Journal of Basic and Environmental Sciences

ISSN
Online:2356-6388

Print:2536-9202

Research Paper Open Access

# Exploring the Antimicrobial Potential of Streptomyces Isolates from Padina pavonica: A Marine Bioprospecting Study

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## Abstract

This study focuses on the exploration of bioactive compounds from the brown macroalgae Padina pavonica collected from the deep-sea waters of Hurghada, Red Sea, Egypt. The distinct morphological features of Padina pavonica, including slender, foliate, and planar fronds in the young stage, and increased thickness with a concave form in mature fronds, were characterized. Delicate hairs on external surfaces, slime-coated internal surfaces, and sparse calcification with inward-curving edges further define this alga.

Endophytes, specifically twelve streptomyces strains, were isolated from the sterilized surfaces of sponge and algae fragments collected from the Red Sea. Isolation techniques involved immediate tagging and transportation to the laboratory, followed by storage in sterile polyethylene bags at 4°C. Actinomycetes were isolated within 24 hours using a serial dilution method based on distinct colony morphology and color variations. Purification was achieved through repeated streaking on ISP-2 medium subculture plates, leading to the isolation of strains coded as 36X, 37X, 38X, and 39X.

The subsequent fermentation of isolated actinomycetes on rice medium in Erlenmeyer flasks yielded bioactive components. Ethyl acetate was employed for the extraction of crude extracts, which were then subjected to antimicrobial screening using the agar disc diffusion method. Isolate 36X exhibited significant antimicrobial activity, particularly against Staphylococcus aureus and Pseudomonas aeruginosa. Isolate 37X displayed moderate activity against both bacteria and fungi (Candida albicans and Aspergillus niger). Conversely, Isolates 38X and 39X did not exhibit significant antimicrobial activity. These findings highlight the diverse antimicrobial capabilities of the isolated compounds, emphasizing the potential

applications of Isolates 36X and 37X in combating infections caused by specific microorganisms. Further research, including concentration variations and additional assay methods, is essential to elucidate the specific bioactive components and unlock the therapeutic potential of these isolates in medical and related fields.

## 1. Introduction

*Actinomycetes*, a diverse group of Gram-positive bacteria characterized by their filamentous structure, have garnered considerable attention for their remarkable ability to produce a vast array of bioactive secondary metabolites. These microorganisms, encompassing well-known genera like Streptomyces, Nocardia, and Actinomyces, inhabit a broad range of environments, from terrestrial soils and aquatic ecosystems to extreme habitats such as hot springs and cold deserts **(Bérdy, 2005)**.

The unique biochemistry of *actinomycetes* facilitates the synthesis of secondary metabolites, also known as natural products, which play pivotal roles in ecological interactions. The biodiversity of *actinomycetes*, owing to their adaptation to varied ecological niches, results in the evolution of distinct strains with diverse genetic backgrounds. Consequently, this diversity translates into the production of secondary metabolites with distinct chemical structures and biological activities **(Genilloud, 2017)**.

This inherent capability to adapt and produce an unparalleled variety of natural products positions *actinomycetes* as invaluable resources for drug discovery and development. The exploration of bioactive secondary metabolites from *actinomycetes* has significantly contributed to various scientific domains, particularly in the realm of medicine and biotechnology (Barka *et al.*, 2016). Streptomyces, a well-studied genus within this bacterial group, has yielded an impressive array of antibiotics, including streptomycin, erythromycin, and vancomycin, which have played pivotal roles in combating infectious diseases **(Hopwood, 2007)**.

Beyond antibiotics, *actinomycetes*-derived compounds exhibit anticancer, antifungal, antiviral, and immunosuppressive properties, expanding their potential applications in pharmaceuticals **(Subramani and Aalbersberg, 2013)**. The impact of bioactive secondary metabolites from *actinomycetes* on medicine is historic, with several drugs derived from these natural products forming the foundation of

modern pharmaceuticals **(Demain and Sanchez, 2009)**. Streptomycin, as the first effective treatment for tuberculosis, exemplifies the transformative influence of *actinomycetes*-derived compounds **(Ligon and Hill, 2010)**. Even as challenges persist, such as the risk of rediscovery of known compounds and difficulties in cultivating certain strains, ongoing advancements in genomics, metabolomics, and synthetic biology hold promise for overcoming these obstacles **(Berdy, 2012)**.

In this rich landscape of microbial diversity, *actinomycetes* stand out as nature's pharmacists, offering a vast repertoire of bioactive secondary metabolites that continue to shape the trajectory of scientific inquiry and medical advancements. As we delve into the complexities of *actinomycetes* and their natural products, we uncover a realm of potential with far-reaching implications for the future of medicine and biotechnology.

## Material and methods

### Collection of Marine Samples

Healthy *Padina pavonica* algae, a small brown alga, showed distinct characteristics with delicate hairs, slime-coated internal surfaces, and sparse calcification. The algae was collected from Hurghada, coded as (3abuM), photographed, and stored in the fridge for further analysis.

### *Actinomycetes* Isolation from Algae

In the process of isolating *actinomycetes* from algae, a meticulous approach was undertaken employing a serial dilution technique and starch nitrate agar media. The healthy brown macroalgae, specifically *Padina pavonica* algae (coded as 3abuM), served as the primary source for *actinomycetes* isolation.

Firstly, the algae samples were collected from water sediment in the deep-sea waters of Hurghada, Red Sea, Egypt. These samples were carefully tagged and transported to the laboratory in an icebox to maintain their integrity. Subsequently, the marine samples were stored in sterile polyethylene bags at 4°C until they were ready for further processing.

Within 24 hours of collection, the algae samples underwent a systematic serial dilution method. This involved the dilution of the samples in a stepwise fashion to obtain a range of concentrations, providing a varied spectrum of microbial colonies. The diluted samples were then plated onto starch nitrate agar media, a specialized growth medium known for its ability to selectively support the growth of *actinomycetes*.

The selection of *actinomycetes* colonies was based on distinct colony morphology and color variations observed on the starch nitrate agar plates. To ensure the isolation of active strains, a meticulous purification process was carried out. This involved the repeated streaking of selected colonies on International Streptomyces Project-2 (ISP-2) medium subculture plates. By employing this methodological combination of serial dilution and starch nitrate agar media, the aim was to systematically isolate and purify *actinomycetes* strains from the *Padina pavonica* algae samples, laying the groundwork for further investigations into the bioactive potential of these microbial isolates.

### *Actinomycetes* Fermentation and Extraction

Isolated *actinomycetes* obtained from previous steps were subjected to small-scale fermentation. This process involved culturing the *actinomycetes* on rice medium in Erlenmeyer flasks. The inoculated flasks, containing 250 mL of solid rice media, were incubated for a duration of 15 days.

Following the fermentation period, ethyl acetate was employed as the extraction solvent for the cultures. The ethyl acetate phase was utilized to extract bioactive components from the fermented *actinomycetes* cultures. The extraction process was thorough, ensuring that the ethyl acetate phase was completely evaporated until dry. The resulting dried extracts were then stored for future research and analysis.

### Screening of Antimicrobial Activity Using Agar Disc Diffusion Method

The extracted compounds obtained from the small-scale fermentation were reconstituted in a suitable solvent to form stock solutions. These stock solutions were then standardized and serially diluted to achieve a range of concentrations for the agar disc diffusion assay. The microbial strains, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger*, were cultured and prepared for susceptibility testing. The microbial cultures were adjusted to a specific turbidity to ensure uniform inoculum concentration.

Petri dishes containing solid agar media specific to the respective microbial strains were used. filter paper discs were impregnated with varying concentrations of the reconstituted extracts and placed into the wells.

The Petri dishes were incubated under conditions suitable for the growth of the respective microorganisms. The

incubation period allowed for the diffusion of the extracted compounds from the discs into the agar medium, establishing concentration gradients.

After incubation, the plates were examined for the presence of zones of inhibition around the discs. The diameter of these zones was measured using a calibrated ruler. Larger zones indicated stronger antimicrobial activity, signifying the ability of the extracted compounds to inhibit the growth of the microbial strains.

Results were recorded and analyzed to determine the effectiveness of the extracted compounds against each microbial strain. The agar disc diffusion method provided a qualitative assessment of antimicrobial activity by visually inspecting the zones of inhibition. The data guided the identification of promising extracts with potential antimicrobial properties against *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, and Aspergillus niger*.

## Results and discussion

### Sample collection

The healthy brown macroalgae *Padina pavonica*, coded as 3abuM was randomly collected from water sediment in the deep-sea waters of Hurghada, Red Sea, Egypt. *Padina pavonica*, is a distinctive small brown alga with a maximum diameter of up to 10 cm (4 in). The morphological characteristics include slender, foliate, and planar fronds in the young stage, while mature fronds exhibit increased thickness and a concave form. External surfaces feature delicate hairs, and the internal surfaces are slime-coated. Sparse calcification is present on both sides, with inward-curving edges.

### Endophytes Isolation Technique

Twelve streptomyces strains were isolated from the sterilized surfaces of the sponge and algae fragment samples were collected from the Red Sea, Egypt. The samples were immediately tagged and transported to the laboratory in an icebox. Upon arrival, the marine samples were stored in sterile polyethylene bags at 4°C until further processing. Within 24 hours of collection, the samples underwent processing using a serial dilution method to isolate actinomycetes. The selection of isolates was based on distinct colony morphology and color variations. Purification was achieved through repeated streaking on International Streptomyces Project-2 (ISP-2) medium subculture plates to obtain active strains. Isolation steps led to obtaining the isolate coded 36x, 37x, 38x and 39x, shown in Table (1).

**Table (1).** Isolated actinomycetes from macroalgae Padina pavonica

| Host | Isolate code |
|---|---|
| ***Padina pavonica*** | **36X** |
| | **37X** |
| | **38X** |
| | **39X** |

**Fermentation and Extraction**

To obtain bioactive components, isolated *actinomycetes* strains were cultured on rice medium for small-scale fermentation. Spore suspensions of *actinomycetes* were inoculated into 250 mL Erlenmeyer flasks containing 25 g of solid rice media and incubated for 15 days. Ethyl acetate was employed to extract the cultures. The *actinomycetes* crude extracts were stored.

**Screening of Antimicrobial Activity**

The antimicrobial screening results, conducted through the agar disc diffusion method, are detailed in the **Table (2)**. Isolate 36X demonstrated noteworthy antimicrobial activity, revealing a zone of inhibition measuring 17 mm against *Staphylococcus aureus* and 15 mm against *Pseudomonas aeruginosa*. However, no activity was observed against *Candida albicans* and *Aspergillus niger* for this isolate. Isolate 37X exhibited moderate antimicrobial activity with a 9 mm zone against *Staphylococcus aureus* and 7 mm against *Pseudomonas aeruginosa*. Interestingly, this isolate displayed antimicrobial efficacy against *Candida albicans* and *Aspergillus niger*, both with a zone of inhibition measuring 9 mm. Conversely, Isolates 38X and 39X did not demonstrate observable antimicrobial activity against any of the tested microorganisms.

In terms of potential applications, Isolate 36X emerges as a promising candidate for further investigation, particularly for combating infections caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Isolate 37X showcases versatility, displaying moderate activity against both bacteria and fungi, suggesting a broad-spectrum antimicrobial potential. On the other hand, Isolates 38X and 39X did not exhibit significant antimicrobial activity under the conditions tested as illustrated in **Figure (1)**. These findings emphasize the diverse antimicrobial capabilities of the isolated compounds, highlighting the need for in-depth studies to elucidate the specific bioactive components responsible for the observed effects. Further research, including variations in concentration and additional assay methods, may provide a comprehensive understanding of the therapeutic potential of these isolates,

guiding their future applications in medicine and related fields.

**Table (2).** Antimicrobial activity of crude extracts from *streptomyces's* isolates

| Isolate code | *Staphylococcus aureus* | *Pseudomonas Aeruginosa* | *Candida albicans* | *A. niger* |
|---|---|---|---|---|
| 36X | 17 mm | 15 mm | NA | NA |
| 37X | 9 mm | 7 mm | 9 mm | 9 mm |
| 38X | NA | NA | NA | NA |
| 39X | NA | NA | NA | NA |

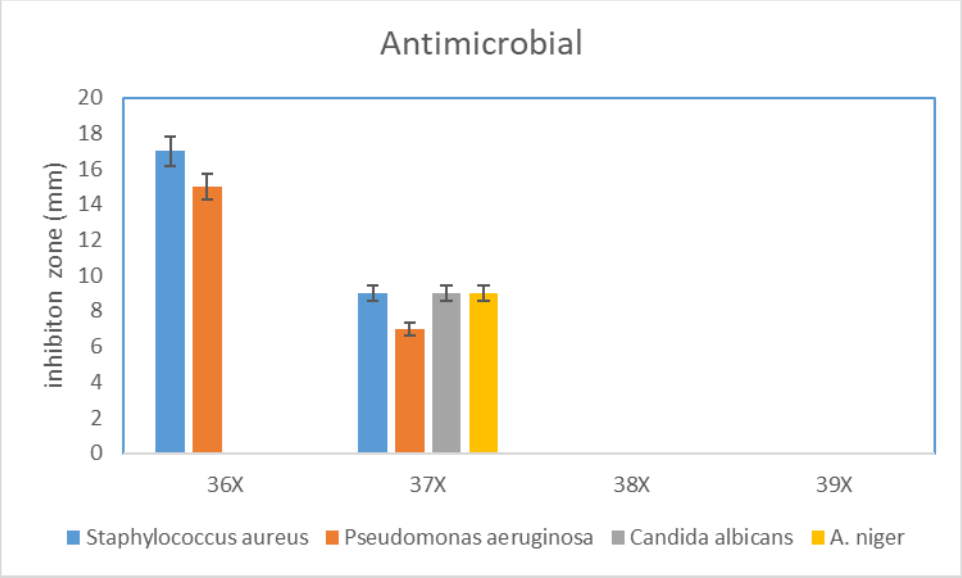


**Figure (1).** Antimicrobial screening of isolated *actinomycetes* extracts

## Conclusion

In conclusion, the investigation into bioactive compounds derived from the brown macroalgae Padina pavonica and the isolation of streptomyces strains from associated marine samples have yielded valuable insights. The morphological characterization of Padina pavonica provided a foundation for understanding its unique features, contributing to the broader understanding of marine biodiversity.

The isolation of streptomyces strains, specifically Isolates 36X, 37X, 38X, and 39X, showcased diverse antimicrobial activities. Notably, Isolate 36X demonstrated significant efficacy against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Isolate 37X exhibited versatility, displaying moderate activity against both bacteria and fungi, including *Candida albicans* and *Aspergillus niger*. However, Isolates 38X and 39X did not exhibit notable antimicrobial activity under the tested conditions. These findings underscore the importance of comprehensively exploring the bioactive potential of microbial isolates, recognizing that each strain may possess distinct properties.

The outcomes of this study open avenues for further research, including the identification of specific bioactive components responsible for the observed effects. The need for additional investigations, such as variations in concentration and the utilization of alternative assay methods, is highlighted. This comprehensive understanding will be crucial in unlocking the full therapeutic potential of these isolates in the fields of medicine and related applications.

Overall, the study contributes valuable data to the realm of marine microbial bioprospecting, emphasizing the potential of streptomyces isolates from *Padina pavonica* in the development of novel antimicrobial agents. These findings not only enrich our understanding of marine ecosystems but also pave the way for future applications in drug discovery and related biomedical research.

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