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Antimicrobial activity of marine derived fungi isolated from soft corals

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Abstract

Marine environment is immensely complex and home to a wide variety of life forms. The water column of the ocean contains around 106 microorganisms per milliliter. Physiologically active chemicals found in marine bacteria and fungi are unusual and numerous, which makes them highly interesting. Over the past few decades, there has been a gradual increase in the amount of secondary metabolites from marine bacteria and fungi that have been reported. Drug-resistant bacterial infections, especially those involving several drug-resistant strains, have become more common due to overuse of antibiotics, and an increasing proportion of drug-unresponsive infectious disease agents is a serious threat to the world's healthcare system. From Red Sea water samples in the form of water, soil, swabs and small pieces from the studied three soft corals were collected. The fungal isolates were isolated from these four different types of samples. Antagonistic activity test was applied between fungal isolates against the two pathogenic bacteria (*Pseudomonas aeruginosa* ATCC-9027, *Staphylococcus aureus* ATCC-6538*)* and the diameter of inhibition zone determined. The crude extract of four chosen fungal isolates has been obtained by cultivation of the fungi on PDB broth medium for 15 days. The crude extract was tested for antibacterial activity against pathogenic bacteria**.** our study revealed various marine fungi from different soft corals species, the foremost report of different *Aspergillus sp.* The result demonstrate that these marine fungi serve as potent producers of antibacterial secondary metabolites. It also evident that they can serve as potential antibacterial mediators against pathogenic bacteria.

Key words: Soft corals, Marine fungi, Secondary metabolites, Antibacterial activity.

1. Introduction

Nature has been a major source of bioactive compounds, which can be directly or indirectly used as inspiration for synthesis or semi-synthesis by being isolated from natural matrices. Natural compounds are still extremely significant in aquaculture, agriculture, and medicine today [10]. Despite encouraging developments in this field, the potential of natural chemicals is currently unexplored [2].

Fungi that are derived from the sea are a fascinating source of bioactive substances, some of which have antibacterial properties. Given the increasing prevalence of antibiotic resistance, these take on particular significance [3].

The overuse of antibiotics has contributed to the rise in drug-resistant bacterial infections, especially the multi drug-resistant strains, and an increasing proportion of drug-unresponsive infectious disease agents have become serious threats to the world health system. Worldwide, *S. aureus* infections that are resistant to antibiotics pose a serious risk to public health. The most frequent Gram-positive organisms that cause infections include drug-resistant *Streptococcus pneumonia*, methicillin-resistant *S.aureus,* mono-to multiple drug-resistant *M. tuberculosis*, and Vancomycin-resistant Enterococci (VRE)species. Staphylococci have been linked to numerous human illnesses, such as implanted device infection, osteomyelitis, endocarditis, periodontitis, and chronic wound infection. [13]. Numerous factors, such as altered cell membrane permeability, an increase in drug efflux pumps, antibiotic enzymatic modification or inactivation, target site modification, alternative metabolic pathways, and biofilm formation, can lead to antimicrobial resistance in microorganisms. With a vast array of biological activity, the marine environment is a great source of varied natural goods. Marine natural products are a highly promising resource in the quest for novel antimicrobial drugs, as antimicrobial substances among them have potential biological actions against a variety of drug-resistant bacteria and fungi [1].

The main goal of this study was to isolate endophytic fungi living within marine soft corals, followed by thorough investigation to theses fungi and their ability to produce bioactive compounds with antimicrobial activity against pathogenic bacteria.

2. Materials and methods

2.1. Studying area

Red Sea was formed about 24 million years ago when the African and Arabian plates separated. Ever then, Red Sea has been distinguished by unique features like its geographic position, comparatively high temperature, and very young geological age. The Red Sea and Mediterranean Sea are joined in the north by the Suez Canal. Bab El-Mandeb Strait connects the Red Sea to the Indian Ocean and the Gulf of Aden in the south. There is a huge diversity of life in all spheres of existence in the Red Sea. (5) .Three soft corals

samples were collected from the Neama bay in Sharm El-Sheikh located in Red Sea.

2.2. Sample collection

The Egyptian research team made collection trips to the Sharm El-Sheikh area. Samples in the form of water, soil, swabs and small pieces from each of the studied three marine organisms. These marine organisms (soft corals) were collected in duplicate, one for cultivation and the other for taxonomic identification by Dr. Tarek (lecturer of marine sciences in the faculty of sciences).Three soft coral species are *Sinularia sp, Sarchophyton.sp , S. compressa*

2.3. Isolation of the marine fungal isolates

The collected samples were in the form of four different types of samples, which were collected as:

2.3.1. Water samples

Water surrounding the chosen soft corals was collected as it may contain the microorganisms which are symbiosis with this corals. The water samples were collected in different volumes of isolation of different concentrations of fungi, these volumes were 200 ml, 100 ml, 50 ml, 25 ml. These water samples were filtered through 0.45 µm membranes (metrical membrane filters 100/PK, presterilized autoclacvable pack. PALL life sciences, Michigan 48103-9019. USA). Using specific filtration instrument in order to exclude bacteria and fungi were retained fungi in up position, on the cultivated media plate.

2.3.2. Sediment samples

Soil under the soft corals was collected for isolation of the fungi living in it . The soil sample handled by two methods, the first by filtration of 10 ml of the diluted sediment as described in the water samples and then plated the same and the second method was the direct plating of 1 ml from the sediment sample on the cultivated media plate by using a sterile glass rod or curved Pasteur pipette.

2.3.3. Tissue samples

To take the tissue samples this method was started by surface sterilization of the collected marine samples; the sponges were initially cleaned with tap water before being surface-sterilized with a series of treatments that included 70% ethanol, sterile distilled water, 2% sodium hypochlorite, and repeated distilled water rinses. The surface-sterilized soft corals was then cut into small pieces and put on a sabouraud dextrose agar (SDA) medium enriched with Lactic acid solution 10% and chloramphenicol to inhibit bacterial growth. (8-9)

2.3.4. Swab samples.

Aseptically swabbing the surface of the sponge was taken under water via sterile closed swab. This swab sample was plated directed by streaking on the surface of the cultivated plate.

2.4. Purification and preservation of marine fungal isolates.

After isolation, the growing colonies were sub-cultured on SDA medium to assure purity, and then the fungal isolates were preserved at 4°C in agar slants of SDA medium(62 gram per liter). The growth was observed for 3 to 7 days and the slants at − 4◦C and glycerol stock at − 20 ◦C were preserved for further study.

2.5. Morphological identification

The SDA medium was used to cultivate the fungal isolates, and morphological analyses were performed once the isolates' mycelium had taken over the entire plate. Both microscopic and macroscopic characterization are included in characteristics. Macroscopic examinations were used to classify the spores' features, morphology, textures, coloration, and form.

2.6. Antagonistic activity test: Primary evaluation through agar plug diffusion technique.

The agar Plug diffusion standard procedure was initially used to screen the pure cultures obtained from coral reefs for antibacterial characteristics. The pure fungal cultures were cultivated for seven days and used to make the agar plugs. The pure fungal cultures were cultivated for seven days and used to make the agar plugs. Next, using a sterile cotton swab, the pure culture plugs were placed onto the Muller Hinton agar medium that had already been seeded by the corresponding bacterial pathogens. The plates were incubated for 16–18 hours at 37 ◦C to promote bacterial growth, and then they were chilled throughout the entire night at 4 ◦C to aid in the diffusion of metabolites. Based on the zone of inhibition seen against the test pathogens, the putative fungal isolates were chosen for secondary screening. (Sadrati, N. et al. 2020-Dhevi V, et al. 2024).

2.7. Preparation of crude extracts from fungal colony culture.

Four dominant fungal species were selected randomly , cultured on PDA medium and culture discs (5mm-dia) from the actively growing margin of colony culture were inoculated on potato dextrose (PD) broth (pH 5.6) and incubated at 28 °C , 150 rpm for 15 days. The culture broth was filtered to separate the mycelial growth . The culture filtrate (CF) was filtered using a three-layered Whatman no.1 filter paper and the filtrate was added with an equal volume (500:500 ml) of ethyl acetate (Himedia, Mumbai), mixed well (10 min.) and kept undisturbed (5 min.) to obtain two clear immiscible layers. The upper layer of ethyl acetate containing the compounds was extracted with ethyl acetate in a separating funnel, The ethyl acetate extract (EtOAc extract) was evaporated

using rotary evaporator (IKA Digital Rotary Evaporator, Sigma-Aldrich,
USA) to obtain concentrated extract. (14- 9- 15).

2.8 Antibacterial assay

Using the agar-well diffusion technique, the antibacterial activity of the metabolites of four chosen fungus species was investigated (Singh, D. et al. 2013) against two bacterial species: Gram negative bacteria *Pseudomonas aeruginosa* ATCC-9027, Gram positive bacteria *Staphylococcus aureus* ATCC-6538).The respective wells were loaded with 100 µL of the sample (1 mg of extract in 1 ml dimethyl sulfoxide (DMSO). The reference DMSO were used as negative controls. The experiments were carried out in triplicate. The plates were incubated at 37 °C overnight and the zone of inhibition (mm) was recorded.

3. Results

3.1. Fungal isolation

In the present study, 22 fungal isolates were isolation from the three soft corals , by using four different methods as mentioned previously . Table 1. represent the data sheet for the fungal isolates.

Table (1) Twenty two fungal isolates from three of different soft corals.

name of coral reef	Type of samples	No of isolated fungi	Co. of isolates
(1)*Sarcophyton. sp*	a)Water	a)2	a) Sar1, Sar2
	b)Sed	b)1	b)Sar3
	c)Tissue	c)2	c)Sar4,Sar5
	d)Swab	d)non	d)non
(2) *Sinularia compressa*	a)Wat	a)4	a) com6,com7,com8,com9
	b)Sed	b)5	b)com10,com11,com12,com13,com14
	c)Tissue	c)3	c)com15,com16,com17
	d)Swab	d)non	d)non
(3) *Sinularia . sp*	a)Wat	a)2	a)sin18,sin19
	b)Sed	b)2	b)sin20,sin21
	c)Tissue	c)1	c)sin22
	d)Swab	d)non	d)non

3.2 Morphological identification:

Four fungal isolates were chosen and identified by macroscopic and microscopic features as *Aspergillus niger , Aspergillus terreus , Aspergillus flavus , Aspergillus fumigatus* as showed in figure 1

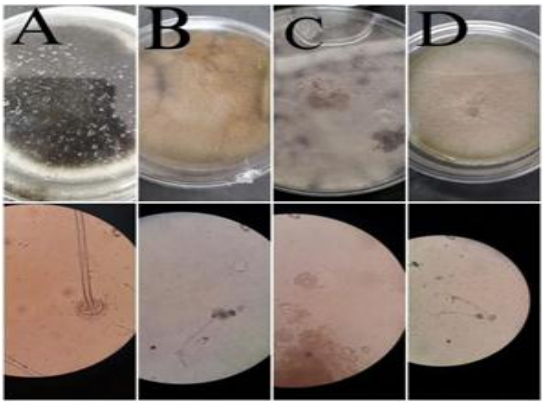

Fig. (1) Characterization and identification of marine fungi *Aspergillus niger* (com9), B) *Aspergillus terreus* (com7), C) *Aspergillus flavus* (com11), D) *Aspergillus fumigatus* (com 13). (Mayar Medhat shots)

3.3 Inhibitory effect of endophytic marine fungi on pathogenic bacteria.

The results obtained revealed that the tested isolates showed varying range of antagonistic effect against pathogenic bacteria. As showed in table (2)

Table (2) The antagonistic effect of chosen fungal isolates agaist bacteria.

Bact / f.code	*Staph.aureus*	*Ps.aeruginosa*
Com 9	2.1±0.1	1.4±0.1
Com 7	2.167±0.15	0

Com 11	0	0
Com 13	1.67±0.05	1.267±0.057

3.4. The antibacterial effect of fungal extract against Pseudomonas aeruginosa and Staphylococcus aureus.

The crude extract of studied fungi showed an inhibitory effect against *Ps. Aeruginosa*(a) and *St.aureus* (b).

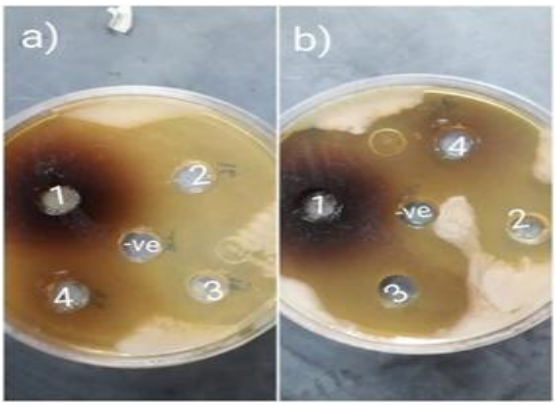

Fig. (2) Diameter of inhibition zone of (a) and (b) bacteria treated with fungal extracts (1) *As.terreus* (2) *As niger* (3) *As . flavus* (4) *As fumigatus* , and DIMSO as negative control.

Table (3) Demonstrate the weight of crude extract , the effect of fungal crude extract against the two tested bacterial strains.

F.code	**wt .(gm)**	***staph. aureus***	***ps.aeruginosa***
Com 7	0.0688	3.967±0.15	4.9±0.1
Com11	0.0778	3.733±0.05	2.567± 0.11
Com 13	0.0685	3.633±0.15	3.33± 0.32

The obtained result showed that the *Aspergillus terreus* (com7) have a highly effect against *Staphylococcus aureus* ATCC-6538 (fig4) and *Pseudomonas aeruginosa* ATCC-9027(fig3) , The *Aspergillus flavus (* com 11) crude extract have antibacterial effect against the two bacteria even though it showed negative antagonistic activity.

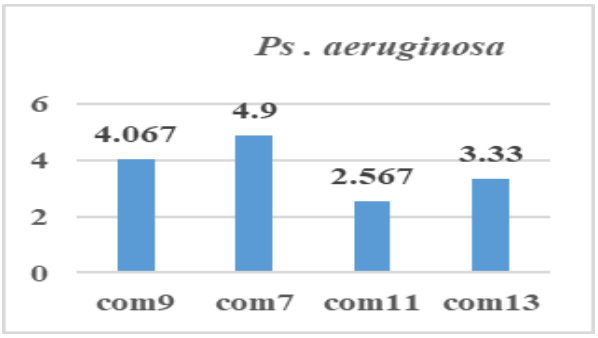

Fig. (3) Antibacterial inhibitory effect of isolated fungal crude extract against *Pseudomonas aeruginosa* ATCC-9027

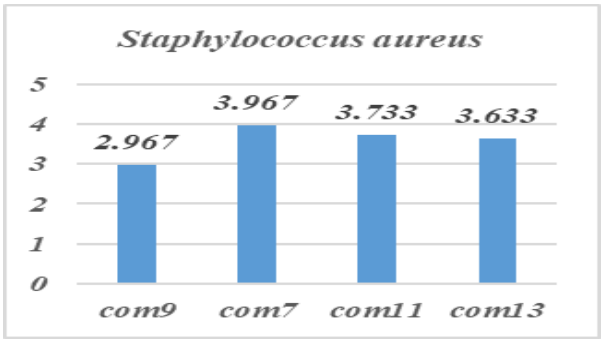

Figure 4. Antibacterial inhibitory effect of isolated fungal crude extract against *Staphylococcus aureus* ATCC-6538.

4. Conclusion

Results show that these marine fungi are strong makers of secondary metabolites with antibacterial activity. It is also clear that they may behave as possible mediators of antibacterial activity against pathogenic bacteria.

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