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Comparative analysis of bacterial communities associated with zooxanthellate jellyfish in indoor and outdoor culture systems

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Original Article

Abstract

The upside-down jellyfish, *Cassiopea* sp., harbours symbiotic photosynthetic dinoflagellates (zooxanthellae) and distinct bacterial communities that contribute to its health and ecological functions. The present study was undertaken to examine the bacterial assemblages associated with *Cassiopea* under indoor and outdoor conditions at the Cnidarian Laboratory of the ICAR-Tuticorin Regional Station of the Central Marine Fisheries Research Institute (CMFRI). Twelve bacterial strains were isolated and identified using biochemical assays and 16S rRNA gene sequencing. The dominant families were Vibrionaceae (seven species), followed by Bacillaceae (four species) and Pseudoalteromonadaceae (one species). In outdoor systems, the dominant bacterial strains in the medusae were *Photobacterium* spp. (three strains) and *Bacillus* spp. (two species), whereas indoor systems were dominated by *Vibrio* spp. (three species) and *Pseudoalteromonas* sp. These findings on the community composition differed significantly ($p<0.01$) between indoor (*Vibrio*-dominated) and outdoor (*Photobacterium*-dominated) culture systems, highlighting system-specific bacterial associations in the upside-down jellyfish. This is the first documentation of system-specific bacterial associations in *Cassiopea* sp. from controlled culture conditions in India, underscoring the novelty of our findings and their implications for understanding host–microbe interactions and optimising jellyfish husbandry.

Keywords: *Upside-down jellyfish, Cassiopea sp., 16S rRNA, Pseudoalteromonadaceae, symbiosis, vbrionaceae*

Introduction

The genus *Cassiopea* (Cnidaria, Scyphozoa: Rhizostomeae), first described by Forsskål in 1775, comprises a group of stationary jellyfish species of the order Rhizostomeae (De Rinaldis *et al.*, 2021). Commonly referred to as upside-down jellyfish, members of this genus exhibit a unique morphology among Scyphomedusae, as they typically rest with their flat exumbrella against the seafloor while orienting their subumbrella and oral arms upward (Ohdera *et al.*, 2018; De Rinaldis *et al.*, 2021). These jellyfish inhabit shallow coastal habitats such as lagoons, mangroves, and seagrass beds across tropical and subtropical regions (Mellas *et al.*, 2014; Lampert, 2016; Ohdera *et al.*, 2018; De Rinaldis *et al.*, 2021).

A defining feature of *Cassiopea* biology is its symbiotic association with photosynthetic dinoflagellates of the family *Symbiodiniaceae* (commonly referred to as zooxanthellae). These algae reside in host tissues, including the oral arms, proboscis, and sustentacular regions (Hofmann *et al.*, 1978; Colley and Trench, 1983; Lampert, 2016; Motone *et al.*, 2020; De Rinaldis *et al.*, 2021). Through photosynthesis, zooxanthellae convert light energy, carbon dioxide, and water into organic compounds such as glucose, which are released and assimilated by the jellyfish host to support metabolic processes, including growth, development, and reproduction (Holcomb *et al.*, 2014; Djeghri *et al.*, 2019). Consequently, *Cassiopea* species depend heavily on light availability and

symbiont photosynthetic efficiency for survival (Verde and McCloskey, 1998; Djeghri *et al.*, 2019; Mammone *et al.*, 2021).

In addition to algal symbionts, an increasing number of studies have highlighted the importance of the bacterial communities associated with *Cassiopea*. These bacteria are thought to influence the acquisition and maintenance of specific Symbiodiniaceae clades (Djeghri *et al.*, 2019). Supporting evidence shows that jellyfish harbour distinct bacterial populations that differ significantly from those in the surrounding seawater (Sipkema *et al.*, 2005; Cleary *et al.*, 2016), and that certain bacteria may be selectively cultivated in specific host tissues to perform site-specific functions (Tinta *et al.*, 2019). For example, bacterial communities on exumbrella and oral arms are believed to protect the host against pathogens and fouling organisms (Harder *et al.*, 2003). The taxa frequently reported in these assemblages include Rhodobacteraceae, Vibrionaceae, and Flavobacteriaceae (Cleary *et al.*, 2016; Frommlet *et al.*, 2018). Beyond their defensive roles, these bacterial partners may also provide nutrients or enhance zooxanthellae survival, thereby reinforcing *Cassiopea*–zooxanthellae symbiosis (Moreira *et al.*, 2019).

Despite these insights, the relationships between different *Cassiopea* species, their associated bacterial communities, and the role of environmental factors in shaping these interactions remain poorly understood (Kaur *et al.*, 2016; Karunarathne *et al.*, 2020). Moreover, the diversity (Ranjith *et al.*, 2014) and functional roles of *Cassiopea*-associated bacteria in Indian waters remain largely unknown. To address this knowledge gap, the present study investigated the bacterial community composition in the zooxanthellate upside-down jellyfish *Cassiopea* maintained under different culture systems. This approach aims to provide new insights into the microbial basis of symbiosis and its ecological implications for jellyfish biology.

Material and methods

Sample collection and maintenance

Cassiopea sp. medusae were maintained under indoor and outdoor culture conditions at the Cnidarian Laboratory, Tuticorin Regional Station, ICAR-Central Marine Fisheries Research Institute (CMFRI). In the indoor system, the medusae (2–3 cm in diameter; Fig. 1a and b) was housed in a recirculating aquarium unit consisting of glass tanks (20 × 20 × 20 cm) supplied with controlled seawater conditions (salinity: 33 ± 2 ppt; temperature: 30 ± 2 °C; light intensity: 60 ± 20 PAR; photoperiod: 18 L/6D). The medusae in the outdoor culture (Fig. 1c and d) were maintained in cemented tanks exposed to ambient atmospheric conditions (with diurnal changes), closely resembling natural seawater parameters. Random sampling was employed, and three individual medusae were collected from each culture system. To minimise external contamination, the medusae were rinsed thoroughly with sterile, filtered, autoclaved seawater before handling. Sterile swabs were used to collect microbial samples from the exumbrella and the oral arms. The swabs were immediately streaked onto Zobell Marine Agar (ZMA) plates for culturing (Kramar *et al.*, 2018), which supported the growth of a broad range of marine heterotrophic bacteria. Plates were incubated at 30 ± 2 °C (matching seawater temperature) for 24–48 h to allow for visible colony formation (Bergey, 1934; Taylor *et al.*, 1983).

Isolation and culture of bacteria

Bacterial isolates obtained using the spread plate method on the ZMA were incubated at 37 °C for 24 h to allow visible colony development. Distinct colonies were purified through repeated sub-culturing. Pure cultures were obtained by the quadrant streak method, and colonies were aseptically re-streaked until a single colony type was isolated and subsequently

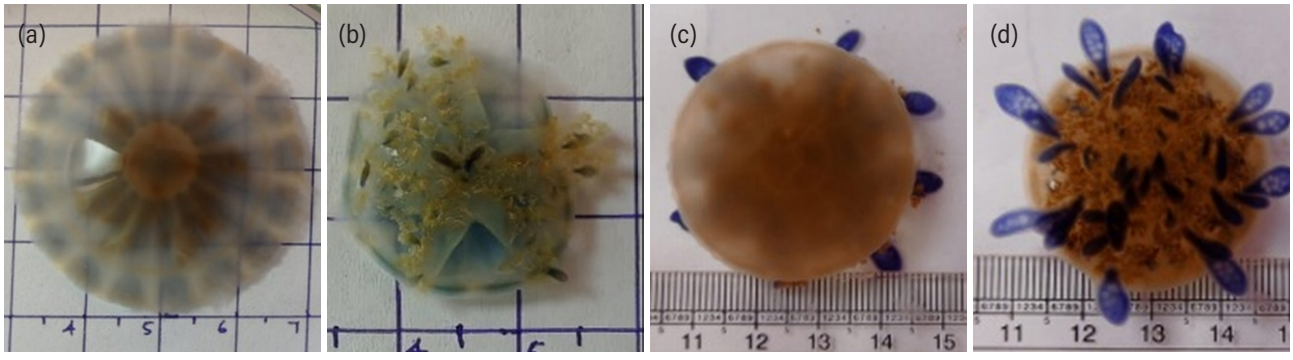

Fig. 1. Oral and aboral view of *Cassiopea* sp. used for the experimental studies from the (a) aboral view (Indoor culture systems), (b) oral view (indoor culture systems), (c) aboral view (outdoor culture systems), (d) oral view (outdoor culture systems)

grown in the medium from which it was obtained. Plates were incubated for 24–48 h, allow visible colony formation (Betts, 2006), and macroscopic morphological features (size, colour, margin, elevation, and texture) were observed and recorded as preliminary identifiers. Isolates were maintained at room temperature for short-term use, while glycerol stocks of bacterial cultures were preserved at -20 °C for long-term storage (Sanders, 2012).

Biochemical characterization

Biochemical profiling of the bacterial isolates was performed following standard microbiological protocols and Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984). The tests included Gram staining for cell wall characterisation, carbohydrate fermentation assays for metabolic profiling, and enzyme-based tests for catalase and indole production. Additional biochemical assays, including citrate utilisation, Methyl Red (MR), and Voges-Proskauer (VP) tests, were conducted to provide further insights into the physiological and taxonomic characteristics of the isolates.

DNA extraction and PCR amplification

Genomic DNA was extracted from overnight cultures (in triplicate) using a modified phenol–chloroform method (Marmur, 1961). The 16S rRNA gene was amplified by PCR in a 20 µl reaction mixture containing 10 µl of master mix, 1 µl of forward primer, 1 µl of reverse primer, 7 µl of nuclease-free water, and 1 µl of DNA template. Amplification was carried out in a Thermal Cycler (Agilent SureCycler 8800) under the following conditions: initial denaturation at 94 °C for 5 min, followed by 32 cycles of denaturation at 94 °C for 30 s, primer annealing at 58 °C for 1 min, and extension at 72 °C for 1.5 min; with a final extension at 72 °C for 10 min. The PCR products were visualised using agarose gel electrophoresis (Bio-Rad documentation system), purified, and subjected to Sanger sequencing.

Phylogenetic and statistical analysis

Sequences were edited using BioEdit (Hall, 1999) and compared with GenBank entries using BLAST (Benson *et al*., 2010). Multiple sequence alignments were performed using Clustal Omega (Thompson *et al*., 1997). Phylogenetic trees were constructed in MEGA v11 (Kumar *et al*., 2018) using the neighbour-joining (NJ) algorithm, with evolutionary distances estimated using the Kimura two-parameter model, and bootstrap analyses were conducted with 1,000 replicates to assess branch support in MEGA v11.0 (Kimura, 1980). Consensus sequences were deposited in GenBank in the NCBI Database, and the Maximum Likelihood tree was rooted with outgroup sequences (Liang *et al*., 2019; Tamura *et al*., 2021). Comparative analyses of the bacterial community composition between the indoor and outdoor culture systems were performed using the chi-square test (χ^2 test) in SPSS version 20 to determine the significance of the relationship between the two variables.

Diversity indices

The Shannon (Shannon and Weaver, 1963) and Simpson (Simpson, 1949) diversity indices were used to measure biodiversity and evaluate species heterogeneity between the culture systems.

Results

Bacterial isolation and morphological characterisation

Twelve morphologically distinct bacterial isolates were obtained from *Cassiopea* sp. medusae, comprising six from indoor (isolates 1, 2, 3, 4, 5, and 6) and six from outdoor (isolates 7, 8, 9, 10, and 12) culture systems. The colony characteristics, including size, colour, texture, elevation, form, and margin, are summarised in Table 1.

Biochemical characterization

Standard biochemical assays (Gram staining, methyl red, Voges–Proskauer, citrate utilisation, carbohydrate fermentation, catalase, indole, and oxidase) differentiated the isolates according to metabolic traits (Table 2).

Table 1. Colony morphology of twelve isolated bacterial strains from the two culture systems

Isolates	Size	Colour	Texture	Elevation	Form	Margin
Indoor culture						
Isolate 1	Moderate	Yellow white	Mucoid	Convex	Round	Entire
Isolate 2	Large	Yellow white	Dry	Draughtsman colony	Round	Lobate
Isolate 3	Moderate	Yellow white	Mucoid	Flat	Irregular	Lobate
Isolate 4	Small	Yellow	Mucoid	Raised	Irregular	Lobate
Isolate 5	Large	Yellow white	Mucoid	Flat	Irregular	Lobate
Isolate 6	Moderate	Yellow white	Mucoid	Raised	Round	Lobate
Outdoor						
Isolate 7	Large	Yellow white	Mucoid	Flat	Irregular	Lobate
Isolate 8	Small	Yellow white	Smooth	Convex	Punctiform	Entire
Isolate 9	Small	Pink	Mucoid	Convex	Round	Entire
Isolate 10	Small	Yellow	Smooth	Convex	Round	Entire
Isolate 11	Small	Yellow white	Mucoid	Raised	Round	Entire
Isolate 12	Small	Transparent	Mucoid	Convex	Round	Entire

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Table 2. Biochemical analysis of isolated bacterial strains from the indoor and outdoor systems

Isolates	GS	MR	VP	CU	CF	CT	I	O
Outdoor								
Isolate 1	G-ve rod	+	+	–	–	+	–	+
Isolate 2	G+ve rod	–	–	+	+	+	–	+
Isolate 3	G-ve rod	+	–	–	+	+	–	–
Isolate 4	G-ve rod	+	+	–	–	+	–	+
Isolate 5	G-ve rod	+	–	–	+	+	+	+
Isolate 6	G-ve rod	+	+	–	–	+	–	+
Indoor								
Isolate 7	G-ve rod	+	–	–	+	+	+	+
Isolate 8	G+ve rod	–	–	–	–	–	–	–
Isolate 9	G-ve rod	–	–	–	–	+	–	+
Isolate 10	G-ve rod	+	–	+	–	+	+	+
Isolate 11	G-ve rod	–	+	+	+	+	–	–
Isolate 12	G-ve rod	+	–	+	–	+	+	+

CM; Gram staining–GS; Methyl red–MR; Voges Proskauer -VP; Citrate utilisation - CU; Carbohydrate fermentation–CF; Catalase–CT; Indole–I; Oxidase–O.

Phylogenetic identification

Amplification of 16S rRNA genes (>1450 bp), followed by BLAST analysis, confirmed the taxonomic identity of the isolated bacterial species (Table 3). Isolate 1 showed 99.12% similarity with *Photobacterium* sp. (Accession No. KP689587.1), isolate 2 showed 100% similarity to *Priestia flexa* (MG407663.1), isolate 3 showed 100% similarity to *Bacillus infantis* (OR268553.1), isolate 4 showed 99.33% similarity to *Photobacterium* sp. (KP689587.1), isolate 5 showed 99.93% similarity to *Vibrio alginolyticus* (ON287250.1), and isolate 6 showed 99.33% similarity to *Photobacterium* sp. (KP689587.1). Similarly, six isolates from the indoor system were identified as follows: isolate 7 with 99.8% similarity to *Vibrio alginolyticus* (ON287250.1), isolate 8 with 99.19% similarity to *Mangrovibacillus cuniculi* (MT146882.1), isolate 9 with 99.07% similarity to *Pseudoalteromonas* sp. (AJ551143.1), isolate 10 with 99.86% similarity to *Vibrio owensii* (OM988202.1), isolate 11 with 99.4% similarity to *Bacillus cereus* (MT103054.1), and isolate 12 with 97.75% similarity to *Vibrio hispanicus* (NR042806.1). The constructed phylogenetic tree of the 12 different isolates is shown in Fig. 2 a-l, and the pooled sequence is shown in Fig. 2 m.

Microbial abundance and diversity

Outdoor medusae harboured significantly higher microbial loads than did indoor samples (ANOVA: F = 31.00, p = 0.00024). Taxonomically, the isolates belonged to Vibrionales (58.3%), Bacillales (33.3%), and Alteromonadales (8.3%), spanning five genera. *Vibrio* (4 isolates), *Photobacterium* (3 isolates), and *Bacillus* (3 isolates) were predominant, whereas *Mangrovibacillus* and *Pseudoalteromonas* were unique to indoor systems.

Table 3. Identified jellyfish-associated bacterial community composition taxon and its NCBI GenBank accession no, % similarity and % query cover

No.	Organism name	Accession no	Similarity (%)	Query cover %	Sequence length
	Class: Gammaproteobacteria, Order: Alteromonadales, Family: Pseudoalteromonadaceae)				
1	*Pseudoalteromonas* sp.	OR691617	99.07	99	1512
	Class: Gammaproteobacteria, Order: Vibrionales, Family: Vibrionaceae				
2	*Photobacterium* sp. 1	OR691393	99.12	100	1483
3	*Photobacterium* sp. 2	OR691721	99.33	99	1490
4	*Photobacterium* sp. 3	OR691719	99.80	100	1489
5	*Vibrio alginolyticus* 1	OR691720	99.80	100	1484
6	*Vibrio alginolyticus* 2	OR691673	99.93	100	1503
7	*Vibrio owensii*	OR691407	99.86	100	1476
8	*Vibrio hispanicus*	OR691409	97.75	99	1533
	Class: Firmicutes, Order: Bacillales, Family: Bacillaceae (4 species)				
9	*Priestia flexus*	OR691363	100	100	1484
10	*Bacillus infantis*	OR691661	100	99	1502
11	*Bacillus cereus*	OR691408	99.40	100	1497
12	*Mangrovibacillus cuniculi*	OR691616	99.19	100	1481

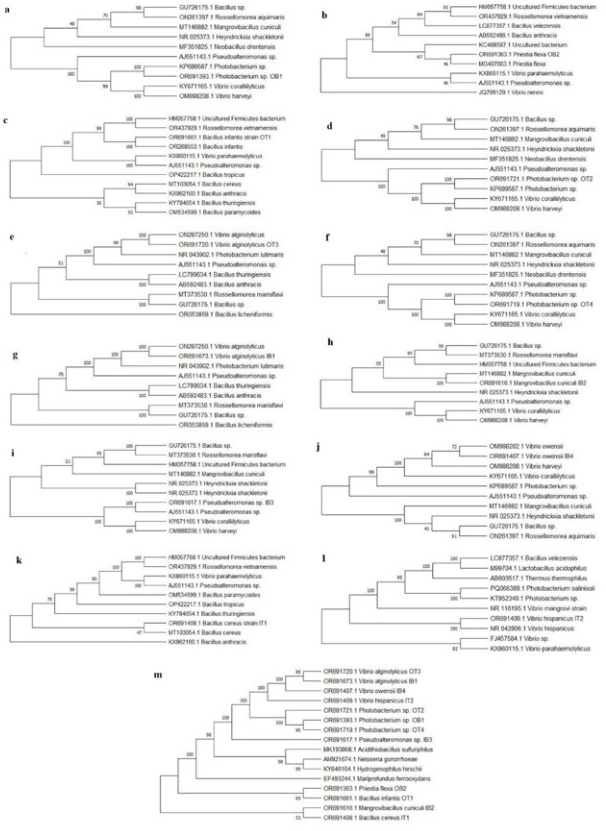

Fig. 2. Maximum likelihood tree based on bacterial 16S rRNA sequences showing phylogenetic relationship for isolated bacterial species from *Cassiopea* sp. Combined phylogenetic tree of a) Isolate 1, b) Isolate 2, c) Isolate 3, d) Isolate 4, e) Isolate 5, f) Isolate 6, g) Isolate 7, h) Isolate 8, i) Isolate 9, j) Isolate 10, k) Isolate 11, l) Isolate 12, m) pooled sequence

Community composition and ecological indices

At the family level, Vibrionaceae (7 isolates) was dominant, followed by Bacillaceae (4 isolates) and Pseudoalteromonadaceae (1 isolate). Chi-square tests showed no significant differences in distribution between the systems (order: $\chi^2 = 1.14$, p = 0.565; genus: $\chi^2 = 6.33$, p = 0.176). However, the diversity indices were higher indoors (Shannon = 1.24; Simpson = 0.67) than outdoors (Shannon = 1.01; Simpson = 0.61), indicating greater taxonomic evenness under controlled conditions. Overall, Gammaproteobacteria (66.7%) and Firmicutes (33.3%) were the dominant groups, with indoor systems supporting higher diversity and outdoor systems exhibiting higher abundance.

Discussion

This study provided preliminary insights into the bacterial communities associated with *Cassiopea* sp. medusae maintained under controlled indoor and outdoor culture conditions. Consistent with earlier findings in scyphozoan jellyfish (Apprill *et al*., 2009; Tinta *et al*., 2012; Tinta *et al*., 2019), the dominant taxa across both environments were members of *Gammaproteobacteria*, particularly *Vibrio* spp. These findings reinforce the notion that certain bacterial groups, including *Vibrio*, *Photobacterium*, and *Pseudoalteromonas*, represent the core components of jellyfish-associated microbiomes across diverse environmental conditions. A statistically significant difference in microbial abundance between the two culture systems (F = 31.00; $p = 0.00024$) underscored the influence of environmental conditions on microbial colonisation. Outdoor medusae exhibited higher colony-forming units, likely because of increased microbial exposure and environmental complexity. In contrast, indoor systems supported a more diverse and taxonomically even community, as reflected by higher Shannon (1.24 vs. 1.01) and Simpson (0.67 vs. 0.61) diversity indices (Li *et al*., 2024). Although chi-square tests revealed no significant compositional differences at the order or genus levels, indoor environments harboured unique taxa that were not detected outdoors, suggesting greater niche availability or stability under controlled conditions.

The dominance of *Vibrio* spp. in both systems is ecologically significant, wherein members of this genus are well-known surface colonisers of marine invertebrates and are equipped with functional traits such as chitin degradation, quorum sensing, and antimicrobial production (Long and Azam, 2001; Bruhn *et al*., 2005; Dang and Lovell, 2016). These capabilities may facilitate microbial competition and host interactions with potential implications for host immunity and nutrient cycling. Some *Vibrio* strains also produce collagenases (e.g., *V. alginolyticus*), which can influence tissue remodelling or medusa development (Hofmann and Brand, 1987). Indoor cultures also support *Pseudoalteromonas* spp., which are prolific producers of bioactive metabolites with antifouling and antimicrobial properties (Holmström and Kjelleberg, 1999; Vera *et al.,* 1998). Their presence may offer protective benefits to hosts under culture conditions, possibly enhancing resilience to opportunistic infections. In outdoor systems, *Photobacterium* spp. were more abundant, (Hendrie *et al*., 1970; Labella *et al*., 2017), which is consistent with previous reports on their bioluminescent traits (Urbanczyk *et al*., 2011; Motone *et al*., 2020) and metabolic roles in nitrogen, sulfur, and vitamin cycling (Cleary *et al*., 2016; Frommlet *et al*., 2018). This suggests that outdoor light regimes and nutrient fluxes may selectively favour functionally specialised taxa. *Bacillus* spp., found in both indoor and outdoor environments but slightly enriched in the latter, further adds to the functional and ecological diversity of the jellyfish microbiome. These bacteria are known for their metabolic versatility, resilience to environmental stress, and production of antimicrobial peptides (Raddadi *et al*., 2008), which may support host defence mechanisms, particularly under fluctuating or nutrient-rich conditions that are typical of outdoor systems. Their presence across multiple jellyfish species, including *Aurelia aurita*, *Chrysaora plocamia*, and *Rhopilema nomadica* (Weiland-Bräuer *et al*., 2015; Viver *et al*., 2017), suggests that *Bacillus* may be a widespread, though perhaps opportunistic, component of jellyfish-associated microbiota. Similarly, Weiland-Bräuer *et al.* (2015) found *Bacillus* species among culturable isolates from *Aurelia aurita* polyps, which are often linked to biofilm formation and antimicrobial activity. Viver *et al.* (2017) reported *Bacillus* and other Firmicutes as transient yet functionally significant members of the microbiome in wild *Cotylorhiza tuberculata*, particularly during bloom periods when environmental conditions change rapidly. These findings imply that *Bacillus* and related taxa may play adaptive roles in mediating host-microbe-environment interactions, particularly in dynamic marine ecosystems or culture systems.

Despite revealing key taxa with potential ecological and functional roles, the present study is limited by its reliance on culturable isolates, which likely capture only a fraction of total microbial diversity. High-throughput sequencing studies have demonstrated that jellyfish-associated microbiomes are often dominated by uncultured or rare taxa, many of which remain functionally uncharacterised (Tinta *et al*., 2012; Viver *et al*., 2017). Moreover, taxa such as *Endozoicomonas*, *Ruegeria*, and *Marinobacter*, which are frequently detected in molecular surveys (Cleary *et al*., 2016; Weiland-Bräuer *et al*., 2015), were absent from our culture-based results. This highlights the need to integrate culture-independent methods, such as 16S rRNA gene sequencing, metagenomics, and metatranscriptomics, to

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obtain a more comprehensive and ecologically meaningful view of jellyfish holobionts. The lack of accompanying environmental metadata (*e.g.*, temperature, salinity, dissolved oxygen, and nutrient concentrations) also constrains our ability to interpret the environmental drivers of microbiome composition. Studies have shown that jellyfish-associated microbiota are responsive to environmental gradients and seasonal cycles (Viver *et al.*, 2017; Tinta *et al.*, 2019), underscoring the importance of linking microbiome dynamics to abiotic and biotic factors for a holistic understanding of host–microbe–environment interactions.

The findings of this study have significant implications for both ecological research and jellyfish aquaculture. The observed differences in microbial abundance and diversity between indoor and outdoor culture systems suggest that microbiomes can be shaped by manipulating the environmental conditions. This opens avenues for microbiome engineering in controlled settings, where beneficial taxa such as *Pseudoalteromonas* can be promoted for their bioactive and protective functions (Holmström and Kjelleberg, 1999), potentially enhancing medusa health, larval settlement, and survival rates. Similarly, the roles of *Vibrio* and *Photobacterium* in nutrient cycling, bioluminescence, and antimicrobial compound production indicate broader ecological functions. For instance, *Photobacterium* species may aid feeding efficiency under ambient light through bioluminescence (Urbanczyk *et al.*, 2011), whereas *Vibrio* spp. contributes to nitrogen and carbon cycling through their enzymatic activities (Zhou *et al.*, 2016). These microbial functions can be incorporated into models of jellyfish bloom dynamics or nutrient fluxes in coastal ecosystems, particularly under scenarios of climate change and eutrophication.

Furthermore, understanding host-associated microbiomes offers potential for developing microbiota-based indicators of jellyfish health and environmental stress. As jellyfish become increasingly abundant in coastal regions due to anthropogenic pressures, the microbial dimension of their ecology deserves greater attention not only for understanding bloom formation but also for monitoring marine ecosystem health and resilience. In summary, this study underscores the dynamic and responsive nature of jellyfish–microbe associations and the critical influence of the environmental context. Although preliminary and limited in scope, these findings lay the groundwork for future research exploring the functional roles, evolutionary stability, and biotechnological potential of jellyfish-associated bacteria in both natural and engineered systems.

Conclusion

This study revealed distinct differences in bacterial communities associated with *Cassiopea* sp. medusae cultured under both indoor and outdoor conditions. While outdoor systems supported significantly higher microbial abundance, indoor systems exhibited greater taxonomic diversity and evenness, including unique genera, such as *Mangrovibacillus* and *Pseudoalteromonas*. Overall, the isolates were dominated by Vibrionaceae and Bacillaceae, with *Vibrio*, *Photobacterium*, and *Bacillus* being the most frequent taxa. These findings highlight the strong influence of environmental context on structuring jellyfish-associated bacterial assemblages and suggest that controlled indoor environments favour microbial diversity, whereas outdoor conditions promote colonisation by abundant but less diverse taxa. The results provide a baseline framework for understanding jellyfish–microbe interactions and their potential ecological and biotechnological implications.

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Author contributions

Conceptualisation: RL, ARS, RS, KC, LPD; Methodology: ARS, RL; Data Collection: ARS, RL, SD; Data Analysis: ARS, RL, SD; Writing the Original Draft: ARS, RL; Writing Review and Editing: RL, RS, KC, LPD; Supervision: RL, APS.

Data availability

The data supporting this study are available in the NCBI GenBank repository, and the accession numbers are provided in the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

Ethical statement

This study was conducted in compliance with ethical standards, and no specific ethical approval was required as the research did not involve protected organisms or sensitive environments.

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