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Marine Endophytic Fungi and the Production of Bioactive Compounds with Antimicrobial Properties

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Abstract: Marine sponges, renowned for their exceptional biodiversity, serve as natural reservoirs for a wide array of microorganisms, particularly endophytic fungi, which have demonstrated a propensity for synthesizing bioactive compounds with remarkable potential in the realms of antimicrobial and anticancer therapeutics. In the current investigation, marine sponges were meticulously procured from two distinct locales in the coastal waters of Hurghada, Egypt, and their internal tissues were harnessed as a source of endophytic fungi.

Isolation techniques were employed to extract and cultivate these endophytic fungi from the sponge tissues. Subsequently, the isolated fungal strains underwent a rigorous screening process to assess their proficiency in generating bioactive compounds possessing potent antimicrobial properties. The evaluation encompassed the efficacy of these compounds against a panel of four pathogenic microorganisms: *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger*.

This study represents a critical exploration of the endophytic fungal communities dwelling within marine sponges, shedding light on their potential to yield novel bioactive molecules capable of combatting a diverse spectrum of pathogenic microorganisms. The findings offer promising insights into the untapped reservoir of natural products from marine environments, further underscoring their significance in the ongoing pursuit of innovative antimicrobial agents.

Keywords: Marine sponges fungi, isolation, Extraction, antimicrobial activity.

1. Introduction

Marine ecosystems, which cover more than two-thirds of our planet, are renowned for their remarkable biodiversity and unexplored potential for natural product discovery **(Pawlik, 2011)**. Within these aquatic environments, a group of microorganisms known as marine endophytic fungi has gained increasing attention due to their ability to inhabit the internal tissues of marine plants and animals. These fungi have developed unique strategies to adapt to the harsh and dynamic conditions of the marine environment, making them a promising source of novel bioactive compounds with diverse biological activities **(Bugni and Ireland, 2004)**.

Endophytic fungi, originally described in terrestrial plants, are now recognized as a significant and relatively untapped resource in marine biology. These fungi establish symbiotic relationships with their host organisms, where they reside within the plant or animal tissues without causing apparent harm. This coexistence allows them to synthesize secondary metabolites with various ecological functions, such as defense against pathogens and predators **(Schulz and Boyle, 2005)**.

One of the most intriguing aspects of marine endophytic fungi is their potential to produce bioactive compounds with antimicrobial properties. Antimicrobial agents are crucial in the fight against infectious diseases caused by bacteria, fungi, and other pathogens. With the growing threat of antibiotic resistance, the search for novel and effective antimicrobial agents has become an urgent priority in the field of pharmaceutical research **(Rice, 2008)**.

Marine endophytic fungi have demonstrated the ability to produce a wide array of bioactive molecules, including antibiotics, antifungal agents, and compounds with antiviral activity. These bioactive compounds have shown promise in combating a range of pathogenic microorganisms. They are not only valuable as potential pharmaceutical agents but also as a resource for developing new drugs to address emerging infectious diseases and multidrug-resistant pathogens **(Saleem *et al*., 2007)**.

In this context, exploring the diversity of marine endophytic fungi and their bioactive metabolites has become a focus of interest for researchers in the fields of microbiology, marine biology, and pharmacology. By delving into the mechanisms of bioactive compound production and their antimicrobial potential, we aim to uncover new solutions to the global challenge of microbial infections and contribute to the development of novel pharmaceutical agents **(Bhadury *et al*., 2006)**.

2.Materials and Methods

2.1.Sample Collection of Marine Sediment from Hurghada:

Marine sediment samples were systematically collected from the coastal region of Hurghada, Egypt, which is renowned for its rich diversity of marine microorganisms. A total of three independent sampling events were carried out at a depth ranging from 1 to 3 meters, employing a Van Veen grab sampler. To ensure the representativeness of the samples, sediment-rich substrates were gathered from multiple locations. Subsequently, these samples were carefully transferred into sterile containers to maintain the integrity of their natural microbial composition. They were then transported to the laboratory under conditions that preserved them in a cool and dark environment.

2.2.Isolation of Fungi Using Serial Dilution Method

Upon arrival at the laboratory, the collected marine sediment samples underwent processing for the isolation of fungi, adhering to stringent aseptic techniques. Each sediment sample was meticulously weighed and aseptically transferred to individual sterile containers. To facilitate the isolation process, a series of serial dilutions were prepared. Known quantities of sediment were suspended in sterile saline solution (0.85% NaCl), resulting in dilutions extending to 10^(-6) to encompass a broad range of fungal concentrations. Subsequently, 100 µL aliquots from the appropriate dilutions were systematically spread-plated onto selective agar media conducive to fungal isolation. To impede the growth of bacteria, Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (50 µg/mL) was employed. Incubation of the plates was carried out at 25°C over a duration of 7-10 days, during which fungal colony development was routinely observed. Distinct fungal colonies were subcultured onto fresh SDA plates, ensuring the acquisition of pure cultures. These pure cultures were then

preserved at 4°C to facilitate subsequent analysis.

2.3. Fermentation on Potato Dextrose Broth Media

Pure fungal cultures derived from the marine sediment samples were further subjected to assessment to gauge their potential for generating secondary metabolites and bioactive compounds. Each fungal isolate was meticulously inoculated onto Potato Dextrose Broth (PDB). The PDA plates containing the isolates were incubated at 25°C for a duration spanning 14-21 days to facilitate fungal growth and the potential production of bioactive compounds. Monitoring during this incubation period encompassed the assessment of fungal growth, sporulation, and the development of any distinctive pigments or metabolites.

2.4. Extraction of Bioactive Metabolites

Following the incubation period, a clear demarcation was made between the fungal mycelia and broth by means of vacuum filtration through sterile filter paper, yielding a filtrate enriched with fungal metabolites within the PDB. Ethyl acetate was selected as the organic solvent for the extraction process due to its efficiency in partitioning a wide range of fungal metabolites. The filtrate was subsequently transferred to a separatory funnel, and an equal volume of ethyl acetate was introduced. Vigorous agitation was employed to ensure thorough mixing, and the resulting mixture was allowed to naturally separate into two distinct layers. The ethyl acetate layer, harboring the extracted crude fungal metabolites, was meticulously separated from the aqueous layer and transferred to a clean flask. To eliminate any residual traces of water, the ethyl acetate extract was subjected to drying using anhydrous sodium sulfate, followed by filtration. Subsequently, the solvent was removed from the ethyl acetate extract under reduced pressure using a rotary evaporator, resulting in a concentrated crude extract of the fungal metabolites. To ensure the complete removal of any remaining solvent, the extract was subjected to further drying within a desiccator for approximately 24 hours. The obtained crude extract was then securely stored at -20°C in airtight containers until it was ready for further analysis. This meticulous extraction process served to concentrate the bioactive compounds produced by the fungi in liquid culture, thus providing a valuable resource for subsequent chemical characterization and bioactivity screening.

2.5. Antimicrobial Results

To assess the antibacterial activity of both the crude and pure compounds, a panel of Gram-negative and Gram-positive bacteria were employed as test organisms. The experimental setup was conducted in 96-well flat polystyrene plates. Each well contained 10 µl of silver nanoparticles (at a final concentration of 500 µg/ml), followed by the addition of 80 µl of Lysogeny Broth (LB broth) and 10 µl of bacterial culture suspension in the logarithmic phase. The plates were subsequently incubated overnight at 37°C. The presence of a positive antibacterial effect was observed as clearance in the wells, while compounds that did not influence bacterial growth were characterized by the opaqueness of the growth media within the wells. The control group consisted of pathogens without any treatment. Following the incubation period, absorbance measurements were recorded at OD600 using a Spectrostar Nano Microplate Reader (BMG LABTECH GmbH, Allmendgrün, Germany).

2.6. Biofilm Inhibitory Activity

To assess the biofilm inhibitory activity of the silver nanoparticles, a microtiter plate assay (MTP) was executed in 96-well flat-bottom polystyrene microtiter plates, employing four clinical microbes (*Pseudomonas aeruginosa*, *Staphylococcus aureus, Escherichia coli*, and *Bacillus subtilis*). Each well was initially filled with 180 µL of LB broth, followed by inoculation with 10 µL of overnight pathogenic bacterial culture. Subsequently, 10 µL of the desired samples were introduced, alongside a blank control. The plates were then incubated at 37°C for 24 hours. After the incubation period, the contents within the wells were carefully removed and subjected to washing with 200 µL of phosphate buffer saline (PBS) at pH 7.2, to eliminate free-floating bacteria. Sessile bacteria adherence was fixed using a 2% sodium acetate solution and stained with 0.1% crystal violet. Excess stain was eliminated through deionized water washes and allowed to dry. Subsequently, dried plates were rinsed with 95% ethanol, and optical density (OD) readings were obtained at 595 nm using a microtiter plate reader (BMG LABTECH GmbH, Allmendgrün, Germany) for further analysis **(Hamed *et al.*, 2020)**.

3. Results and Discussion:

3.1 Isolation of *Penicillium rubens* from Marine Sediment in Hurghada:

In our study, we identified the most potent fungus, which exhibited exceptional bioactive properties. This fungus was subjected to a comprehensive characterization, encompassing both morphological and genetic aspects. Morphologically, it displayed distinctive features consistent with the genus Penicillium, such as the characteristic conidiophores and conidia formation. Moreover, through genetic analysis, specifically DNA sequencing, we confirmed its identity as Penicillium rubens, a species known for its remarkable metabolic potential. This dual approach of morphological and genetic characterization not only elucidated its taxonomic classification but also provided valuable insights into its potential for the production of bioactive compounds, positioning it as a promising candidate for further investigation and potential biotechnological applications.

3.2. Extraction of Crude Extract from *Penicillium rubens* Cultivated in Potato Dextrose Broth

In order to comprehensively evaluate the bioactive potential of Penicillium rubens, an extensive cultivation process was undertaken. The fungus was carefully cultured in a specialized growth medium, namely Potato Dextrose Broth (PDB), which is renowned for its richness in nutrients, rendering it highly conducive to fungal growth and the production of secondary metabolites. This culture proved to be a crucial step in harnessing the fungal metabolites for subsequent analysis. To efficiently extract and concentrate the bioactive compounds generated by the fungus, ethyl acetate was judiciously selected as the extraction solvent. This choice was deliberate, as ethyl acetate has demonstrated a remarkable ability to selectively partition a diverse spectrum of fungal metabolites, allowing for the preservation and concentration of these valuable compounds for further investigation.

3.3. Antibacterial Activity Assessment:

The investigation involved subjecting the crude extract derived from Penicillium rubens to a battery of antibacterial assays, meticulously assessing its effectiveness against a panel of clinically relevant bacterial strains, namely *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. The primary objective was to discern the extent to which this extract could inhibit the growth of these pathogenic bacteria. In order to accomplish this, precise measurements of inhibition ratios were taken and diligently recorded.

The results yielded intriguing insights into the antibacterial potential of the crude extract. It was evident that this fungal extract possessed

remarkable antibacterial activity, albeit with varying degrees of potency against the different bacterial strains. Specifically, it exhibited robust inhibition against *Staphylococcus aureus* and *Bacillus subtilis*, both of which are notorious pathogens in clinical settings. This observation underscores the extract's substantial promise as a source of potent antibacterial compounds with the potential for therapeutic applications. While the extract displayed significant antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli*, it was noteworthy that the inhibitory effects against these Gram-negative bacteria were relatively milder in comparison to the Gram-positive strains. Nevertheless, the demonstrated inhibitory activity against a range of bacterial strains highlights the potential versatility of the crude extract as a source of bioactive molecules. A comprehensive summary of these results, including inhibition ratios for each bacterial strain, is presented in Table 1, and a visual representation of these findings can be observed in Figure 1. These results collectively underscore the extract's selective and efficacious antibacterial properties, offering promise for further exploration and potential drug development.

Table 1. antimicrobial activity against test microbes

Test microbes	Inhibition ratio (%)			
	P. aeruginosa	*E. coli*	*B. subtilis*	*S. aureus*
Crude	35.23	50.46	10.22	63.32

100:70: potent 70:50: active 50:30: moderately active 30: 10 : weak

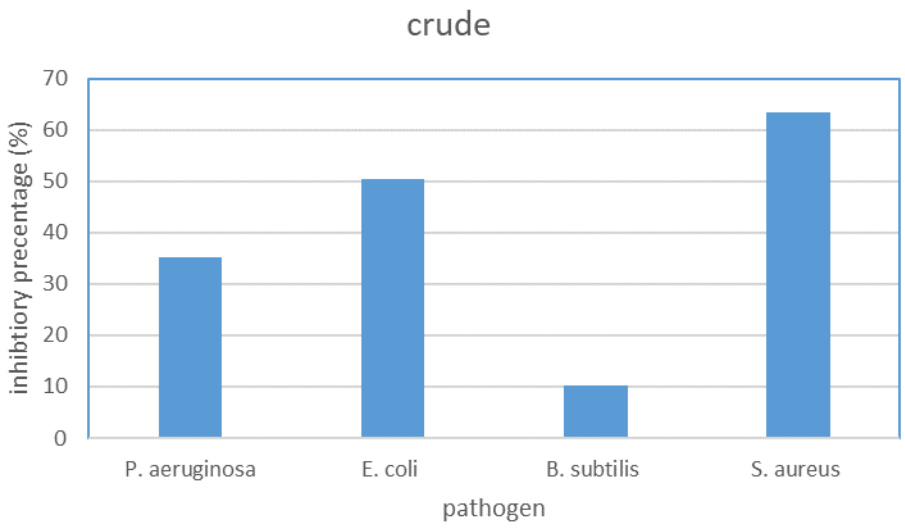

Figure 1. Antimicrobial activity

References:

1. Pawlik, J. R. (2011). The chemical ecology of sponges on Caribbean reefs: Natural products shape natural systems. BioScience, 61(11), 888-898.

2. Bugni, T. S., & Ireland, C. M. (2004). Marine-derived fungi: a chemically and biologically diverse group of microorganisms. Natural Product Reports, 21(1), 143-163.

3. Schulz, B., & Boyle, C. (2005). The endophytic continuum. Mycological Research, 109(6), 661-686.

4. Rice, L. B. (2008). Federal funding for the study of antimicrobial resistance in nosocomial pathogens: No ESKAPE. Journal of Infectious Diseases, 197(8), 1079-1081.

5. Saleem, M., Ali, M. S., Hussain, S., Jabbar, A., Ashraf, M., & Lee, Y. S. (2007). Marine natural products of fungal origin. Natural Product Reports, 24(5), 1142-1152.

6. Bhadury, P., Mohammad, B. T., & Wright, P. C. (2006). The current status of natural products from marine fungi and their potential as anti-infective agents. Journal of Industrial Microbiology & Biotechnology, 33(5), 325-337.

7. Hamed, A.A.; Kabary, H.; Khedr, M.; Emam, A.N. Antibiofilm, antimicrobial and cytotoxic activity of extracellular green-synthesized silver nanoparticles by two marine-derived actinomycete. RSC Adv. 2020, 10, 10361–10367.