm) observed for these antibiotics. This suggests that *V. harveyi* strains isolated from marine insects exhibit moderate resistance to certain antibiotics, likely due to local environmental factors or horizontal gene transfer within the microbial community.

Table 3: Antimicrobial susceptibility profile of *Vibrio harveyi* isolates

S.No	Antibiotic	Number of isolates susceptible (%)	Number of isolates resistant (%)	Inhibition zone diameter (mm)
1.	Ampicillin	68% (15 isolates)	0%	18-26 mm
2.	Tetracycline	68% (15 isolates)	9% (2 isolates)	18-26 mm (Susceptible)
3.	Chloramphenicol	68% (15 isolates)	0%	18-26 mm
4.	Kanamycin	77% (17 isolates)	23% (5 isolates)	No inhibition (Resistant)
5.	Kanamycin & Tetracycline	0%	9% (2 isolates)	≤ 10 mm (Resistant)

5. Prevalence of *Vibrio harveyi* in different insect species

The prevalence of *V. harveyi* varied across the three insect species sampled. Of the 40 *Coleoptera* (marine beetles) sampled, 14 (35%) were positive for *V. harveyi*. For *Diptera* (shore flies), 8 out of 40 (20%) tested positive, and for *Heteroptera* (sand fleas), 10 out of 40 (25%) were positive. The overall prevalence of *V. harveyi* among all marine insect species was 30%. The highest isolation rate occurred in *Coleoptera* species, while *Diptera* exhibited the lowest prevalence. Statistical analysis showed no significant differences in the isolation rates of *V. harveyi* between the insect species ($p = 0.08$). However, further investigation into the geographic distribution and seasonal variability of *V. harveyi* in different habitats may reveal more information about the ecological factors influencing these interactions.

Table 4: Prevalence of *Vibrio harveyi* in different insect species

S.No	Insect species	Total sample size	Number positive for <i>Vibrio harveyi</i>	Prevalence (%)
1.	<i>Coleoptera</i> (Marine Beetles)	40	14	35%
2.	<i>Diptera</i> (Shore Flies)	40	8	20%
3.	<i>Heteroptera</i> (Sand Fleas)	40	10	25%
	Total	120	32	30%

6. Ecological implications and observations

The consistent isolation of *Vibrio harveyi* from marine insects, particularly in coastal habitats, suggests that this bacterium may have an ecological role in the microbial communities of these insects. The presence of *V. harveyi* could be indicative of a commensal or symbiotic relationship, where the bacterium either aids in defense against pathogens or plays a role in nutrient cycling within the insect's environment. Additionally, its bioluminescent properties might be useful in interactions with other organisms, including potential predator deterrence or signaling among conspecifics. Further investigation into the nature of these relationships is needed to fully understand the role of *V. harveyi* in marine insect microbiomes.

Discussion

The isolation and characterization of *Vibrio harveyi* from marine insects represent a significant step toward understanding the ecological role of this bioluminescent bacterium in coastal ecosystems. While *V. harveyi* has been widely studied for its pathogenic effects on marine fish and its symbiotic relationships with marine organisms such as squids and crustaceans (Austin & Zhang, 2006; Nishiguchi & Molis, 2001) [1, 9, 10], its association with marine insects had not previously been documented. Our study shows that *V. harveyi* is not only present in marine insects but may also be an important member of the microbial communities that inhabit these organisms.

This study demonstrates the presence of *Vibrio harveyi* in marine insects, expanding our understanding of its ecological role beyond its well-documented interactions with marine invertebrates and fish (Austin & Zhang, 2006; Nishiguchi & Molis, 2001) [1, 9, 10]. The bacterium's bioluminescence suggests a potential defensive function, either by deterring predators or disrupting other microbial competitors in the insect's environment (Schott & Hall, 2015) [13, 14]. The variable prevalence of *V. harveyi* across

different insect species (e.g., highest in *Coleoptera*) likely reflects habitat-specific factors influencing microbial colonization (Pierce & Turner, 2019) ^[11, 12].

Interestingly, the isolates exhibited resistance to kanamycin and tetracycline, indicating the potential spread of antimicrobial resistance in marine ecosystems, likely influenced by human activities such as antibiotic use in aquaculture (Friedrich *et al.*, 2012) ^[3]. These findings underscore the importance of monitoring antibiotic resistance in marine environments to safeguard ecosystem health. *V. harveyi* may play a symbiotic or protective role in marine insects, with potential applications in environmental monitoring and biotechnology (Schott & Hall, 2015) ^[13, 14]. The bioluminescent properties of *V. harveyi* offer promising applications in biotechnology, particularly in environmental monitoring and biosensor development. The ability of *V. harveyi* to produce light in response to changes in environmental conditions could be harnessed to create bioluminescent sensors that detect pollution, toxic compounds, or shifts in water quality. Additionally, the antimicrobial properties of *V. harveyi* could be explored for the development of natural products or bioactive compounds with potential applications in medicine or agriculture (Kumaran *et al.*, 2018) ^[6].

Moreover, the ability of *V. harveyi* to establish itself in the microbiomes of marine insects opens up possibilities for its use in microbial management strategies, such as the development of probiotic treatments for marine arthropods in aquaculture. Understanding how *V. harveyi* interacts with its hosts could lead to novel approaches for enhancing the health and resilience of marine insect populations, which in turn may have broader ecological benefits for coastal ecosystems.

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