

ORIGINAL RESEARCH ARTICLE

Exploring the antimicrobial potential of marine actinobacteria from the Fijian hard corals

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Abstract

Marine ecosystems, particularly corals and their microbiomes, are recognized as prolific sources of unique natural products with diverse bioactivities. Despite the evident paucity of literature on microbial compounds associated with Fijian corals, this study aims to explore and elucidate the potential of actinomycetes associated with Fijian hard corals as sources of bioactive compounds. To highlight, actinomycete strains were isolated from five hard coral genera using three distinct isolation media. Among these, all 41 strains are facultative salt-tolerant, with 16 demonstrating biological activity by inhibiting the growth of three or more test pathogen strains. Interestingly, all ethyl acetate extracts of putative strains exhibited varying degrees of antimicrobial activity, with isolates FJC 16, 28, 33, and 41 being the most active. Moreover, the brine shrimp lethality test confirmed that actinomycete strain FJC 16 exhibited the highest toxicity toward brine shrimps with a lethal concentration of 37.50 µg/mL for 50% of the test population. The International Streptomyces Project medium-2 demonstrated high efficiency in isolating total actinomycetes. With a focus on cultivation techniques, the applications of pretreatment practices contributed to a successive recovery of actinomycetes. The current study serves as a foundational effort to evaluate the biosynthetic capabilities of Fijian hard corals, aiming to advance the current frontier of microbial natural product research within coral-associated microbiomes. Overall, as the footprint of microbial metabolites grows, Fijian hard corals represent a valuable prospect for further advanced studies of microbial designer molecules or analogs.

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1. Introduction

Actinobacteria, also known as actinomycetes, are Gram-positive bacteria that contain sufficient G+C content in their genome. They are a diverged group of bacterial lineages extensively studied for their antibiotic-producing capabilities.^{1,2} Hierarchically, they are classified under the phylum Actinomycetota (Actinobacteria), a clade that is intermediary between fungi and bacteria.^{2,3} Actinomycetes can be unicellular (e.g., *Arthrobacter* spp.) and multicellular (e.g., *Streptomyces* spp.). They exist as both symbionts (e.g., *Frankia* spp.) and pathogens (e.g., *Mycobacterium* spp.), and contribute substantially to nutrient

turnover and bioremediation processes.^{4,5} Actinobacteria are highly competitive under adverse environmental conditions due to their inherent adaptive mechanisms. These mechanisms involve the production of spores and secondary metabolites, and the efficient coordination of nutrients through mycelial growth.⁶ Actinomycetes are widely endowed with antibiotic machinery and are responsible for a growing number of antibiotic discoveries and productions. It is worth noting that actinomycetes possess antibiotic resistance genes and are therefore immune to their own antibiotics. In spite of this, they can transfer these resistant genes through horizontal gene transfer to other pathogens.^{7,8} The genus *Streptomyces* has contributed about 80% of pharmaceutical drugs.^{9,10} It comprised over 500 species responsible for the bioactive compounds discovered.¹¹ The advent of improved high-end technologies has unveiled the large genomes of actinomycetes and encodes their ability to produce 10–20 bioactive compounds per strain.¹² Actinobacteria have a complex and unusual life cycle in which most spend much of their life cycle as semi-dormant spores under adverse conditions, but develop into mycelium when conditions are favorable.^{2,13,14} Actinomycetes are omnipresent within the ocean boundaries and comprise a tenth of all marine microbes.³ However, the majority remain elusive due to the complexity of the microbial interactions.^{15–17}

Hard corals are hypercalcifiers due to their ability to host unicellular dinoflagellate zooxanthellae, and thus are considered the main architecture of coral reefs.^{18,19} They belong to the order Scleractinia within the class Anthozoa²⁰, which has an ancient fossil lineage of over 230 million years.²¹ Scleractinian corals have different morphological shapes or growth forms regulated by environmental parameters, geographical location, or ocean depth.²² Actinobacteria were first recovered from corals via a culture-dependent method.²³ Further phylogenetic analyses showed variation in abundance and diversity of actinomycetes among different species of coral.²⁴ Actinobacteria live in symbiosis with corals and other marine organisms.^{25–27} A significant number of natural products obtained from the marine environment are produced by corals.^{28,29} Studies have shown that most bioactive compounds derived from corals are produced by symbiotic microbes.³⁰ Actinobacteria associated with hard corals are capable of producing potent specialized molecules with antibiofilm activity³¹, antimicrobial activity^{32–34}, potent biosurfactant-anticancer activity³⁵, antifungal activity^{36,37}, and plant biostimulant activity.³⁸ Furthermore, they are able to degrade petroleum, oil, and pesticides.^{39,40}

Fiji's ocean houses a huge reservoir of marine flora and fauna. It was reported that over 50% of metabolites isolated and tested from Fiji's marine flora and fauna displayed anticancer activity, and more than 25% showed antimicrobial activity.⁴¹ Fiji has one of the most diverse coral reefs in the Pacific region. To date, Fiji has recorded about 1,000 coral reefs with over 340 coral species, of which 219 species are stony corals. These stony corals are classified under the genera *Acropora*, *Pocillopora*, *Porites*, *Montipora*, and others.⁴² Fiji contributed 10% of its coral exports to international trade from 2000 to 2010⁴³ and is the largest exporter within the Pacific.⁴⁴ Fiji's corals are resilient, with higher recovery capacity observed over two to five years. Studies have suggested that coral resilience results from good water quality (not exposed to large-scale pollution), higher coral biodiversity, and rare coral-damaging events that simultaneously affect all corals during the same period.^{42,45} Overall, Fiji's coral reefs are heterogeneous and mainly affected by the cumulative impacts of sediment inundation, pollution, coastal development, and destructive fishing practices.⁴⁶ The published literature on the microbial bioassay of Fijian hard corals remains scarce. Notably, previous studies have identified distinct secondary metabolites from the Fijian marine environment^{47,48}, highlighting the region's potential for discovering therapeutic agents. This underscores the significance of exploring Fijian corals as a distinct and promising niche for natural product discovery, making our work particularly relevant and innovative in this underexplored marine biodiversity hotspot. Therefore, this research aims to address the paucity of literature by reporting the isolation and antimicrobial activity of actinomycetes from Fijian hard corals within the Order Scleractinia. It is hypothesized that Fijian coral-derived actinobacteria exhibit notable antimicrobial activity potentially influenced by marine environmental adaptations. The outcome of this research serves as a baseline for further studies on the diversity and antimicrobial compounds of coral-derived actinomycetes in Fiji.

2. Materials and methods

2.1. Study area

The study area is located in the coastal environment southeast of Viti Levu along the Suva peninsula with geographic coordinates of 18° 10' 592" S and 178° 26' 778" E (Figure 1). The site is approximately 1.5 km from the nearest land mass and about 3 km from the University of the South Pacific lower campus foreshore. The surveyed coral reef lies within the confines of Suva lagoon, an area subject to frequent shipping traffic, substantial nutrient

influxes, pollution runoff through a vast network of conduits, and intensified fishing practices. Despite these circumstances, previous studies have documented the resilience and adaptability of inshore coral reefs in the greater Suva lagoon to periodic sediment smothering and light-limited marine conditions.⁴⁹ Such adaptive capacities are often species-specific, contributing to the persistence and productivity of coral communities in this environment.

2.2. Collection, transportation, and storage

The coral species, *viz.* *Goniastrea* sp., *Porites* sp., *Stylophora* sp., *Acropora* sp., and *Psammocora* sp., as shown in Figure 2, were collected along Suva reef, Fiji, on April 11, 2022, at a depth of < 5 m. These corals were identified with their respective genera. Coral fragments (approximately 10 × 10 cm) were obtained in triplicate via scuba diving, either by hand or with a chisel and punch tool. The fragments were placed in a labeled zip-lock bag within an ice-cooled box and transported to the laboratory immediately (2–3 h) to minimize experimental errors.⁵⁰ To maintain the integrity of the samples, the entire sampling process was completed in a limited timeframe. In the laboratory, the coral samples underwent surface sterilization, during which they were rinsed with filtered sterile seawater to remove contaminants from the surrounding seawater and associated microbes. This was followed by immersion in sterile or filtered seawater for five minutes, with gentle agitation to dislodge surface microorganisms. Prior to storage, the coral fragments were identified and maintained at 4 °C until further analyses. This preservation step is crucial, as it allows for the suspension of metabolism at an optimal rate.⁵¹ To minimize research errors and ensure sample integrity, it is essential to maintain the natural and viable state of the corals or isolated bacteria without compromising their morphological, physiological, or genetic properties. Coral collection was conducted with the necessary authorization from the Fiji Ministry of Fisheries. Moreover, this research was undertaken in accordance with the University of the South Pacific Research Ethics Committee regulations.

2.3. Field parameters

During the sampling expedition, key physicochemical parameters, including temperature, pH, dissolved oxygen, and salinity, were measured *in situ* using a conductivity meter (YSI Pro 30, Xylem Inc., United States of America [USA]). These measurements were conducted to analyze the physical and chemical features of the reef water.

2.4. Isolation, purification, and preservation of actinobacteria

To prevent contamination, all experimental procedures were conducted within a sterile biosafety cabinet in

accordance with rigorous aseptic protocols. All media and materials were sterilized by autoclaving at 121 °C for 20 min. The working environment, including the laboratory bench, was routinely disinfected with 70% ethanol. These aseptic techniques were stringently adhered to throughout experimental procedures to ensure the integrity and reliability of the results.

Actinomycetes from the sampled hard corals were isolated according to the method described by Mahmoud and Kalendar.³² The frozen coral fragments were thawed, rinsed several times with filtered sterile seawater, and then cut into 1 cm³ pieces and thoroughly homogenized using a sterile mortar and pestle. To maximize and facilitate actinomycete isolation, approximately 10 g of coral homogenate was dissolved in 90 mL of purified seawater and subjected to wet heat treatment; the resulting amalgam was heated in a water bath at 60 °C for 15 min prior to serial dilutions. Three prominent types of media were employed to isolate actinomycetes from corals, *viz.* starch casein agar (SCA; soluble starch, 10 g; dipotassium hydrogen phosphate, 2 g; potassium nitrate, 2 g; casein, 0.3 g; magnesium sulfate heptahydrate, 0.05 g; calcium carbonate, 0.02 g; ferrous sulfate heptahydrate, 0.01 g; agar, 15 g; filtered sea water, 1,000 mL; pH 7.0), International Streptomyces Project medium-2 (ISP2; malt extract, 10 g; yeast extract, 4 g; glucose, 4 g; agar, 20 g; filtered sea water, 1,000 mL; pH 7.0), and actinomycete isolation agar (AIA; sodium caseinate, 2 g; L-asparagine, 0.1 g; sodium propionate, 4 g; dipotassium phosphate 0.5 g; magnesium sulphate, 0.1 g; ferrous sulphate 0.001 g; agar, 15 g; filtered sea water, 1,000 mL; pH 7.0). All media were supplemented with nystatin (50 µg mL⁻¹) to inhibit fungi and with nalidixic acid (15 µg mL⁻¹) to inhibit fast-growing Gram-negative bacteria. Furthermore, 10-fold serial dilutions were made from 10⁻¹ to 10⁻⁴, and 0.1 mL of each dilution was plated onto the designated culture media in triplicate. The inoculated plates were aerobically incubated at 28 °C for 3–6 weeks and observed periodically. In addition, morphologically distinct colonies and other characteristics, such as pigmentation, were selected, sub-cultured, and purified on modified ISP2 agar plates (50% seawater and 50% distilled water, pH 7.0). All purified strains were stored at 4 °C and as 25% (v/v) glycerol suspension at –80 °C for long-term preservation.

2.5. Seawater requirement test

All isolates were evaluated for dependence on seawater for growth using the ISP2 medium prepared with distilled water instead of seawater. The inoculated plates were incubated at 28 °C for 4–6 weeks and monitored constantly throughout this period. Based on their growth responses, the strains were categorized as either facultative

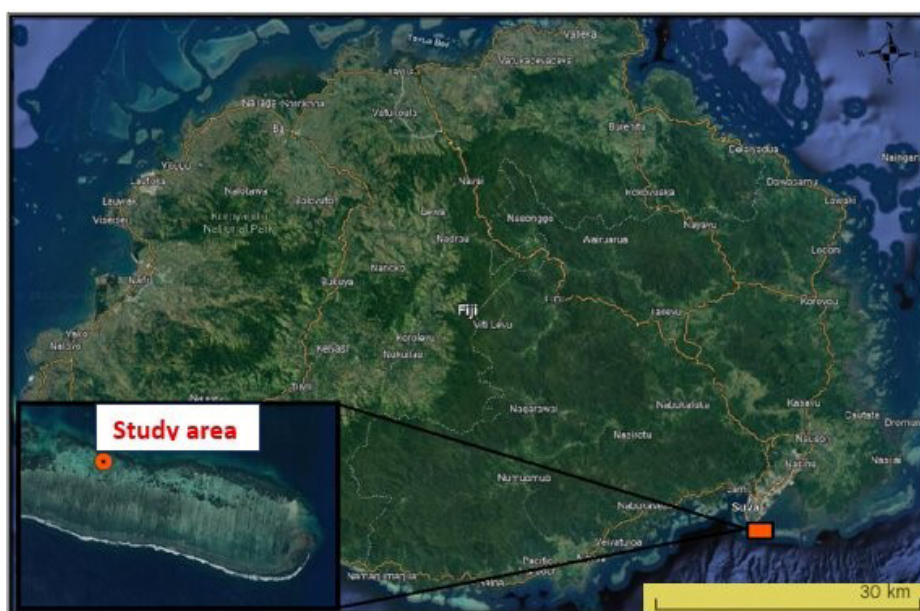


Figure 1. Map of the study area, Suva, Fiji

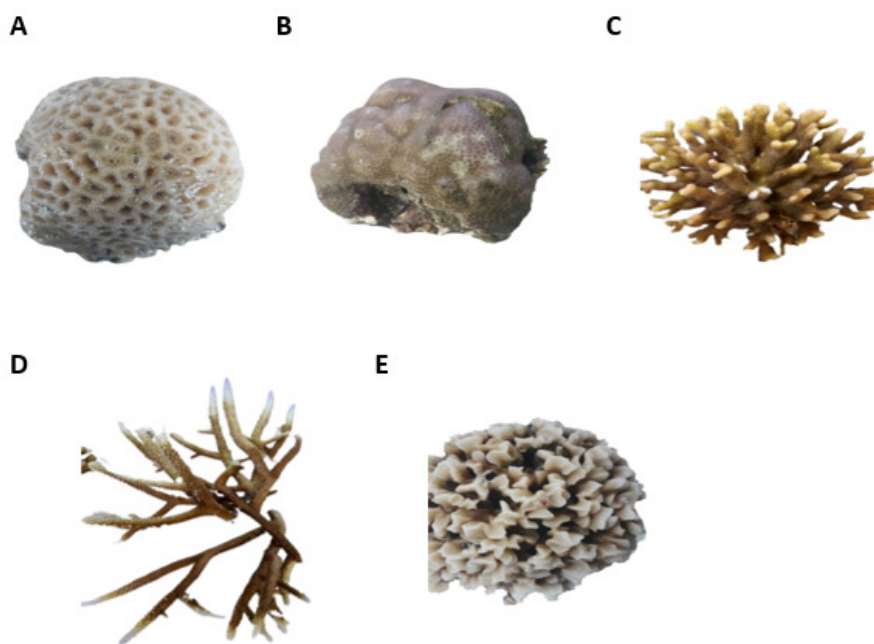


Figure 2. Image of studied coral species. (A) *Goniastrea* sp. (B) *Porites* sp. (C) *Stylophora* sp. (D) *Acropora* sp. (E) and *Psammocora* sp.

salt-tolerant (demonstrating growth on the medium) or obligate salt-tolerant (showing no growth under these conditions).⁵²

2.6. Method for preparation of pathogenic bacteria and fungal strains

Bacterial cultures (10 μ L) were cultured into 10 mL tryptic soy broth (pancreatic digest of casein, 17 g; papaic digest

of soya bean, 3 g; sodium chloride, 5 g; dipotassium phosphate, 2.5 g; glucose, 2.5 g; distilled water, 1,000 mL; pH 7.3) and incubated at 37 °C for 24 h until visible turbidity and density equal to that of 0.5 McFarland. While 100 μ L of fungal culture was cultured in 10 mL potato dextrose broth (potato infusion, 200 g or potato extract, 4 g; dextrose, 20 g; distilled water, 1,000 mL; pH 5.1) and incubated at 30 °C for 24–48 h.

2.7. Bioactivity testing: Test microorganisms

The preliminary recovered actinomycetes and their subsequent crude extracts were tested against a panel of seven human pathogens, including *Shigella sonnei* (ATCC 29930), *Escherichia coli* (ATCC 11775), *Salmonella enterica* (ATCC 43974), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 12600), *Enterococcus faecium* (ATCC 19434), and *Candida albicans* (ATCC 10231). All test pathogens were procured from the University of the South Pacific Institute of Applied Science Laboratory and cultivated prior to antimicrobial assays.

2.8. Primary screening assay for antimicrobial activity

Antimicrobial activity was assessed using the conventional cross-streaking method⁵³, a straightforward preliminary screening undertaken to rapidly evaluate the antimicrobial profile of the investigated strains. Briefly, a single colony of each purified actinobacterial strain was streaked onto nutrient agar plates (peptone, 5 g; beef/yeast extract, 3 g; sodium chloride, 5 g; agar, 15 g; distilled water, 1,000 mL; pH 7.4). The inoculated plates were incubated at 28 °C for 7–14 days until the bacterial growth was visible and fully fledged. Subsequently, different test pathogens were streaked perpendicularly to the actinobacterial streaks and incubated at 37 °C (bacteria) and 30 °C (fungus) for 24 h. Antagonistic strains were identified and selected for secondary screening based on their zone of inhibition. A control plate containing only the test pathogens, with no actinomycete propagules, was included to serve as a baseline for comparison and to confirm the antagonistic effects.

2.9. Small-scale fermentation and extraction of metabolites

The antagonistic strains were subjected to small-scale submerged fermentation following the procedure described by Darabpour *et al.*⁵⁴ Briefly, actinobacterial strains were inoculated in 100 mL of ISP2 broth (malt extract, 10 g; yeast extract, 4 g; glucose, 4 g; filtered sea water [70%] and distilled water [30%], 1,000 mL; pH 7.0) within 100 mL culture flasks, then it was incubated on a rotary shaker incubator at 28 °C for 7–14 days to facilitate optimal growth and secondary metabolite production. Following incubation, the broth cultures were centrifuged at 10,000 rpm for 15 min at 4 °C to separate the cell biomass from the supernatant. For extractions of secondary metabolites, ethyl acetate was employed as the organic solvent, consistent with preferred extraction methods for nonpolar compounds.⁵⁵ Equal volumes (50 mL) of the clarified supernatant and ethyl acetate were thoroughly

mixed for 1 h using a separatory funnel to facilitate solvent-solute interaction, then allowed to separate for 30 min. The organic phase was collected and evaporated under reduced pressure at 40 °C using a rotary evaporator (Rotavapor R-210, Buchi, Switzerland). The resulting residues were either stored at 4 °C for further analysis or redissolved in dimethyl sulfoxide (DMSO) for subsequent bioassays.

2.10. Secondary screening assay for antimicrobial activity

The antimicrobial activity of the crude extract was evaluated using the agar disc diffusion method as described by Darabpour *et al.*⁵⁴ Mueller–Hinton agar (beef extract, 2 g; acid hydrolysate of casein, 17.5 g; starch, 1.5 g; agar, 17 g; pH 7.3) was used in this analysis. All petri plates were autoclaved prior to use. Sterile paper discs with approximately 6 mm diameter were impregnated with 10 µL of 25 mg/mL of crude extract. Subsequently, the paper discs were dried aseptically and placed onto the agar plates inoculated with pathogenic bacteria and fungi. The inoculated agar plates were left undisturbed for 15 min to allow complete absorption of crude extracts, then incubated at 37 °C (bacteria) and 30 °C (fungus) for 18–24 h. The zone of inhibition was measured and recorded after incubation. The antimicrobial activity was compared to known commercial antibiotics, including ampicillin and penicillin (HP053A, Thermo Scientific, USA), as positive controls, and DMSO (10 µL/disc) was used as the negative control.

2.11. Test for cytotoxicity using the brine shrimp lethality assay

This test followed the method described by Sahgal *et al.*⁵⁶ with some modifications to verify the cytotoxic activity of the crude metabolites. Four representative actinomycete strains with highly promising antimicrobial activities were employed for this analysis. Brine shrimp cysts were hatched in a small aquarium with natural filtered seawater (pH 8.0) for approximately 48 h under aeration and continuous light at 28 °C. Active nauplii were attracted to one side of the vessel using a light source for isolation and subsequently collected with a plastic pipette. After 48 h of development, 15 phototrophic nauplii were transferred to each test vial containing 20 µL of crude extract solutions at concentrations of 100, 75, and 50 µg/mL. Incubation was conducted at 28 °C for 24 h. Following incubation, the vials were examined under a light background to accurately quantify the number of live and dead larvae.

The control vials included a positive control with phototrophic nauplii exposed to DMSO, and a negative control with phototrophic nauplii in normal saline. The

mortality proportion was calculated relative to the control at each dose level. Toxicity was expressed as the concentration of crude extracts that kills 50% of brine shrimps (LC_{50}). A graph was plotted to illustrate the relationship between the brine shrimp's mortality rate and the logarithm of the concentration, from which regression equations and the LC_{50} values ($\mu\text{g/mL}$) were derived. The percentage mortality was determined using Equation 1:

$$\text{Percentage mortality} = \frac{\text{Number of dead shrimp}}{\text{Total number of shrimp}} \times 100\% \quad (1)$$

2.12. Statistical analysis

Antimicrobial activity analyses were performed using GraphPad Prism (Version 10.0.2, GraphPad Software, Inc., USA). Cytotoxicity assays on brine shrimp were analyzed using Excel (2019, Microsoft Corporation, USA). Statistical analysis of antimicrobial activity was conducted using two-way analysis of variance followed by Tukey's post-hoc test, with multiple comparisons performed as appropriate at a 95% confidence level. All experiments were performed in biological triplicate and repeated three times, with data presented as the mean $\pm$ standard deviation of these three independent replicates.

3. Results

3.1. Physicochemical analysis of the study area

The physicochemical parameters of the study site, as shown in Table 1, were characterized by a shallow depth of less than 5 m, with water temperatures averaging 31.6 °C, indicative of a warm tropical environment. The salinity was 30.1 ppt, and the pH was slightly alkaline at 7.6. Dissolved oxygen levels were measured at 6.2 mg/L, suggesting sufficient oxygen availability for marine life. The geographic coordinates of the site are 18° 10' 592" S and 178° 26' 778" E, accurately locating the sampling area. These environmental conditions provide context for understanding the microbial diversity and coral health observed in the samples.

Table 1. Summary of physicochemical parameters of the study site

Physicochemical parameters	Value
Location	18° 10' 592" S 178° 26' 778" E
Depth (m)	<5
Temperature (°C)	31.6
Salinity (ppt)	30.1
pH	7.6
Dissolved oxygen (mg/L)	6.2

3.2. Selective isolation and morphological features of coral-derived actinobacteria

The study isolated 41 actinomycete-like bacteria, characterized by a chalky appearance, toughness, an earthy odor, and leathery textures, with voucher numbers ranging from FJC 1 to FJC 41 (Table 2). Among them, six (15%) isolates were isolated from *Goniastrea* sp., 12 (29%) from *Acropora* sp., four (10%) from *Porites* sp., 11 (27%) from *Psammocora* sp., and eight (19%) from *Stylophora* sp. (Figure 3). The majority of isolates were recovered from ISP2 (16 isolates, 39%), followed by AIA (15 isolates, 37%), SCA (10 isolates, 24%) (Figure 3). Thus, the ISP2 media favored the isolation of actinobacteria.

3.3. Primary antimicrobial screening

The study assessed the antimicrobial activities of actinomycete isolates against two pathogenic Gram-positive bacteria (*S. aureus* and *E. faecium*), four Gram-negative bacteria (*S. sonnei*, *S. enterica*, *E. coli*, and *P. aeruginosa*), and a fungus (*C. albicans*). These pathogens are renowned for causing various atrocious diseases and their drug resistance. Primary screening via the cross-streak method (Table 3) revealed that 16 of 41 isolates exhibited promising antimicrobial activity. The taxonomic identification of the 16 bioactive strains is detailed by Siro *et al.*⁵⁷ Notably, certain isolates demonstrated broad-spectrum inhibition across multiple bacterial and fungal pathogens, while others showed limited or selective activity.

3.4. Secondary antimicrobial screening

Based on initial screening, isolates with notable bioactivity were selected for further antimicrobial evaluation using their ethyl acetate extracts against the test pathogens. The secondary metabolic profiles of these selected isolates are summarized in Table 4. Most extracts demonstrated significant antimicrobial activity, with inhibition zones of at least 7 mm (typical standard deviation variations of ± 0.6 or ± 0.2) observed consistently across all isolates' extracts at different concentrations (100, 75, and 50 $\mu\text{g/mL}$). The isolates exhibited varying degrees of potency, from moderate to strong. Overall, the percentage of isolates demonstrating inhibitory effects against each pathogen is as follows (irrespective of the separation of extract concentrations): *S. aureus* (56.25%), *S. sonnei* (25.00%), *S. enterica* (100.00%), *E. coli* (100.00%), *P. aeruginosa* (93.75%), *E. faecium* (81.25%), and *C. albicans* (93.75%). All 16 isolates inhibited more than three pathogens, with 18.75% inhibiting four organisms, another 18.75% inhibiting five, 56.25% inhibiting six, and 6.25% inhibiting all seven tested microorganisms.

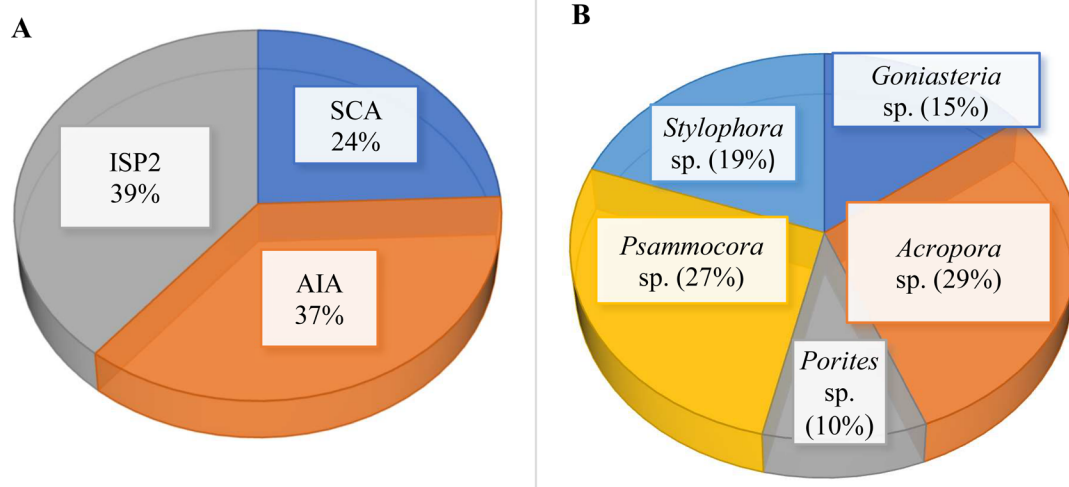


Figure 3. Statistics of total actinobacterial isolates recovered. (A) Different isolation media and (B) Different coral species. Abbreviations: AIA: Actinomycete isolation agar; ISP2: International Streptomyces Project medium-2; SCA: Starch casein agar.

3.5. Brine shrimp lethality analysis

The cytotoxic activity of selected actinomycete isolates was assessed using the brine shrimp lethality assay, with extracts tested at various concentrations. The mortality rates of brine shrimps at different extract concentrations are summarized in Table 5. Four antimicrobial actinomycete strains were evaluated, revealing LC_{50} values ranging from 37.50 to 56.66 $\mu\text{g/mL}$. According to established criteria, these values indicate moderate toxicity. Notably, strain FJC 16 exhibited the lowest LC_{50} (37.50 $\mu\text{g/mL}$), suggesting it has greater cytotoxic potential compared to the other strains. The mortality rate was directly proportional to the extract concentration, demonstrating a dose-dependent response. The positive control, DMSO, showed a higher LC_{50} of 183.38 $\mu\text{g/mL}$, confirming its lower toxicity relative to the test extracts.

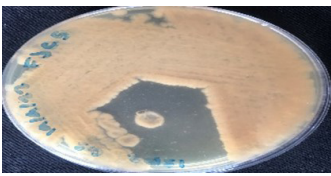
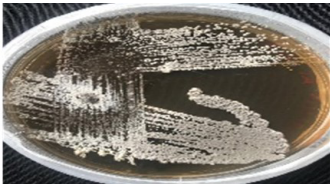
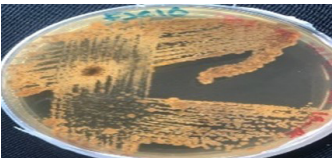
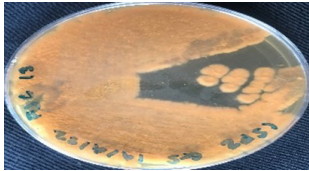
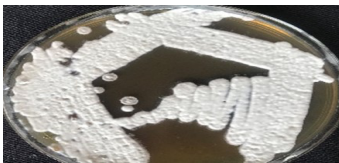
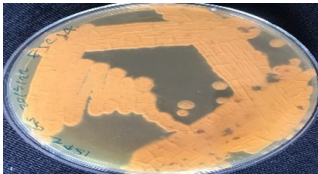
4. Discussion

Interdependence among organisms, ranging from simple to complex relationships, is a fundamental characteristic of all ecosystems, including the marine biosphere.⁵⁸ Symbiotic interactions, in particular, have significantly contributed to the rich biodiversity observed in coral reef environments. Such relationships enhance the collective fitness of the participating species, thereby promoting mutual survival and adaptation.⁵⁹ These symbioses are crucial for maintaining host homeostasis and are increasingly recognized as key drivers of ecosystem health and functionality. Corals form discrete ecological units through symbiotic associations with a diverse and metabolically active microbiome, predominantly bacterial.

The presence of actinomycetes within hard corals has been extensively documented.⁶⁰ These ubiquitous and influential bacteria possess sophisticated biological machinery that underpins various applications, including antibiotic production, bioremediation, and symbiosis facilitation.⁶¹ Our study focused on hard corals as an ecological niche, where the presumed corals were subjected to actinomycete analyses. Coral identification revealed five species of scleractinian coral belonging to the genera *Goniastrea*, *Porites*, *Stylophora*, *Acropora*, and *Psammocora*, and the families Merulinda, Poritidae, Pocillopora, Acroporidae, and Psammocoridae, respectively. These corals exhibited diverse morphological characteristics, ranging from branching to massive forms. They are characterized by rapid growth, wide distribution, and high abundance, all of which contribute significantly to the accretion of calcium carbonate structures, making them vital reef-builders.^{62–64} During sampling, these corals were collected from the back-reef margins where they are exposed to turbid waters and periodic inundation. Increased sedimentation and wastewater plumes are common during the rainy season, affecting water clarity and quality. While turbid nearshore reefs are often perceived as indicators of compromised health, recent studies suggest that such environments may serve as refugia for resilient coral species capable of withstanding future environmental challenges.^{65–67}

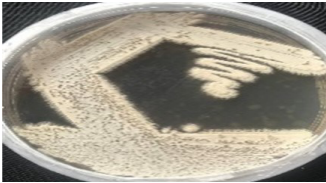
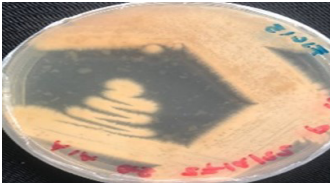
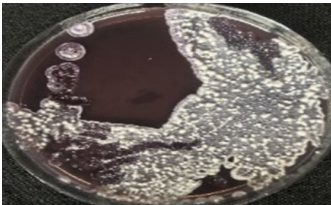
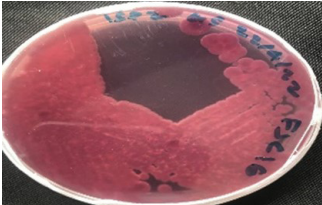
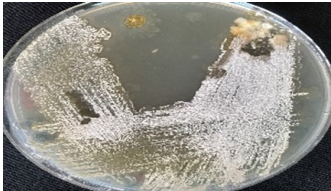
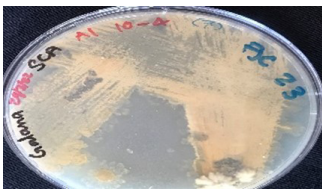
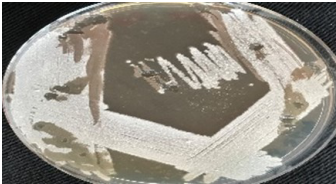
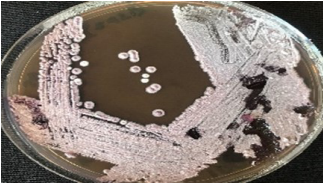
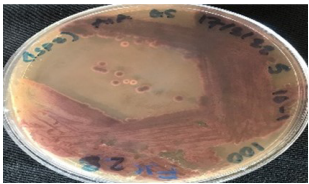
Variations in microbial assemblages exhibit cascading effects, influenced by seasonal and geographical factors.^{68,69} Therefore, analyzing both biotic and abiotic marine factors is highly important as they significantly impact the coral holobiont's structure and function. The

Table 2. Isolation and morphological characteristics of selected actinomycetes demonstrating bioactivity

Original medium	Dilution factor	Coral type ^a	Strain name (FJC)	Character ^b	Aerial mycelium (front view)	Substrate mycelium (bottom view)
AIA	10 ⁻³	M	2	P		
AIA	10 ⁻²	A	5	P		
ISP2	10 ⁻³	M	9	P		
ISP2	10 ⁻¹	A	10	P		
ISP2	10 ⁻³	P	13	P		
AIA	10 ⁻¹	S	14	P		

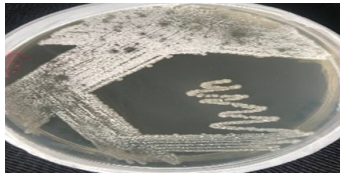
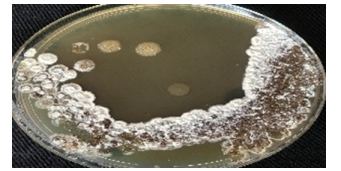
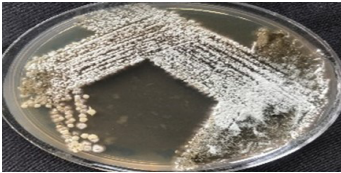
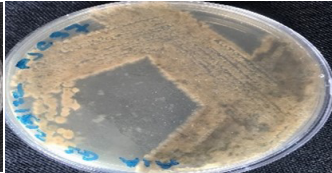
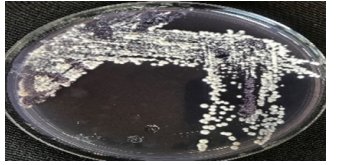
(Cont'd...)

Table 2. (Continued)

Original medium	Dilution factor	Coral type ^a	Strain name (FJC)	Character ^b	Aerial mycelium (front view)	Substrate mycelium (bottom view)
AIA	10 ⁻¹	P	15	P		
SCA	10 ⁻⁴	M	16	L		
ISP2	10 ⁻⁴	A	23	P		
AIA	10 ⁻¹	S	26	P		
ISP2	10 ⁻¹	S	28	P		

(Cont'd...)

Table 2. (Continued)

Original medium	Dilution factor	Coral type ^a	Strain name (FJC)	Character ^b	Aerial mycelium (front view)	Substrate mycelium (bottom view)
ISP2	10 ⁻²	G	30	L		
SCA	10 ⁻¹	A	33	P		
SCA	10 ⁻¹	M	36	L		
ISP2	10 ⁻¹	S	37	P		
AIA	10 ⁻¹	M	41	P		

Notes: ^aCoral type: M: *Psammocora* sp.; S: *Stylophora* sp.; A: *Acropora* sp.; P: *Porites* sp.; G: *Goniastrea* sp. ^bCharacter: P: Powdery; L: Leathery. Abbreviations: AIA: Actinomycete isolation agar; ISP2: International Streptomyces Project medium-2; SCA: Starch casein agar.

Table 3. Antimicrobial assay of isolated actinomycete strains against various pathogens using the cross-streak study

Isolate code (FJC)	Pathogens						
	Gram-negative				Gram-positive		Fungus
	<i>S. sonnei</i>	<i>P. aeruginosa</i>	<i>S. enterica</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. faecium</i>	<i>C. albicans</i>
2	+	+	+	+	+	+	+
5	–	–	+	+	+	+	+
9	–	+	+	+	–	+	+
10	–	+	+	+	+	+	+
13	–	+	+	+	–	+	+
14	–	+	+	+	+	+	+
15	–	+	+	+	–	–	+
16	–	+	+	+	+	+	+
23	+	+	+	+	–	–	+
26	+	+	+	+	–	+	+
28	+	+	+	+	+	+	+
30	–	+	+	+	+	–	–
33	+	–	+	+	+	+	+
36	–	+	+	+	–	–	+
37	–	+	+	+	–	+	+
41	–	+	+	+	+	+	+

Notes: –: No growth inhibition observed; +: Growth of test pathogens was inhibited.

environmental parameters observed at the site (Table 1), including high water temperature (31.6 °C), salinity (30.1 ppt), and slightly alkaline pH (7.6), are characteristic of tropical coral reef ecosystems. The shallow depth (<5 m) likely increases light availability, promoting photosynthetic activity among coral symbionts and associated microbial communities. Dissolved oxygen levels (6.2 mg/L) indicate a healthy aquatic environment capable of supporting diverse biological processes. These physicochemical conditions are vital for coral health, shaping microbial communities and creating varied microhabitats that differ among species and reef areas. Understanding these parameters helps contextualize microbial diversity and coral resilience, highlighting the coral holobiont's complexity as a dynamic, environment-influenced meta-organism.

Differentiation of the actinomycetes was primarily based on colony morphology traits, including their appearance, elevation, margin, texture, and pigmentation. This detailed morphological assessment is essential for evaluating the diversity of culturable actinomycetes present in the samples. One of the key

methods for grouping actinomycetes involves analyzing the chromogenicity of their mycelium.⁷⁰ Consistent with existing literature, the white-colored series of actinobacteria was predominantly observed, indicating their dominance in natural environments.⁷¹ Consistent with our findings, most isolates displayed white aerial mycelium and brown vegetative mycelium upon closer examination of the colonies. A subset of colonies exhibited orange to purple pigmentation, and several strains produced diffusible pigments, which are important characteristics for actinomycete identification. Furthermore, the majority of isolates exhibited a hard, powdery texture, as summarized in Table 2. In this study, particular emphasis was placed on actinomycete strains exhibiting antimicrobial activity, with their potential profiles serving as a primary focus for subsequent analyses and applications.

Culturing microbes in the laboratory presents challenges due to the complex, highly interrelated factors influencing their biology. Microbes perform optimally under ideal conditions⁷², prompting efforts to mimic their natural habitats. In this study, we simulated the coral microhabitat by adjusting media pH to 7.2, incorporating

Table 4. Antimicrobial activity of actinomycetes ethyl acetate crude extracts against pathogenic test organisms using the disc diffusion technique

Antimicrobial activity (zone of inhibition in mm)																												
Strains (FIC)	Gram-negative														Gram-positive										Fungus			
	<i>S. sonnei</i>				<i>P. aeruginosa</i>				<i>S. enterica</i>				<i>E. coli</i>				<i>S. aureus</i>				<i>E. faecium</i>							<i>C. albicans</i>
	100	75	50	C	100	75	50	C	100	75	50	C	100	75	50	C	100	75	50	C	100	75	50	C	100	75	50	C
2	-	-	-	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	-	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	-	8 ± 0.6 ^a	7 ± 0.6 ^a	9 ± 0.6 ^a	8 ± 0.6 ^a	-	7 ± 0.6 ^a	-	7 ± 0.6 ^a	-	7 ± 0.6 ^a	-	7 ± 0.6 ^a	-	7 ± 0.6 ^a	-	-	-
	-	-	-	8 ± 0.2 ^b	7 ± 0.6 ^a	-	-	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	-	8 ± 0.2 ^b	7 ± 0.6 ^a	9 ± 0.6 ^a	7 ± 0.6 ^a	-	7 ± 0.6 ^a	-	7 ± 0.6 ^a	-	7 ± 0.6 ^a	-	7 ± 0.6 ^a	-	7 ± 0.6 ^a	-	-	-
9	-	-	-	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	-	7 ± 0.6 ^a	7 ± 0.6 ^a	-	10 ± 0.6 ^a	8 ± 0.6 ^a	-	8 ± 0.6 ^a	-	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a
	-	-	-	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	10 ± 0.6 ^a	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
10	-	-	-	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	10 ± 0.6 ^a	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
	-	-	-	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	10 ± 0.6 ^a	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
13	-	-	-	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	0.2 ^b	7 ± 0.6 ^a	7 ± 0.6 ^a	-	-	-	10 ± 0.6 ^a	9 ± 0.6 ^a	10 ± 0.6 ^a	9 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
	-	-	-	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	10 ± 0.6 ^a	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
14	-	-	-	8 ± 0.6 ^a	7 ± 0.6 ^a	-	17 ± 0.0 ^d	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	-	10 ± 0.6 ^a	9 ± 0.6 ^a	7 ± 0.6 ^a	38 ± 0.0 ^d	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
	-	-	-	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	0.0 ^d	7 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	25 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
15	-	-	-	9 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	0.0 ^d	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
	-	-	-	9 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	0.0 ^d	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
16	-	-	-	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	0.0 ^d	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
	-	-	-	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	0.0 ^d	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
23	7 ± 0.6 ^a	-	-	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	-	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
	7 ± 0.6 ^a	-	-	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	-	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
26	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
28	8 ± 0.2 ^b	7 ± 0.6 ^a	7 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
	8 ± 0.2 ^b	7 ± 0.6 ^a	7 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
30	-	-	-	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	-	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	
	-	-	-	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	-	8 ± 0.6 ^a	7 ± 0.6 ^a	0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	20 ± 0.0 ^d	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	

(Cont'd...)

Table 4. (Continued)

Strains (FJC)	Antimicrobial activity (zone of inhibition in mm)																	
	Gram-negative						Gram-positive						Fungus					
	<i>P. aeruginosa</i>			<i>S. enterica</i>			<i>E. coli</i>			<i>S. aureus</i>			<i>E. faecium</i>			<i>C. albicans</i>		
	100	75	50	100	75	50	100	75	50	100	75	50	100	75	50	100	75	50
33	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	9 ± 0.0	7 ± 0.0	7 ± 0.0	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	9 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	10 ± 0.6 ^a	9 ± 0.6 ^a	8 ± 0.6 ^a
36	-	-	-	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	-	-	-	-	-	-	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a
37	-	-	-	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	8 ± 0.2 ^b	7 ± 0.6 ^a	7 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	8 ± 0.6 ^a	10 ^d	10 ^d	10 ^d
41	-	-	-	8 ± 0.2 ^b	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	11 ± 0.6 ^a	11 ± 0.6 ^a	11 ± 0.6 ^a	11 ± 0.6 ^a	8 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a	7 ± 0.6 ^a

Notes: “-”: no growth inhibition observed; extract concentrations: 100, 75, and 50 µg/mL; C: control (ampicillin for pathogenic bacteria and penicillin for pathogenic fungus). Values are presented as mean ± standard deviation (n = 3). Different superscript letters indicate statistically significant differences among means, as determined by two-way ANOVA followed by Tukey's post hoc test ($p < 0.001$).

seawater, and using a thick agar layer to support prolonged incubation. Multiple media were employed to enhance actinomycete cultivation, as these bacteria utilize diverse nutrients, including proteins, starch, and amino acids.⁷³ Serial dilutions up to 10⁻⁴ prevented overcrowding. Higher dilutions (10⁻⁶ and above) are commonly used for soil samples⁷⁴, whereas marine samples require lower dilutions due to lower actinomycete density.^{3,75}

Microbial communities in coral mucus, tissue, and skeleton differed both quantitatively and qualitatively, with higher abundances in tissues due to diverse associated niches. Several microbes are habitat-specific⁵⁰, thus we used the entire coral compartments for analysis. One hallmark of actinobacteria cultivation is the routine application of pretreatment techniques.^{13,76} The resistance of most actinobacteria to such treatments is due to their various mode of spore formation, such as endogenous (*Thermoactinomyces* spp.) and exogenous (*Streptomyces* spp.).⁷⁷ This empirical research harnessed the use of supplements, such as nystatin and nalidixic acid, along with wet heat treatment as its pretreatment measures. Consequently, this mandatory step has been shown to reduce the abundance of undesirable microorganisms, thereby increasing the success rate of obtaining diverse actinomycete species. However, the techniques used were purported for a broader range of microorganisms, whereas others were resistant (e.g., *Bacillus* spp.) due to inherent morphological and genetic adaptations.⁷⁸ Although pretreatment techniques reduced the ratio of undesired microbial contamination on isolation plates, the number of actinomycetes may also decrease.⁷⁹ Given the nature of actinomycetes in terms of their sluggish growth, these steps were necessary to compensate for unsolicited resource competition and promote a higher growth rate.

Collectively, three routinely used selective media (ISP2, SCA, and AIA) were strategically modified to recover a total number of 41 actinomycete strains across various hard coral species. These growth media differed in their nutrient compositions, influencing both the number and diversity of isolated actinomycetes, thereby increasing the probability of acquiring profitable cultures. Among the selective media, ISP2 medium yielded the highest number of isolates (39%), followed by AIA (37%) and SCA (24%) (Figure 3). ISP2 medium is considered optimal for actinomycete isolation due to its content of malt extract, yeast extract, and dextrose as carbon sources, which are preferred by most actinobacteria.^{80,81} This suggests the specific nutrient requirements of marine actinomycetes inhabiting hard corals. Although previous studies have indicated that SCA is considered the most effective medium for isolating marine actinobacteria due to its

Table 5. Brine shrimp lethality of putative extracts

Strain	Concentration (µg/mL)	Log concentration	Mortality rate (%)	LC ₅₀ (µg/mL)
16	100	2.00	80.0	37.50
	75	1.88	66.7	
	50	1.70	46.7	
28	100	2.00	100.0	43.75
	75	1.88	80.0	
	50	1.70	53.3	
33	100	2.00	80.0	56.66
	75	1.88	60.0	
	50	1.70	46.7	
41	100	2.00	86.7	47.92
	75	1.88	66.7	
	50	1.70	60.0	
DMSO (control)	100	2.00	40.0	183.38
	75	1.88	33.3	
	50	1.70	33.3	

Abbreviations: DMSO: Dimethyl sulfoxide; LC₅₀: Concentration of crude extracts that kills 50% of brine shrimps.

selective properties⁸², our findings suggest otherwise. This divergence from established norms could be attributed to differences in culturing techniques and other factors. For instance, Studies have demonstrated that non-heat treatment with SCA yielded the highest abundance of actinomycetes, while heat treatment resulted in the lowest recovery.⁸³

Notably, the majority of actinomycete isolates, as depicted in Figure 3, were recovered from *Acropora* sp. (29%), followed by *Psammocora* sp. (27%), *Stylophora* sp. (19%), *Goniastrea* sp. (15%), and *Porites* sp. (10%). During bioactive screening, most strains exhibited attenuated or no antimicrobial activity, likely due to activation or silencing of biosynthetic gene clusters (smBGCs) under test conditions. This suggests that potent strains may produce bioactive compounds when their smBGCs are triggered, whereas inactive strains may harbor silent gene clusters or produce low yields of metabolites.^{84,85} Optimizing and manipulating culture conditions could enhance metabolite production and antimicrobial efficacy at larger scales. Interestingly, mini-isolates (strains FJC 2 and 5) showed inhibitory or no activity in primary screening, whereas in secondary screening, the opposite was observed (Tables 3 and 4). Despite this, the majority of the antimicrobial assays of ethyl acetate extracts were consistent with the activity observed in the primary screening of isolates. This discrepancy may be attributed to differences between filamentous mycelia on solid media and fragmented mycelia in liquid media, which can affect

metabolite production.

Additionally, the loss of certain active compounds during extraction could render several metabolites inactive.^{86,87} The bioactivity results indicated that actinomycete isolates were more potent against pathogenic Gram-positive bacteria (*S. aureus* and *E. faecium*) than Gram-negative bacteria (*E. coli*, *S. enterica*, *P. aeruginosa*, and *S. sonnei*) and fungi (*C. albicans*). This differential susceptibility may be related to morphological differences, particularly in the cell wall components, which influence the permeability of bioactive compounds and toxins.^{86,88} Gram-negative bacteria have an outer membrane and a relatively thin peptidoglycan layer sandwiched between the inner and outer membranes. Furthermore, they have a thick lipopolysaccharide layer on the exterior, forming a complex and resilient barrier. This robust outer membrane impedes penetration of antimicrobial agents, rendering Gram-negative bacteria generally more resistant than Gram-positive bacteria, which lack such a membrane and have a thick peptidoglycan layer.⁸⁹ It is well established that Gram-positive bacteria are highly susceptible to bioactive metabolites produced by actinomycetes.⁸⁸

Inherently, the observed zone of inhibition was indicative of the presence or fingerprint of bioactive compounds associated with the corresponding isolates, which are derived from their antibiotic-producing genes via complex pathways. However, the size of the inhibition zone is largely influenced by the compounds' effectiveness and diffusibility in the medium. In most

cases, a large inhibition zone indicates higher potency and diffusibility.⁹⁰ The compounds analyzed tentatively can be nonpolar or polar in nature, which in this study remains undetermined due to the practicalities of structural elucidation. Of all the isolates characterized, strains FJC 16, 28, 33, and 41 reveal pronounced antimicrobial activity. However, the inhibition zones produced by standard antibiotics (ampicillin and penicillin) were larger than those of the extracts. The inhibition zones of actinomycete isolates against *C. albicans* were measured as 8.0 ± 0.6 mm, 7.0 ± 0.6 mm, 8.0 ± 0.2 mm, 9.0 ± 0.6 mm, 10 ± 0.0 mm, and 10.0 ± 0.6 mm. These values are comparatively lower than those reported in other studies.³⁶ Regarding antibacterial activity, strains 16, 28, and 41 exhibited the highest activity against *S. aureus* and *E. faecium*, with strain 16 demonstrating the most potent inhibition. Specifically, strain 16 showed inhibition zones of 25.0 ± 0.6 mm, 20.0 ± 0.6 mm, and 18.0 ± 0.6 mm against *S. aureus*, and zones of 26 ± 0.2 mm, 23 ± 0.2 mm, and 22 ± 0.2 mm against *E. faecium*. Additionally, statistical analysis revealed a significant difference in the antimicrobial activity among the isolates ($p < 0.001$). The present findings are parallel with other studies regarding the antagonistic activity of marine actinomycetes associated with hard corals against pathogenic bacteria and fungi.^{32,34}

In disk susceptibility testing, several common issues can affect the accuracy of results. For instance, improper disk placement on the media can distort the zone of inhibition, while excessive bacterial inoculum and thick agar gel layers often result in smaller inhibition zones.⁹¹ Additionally, it was observed that in certain petri plates, the pathogenic strains exhibited secondary growth within the zone of inhibition after 24 h of incubation. This phenomenon could be attributed to a hetero-resistant subpopulation, contamination with a non-susceptible strain, or heavy bacterial inoculum.⁹² Furthermore, this study supports existing literature indicating that extended submerged fermentation of actinomycete isolates beyond an optimal period does not necessarily increase the concentration of active agents. Instead, prolonged fermentation may result in decreased yield and potency due to the accumulation of toxins and degradation of specialized compounds.^{93,94} In the current investigation, the seeded broth cultures were incubated for four weeks prior to solvent extraction and antimicrobial assay. It was observed that extending the fermentation period beyond one month led to a significant decline in antimicrobial activity, accompanied by notable physical changes in the fermentation broth, including increased darkness and pigmentation, higher viscosity, and a more pronounced odor.

The seawater requirement assay highlighted the environmental adaptability of marine-derived

actinomycetes. These microbes are genetically versatile, thriving in diverse conditions⁹⁵, with seawater providing essential ions for growth and metabolism.⁹⁶ The analysis demonstrated that none of the strains were obligate seawater-dependent; rather, all exhibited growth on media supplemented with either seawater or distilled water, suggesting their facultative nature. This suggests that they may originate from inland sources as dormant spores, reflecting their resilience. Interestingly, seawater significantly influences their physiological performance and bioactive compound production.⁹⁷ Notably, using a 70% seawater formulation during fermentation yielded notable antimicrobial activity, emphasizing seawater's role in optimizing bioactive synthesis.

Brine shrimp lethality is an indicative bioassay of various therapeutic activities.⁹⁸ Although this preliminary assay does not elucidate the mechanism of action of the extracts, its trajectory has proven convenient for assessing toxicity. Previous studies interpret the brine shrimp lethality test criterion as follows: $LC_{50} < 1$ $\mu\text{g/mL}$ indicates high toxicity; 1–10 $\mu\text{g/mL}$ signifies toxicity; 10–30 $\mu\text{g/mL}$ denotes moderate toxicity; 30–100 $\mu\text{g/mL}$ reflects mild toxicity; and values above 100 $\mu\text{g/mL}$ are considered non-toxic.⁹⁹ The cytotoxic activity of the isolate extracts at different concentrations, as measured by brine shrimp mortality, is outlined in Table 5. The observed LC_{50} values, ranging from 37.50 to 56.66 $\mu\text{g/mL}$, classified the tested actinomycete strains as moderately toxic based on these established criteria. Notably, a lower LC_{50} value indicates higher toxicity, suggesting that strain FJC 16 (37.50 $\mu\text{g/mL}$) is more toxic than others. This suggests that FJC 16 produces or contains higher levels of bioactive compounds that can cause greater damage or stress to the brine shrimp even at lower concentrations. In practical terms, the increased toxicity of FJC 16 makes it a candidate for further investigation, especially in contexts where cytotoxic or antimicrobial activity is desired, as higher toxicity often correlates with stronger biological activity. The positive control, DMSO, exhibited a higher LC_{50} value of 183.38 $\mu\text{g/mL}$, confirming its comparatively lower toxicity and validating the assay's reliability. Moreover, the dose-dependent increase in mortality reinforces the assay's effectiveness in reflecting biological activity.

5. Conclusion

This study demonstrated the significant bioactive potential of actinomycete strains isolated from hard corals, including *Porites* sp., *Stylophora* sp., *Acropora* sp., *Psammocora* sp., and *Goniastrea* sp, affirming the study's hypothesis that Fijian coral-derived actinomycete isolates possess notable bioactive potential. Out of 41 isolates, 16 exhibited varying degrees of activity against common pathogenic strains,

indicating that Fijian hard corals are a promising source of biosynthetic actinomycetes. Notably, isolates FJC 16, 28, 33, and 41 showed broad-spectrum antimicrobial activity against human pathogens. Among these, FJC 16 also exhibited the highest toxicity in brine shrimp assays, suggesting strong bioactivity. All isolates were facultatively salt-tolerant, reflecting adaptation to saline environments.

However, the scope of this study was limited by the relatively small sample size and focus on a specific geographic region, which may not fully represent the microbial diversity of coral-associated actinomycetes. Additionally, bioactive compounds were tested solely using brine shrimp lethality assays without comprehensive chemical characterization, limiting insights into their structures and mechanisms. Moreover, reliance on traditional isolation techniques may have overlooked cryptic or low-abundance species, and environmental factors influencing metabolite production remain underexplored. Future research should employ large-scale, multi-site sampling across diverse coral ecosystems to capture broader microbial diversity. Integrating advanced omics approaches—such as genomics, metabolomics, and transcriptomics—will enable detailed elucidation of biosynthetic pathways and facilitate metabolic engineering. Comprehensive chemical characterization of bioactive metabolites is crucial for structure-activity relationship studies. Additionally, exploring environmental and ecological factors that affect metabolite expression may help optimize bioprospecting strategies.

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Conflict of interest

The authors declared no conflict of interest.

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Consent for publication

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Availability of data

The data generated and analyzed are included in this manuscript.

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