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Exploring the Metabolic Symphony of Fungal-Bacterial Coculturing and Individual Cultures as antimicrobial

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Abstract:

This research delves into the dynamic alterations in antimicrobial activity resulting from the co-cultivation of a fungus and an actinomycete, with a keen focus on discerning the nuanced changes influenced by their interplay. The initiation of the study involved a meticulous isolation process targeting both fungi and actinomycetes from marine samples, specifically Sea water from Hurghada, Ras Sedr sediments, and Ain Sokhna sediment. The isolated microorganisms, coded as the fungus FGH2 and the actinomycete AGH5, were subjected to individual and co-culture conditions. A detailed exploration into the metabolites produced by these microorganisms ensued, involving the preparation of crude extracts from both individual cultures and their co-culture scenarios. Notably, the findings strongly suggest the existence of synergistic effects within the co-culture, influencing the biosynthesis of unique compounds. The varied biological activities associated with these compounds further underscore the potential of the co-cultivation strategy in eliciting novel and enhanced antimicrobial properties.This study, anchored in a thorough investigation of individual and co-cultured microbial metabolites, provides a nuanced understanding of the intricate dynamics shaping antimicrobial activities. The insights gained pave the way for future research exploring the applications of these synergistic interactions in drug discovery and biotechnological advancements.

Keywords: *Fungi*, *Bacteria* **,** Coculturing, secondary metabolites.

Introduction

Microbial interactions within natural environments form the intricate tapestry of life, influencing ecological processes and serving as fundamental contributors to the rich diversity of natural products. The dynamic interplay among microorganisms' shapes ecosystems, modulates nutrient cycling, and significantly impacts the biosynthesis of secondary metabolites, many of which possess valuable bioactivities. Among the microbial kingdom, fungi and actinomycetes stand out as prolific producers of secondary metabolites, showcasing an extensive repertoire of compounds with diverse biological functions [(1)].

Fungi, a kingdom of eukaryotic microorganisms, have long been recognized for their ability to produce an array of secondary metabolites with varying chemical structures and biological activities. From antibiotics to mycotoxins, fungi have played a crucial role in the discovery and development of pharmaceuticals, while simultaneously influencing agricultural practices. Actinomycetes, filamentous bacteria with high G+C content in their DNA, are another group of microorganisms renowned for their secondary metabolite production. With a myriad of compounds, including antibiotics like streptomycin and tetracycline, actinomycetes have been pivotal in revolutionizing medicine and disease control [(2)].

The chemical diversity inherent in the secondary metabolites of fungi and actinomycetes has positioned these microorganisms as promising sources for novel bioactive compounds. Of particular interest are their antimicrobial and anticancer properties, which hold immense potential for addressing global health challenges, combating antibiotic resistance, and developing innovative cancer therapeutics. However, the full spectrum of their metabolic capabilities and the intricate interactions that occur when these microorganisms coexist remain areas of active exploration[(3)].

This study focuses on elucidating the metabolic capabilities of a carefully selected fungus and actinomycete, both individually and in co-culture, employing Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The utilization of GC-MS allows for the identification and characterization of specific compounds, providing a nuanced understanding of the chemical profiles generated by these microorganisms. By delving into the metabolic intricacies of individual cultures and the collaborative dynamics of co-cultures, we aim to unravel the potential

synergies that contribute to the production of unique bioactive molecules[4].

Understanding the chemical diversity of these microorganisms has profound implications for diverse industries, including pharmaceuticals, agriculture, and biotechnology. The exploration of metabolic interactions in co-culture settings opens avenues for the discovery of novel compounds that may not be apparent in individual cultures. The insights gained from this study may pave the way for the development of innovative drugs, biopesticides, and other biotechnological applications, thereby contributing to the sustainable utilization of microbial resources in various fields. As we embark on this journey into the metabolic intricacies of fungi and actinomycetes, we anticipate uncovering hidden potentials and expanding our knowledge of the microbial world's vast and untapped reservoir of bioactive compounds [5].

Material and Methods:

Sample Collection:

Marine samples were collected from three different locations in Egypt (Hurghada, Ain Sokhna and Ras sedr) samples were collected from diverse environments, representing a range of ecological niches. Samples were aseptically transported to the laboratory for subsequent isolation of microorganisms.

Isolation of Fungi and Bacteria: dextrose broth and selective media starch nitrate agar for actinomycetes. standard microbiological techniques. Pure cultures were obtained through successive streaking and isolation of individual colonies. [6].

Small-scale fermentation and extraction of fungal and bacterial crude extracts

Cultures of all isolates were grown on ISP2 agar plates, and after suitable incubation periods, crude extracts were prepared. The extraction involved the use of appropriate solvents to obtain secondary metabolites from the microbial cultures. [8].

Biological evaluation of activity

To measure the antibacterial activity of fungal and actinomycetes crude extract against Gram-negative bacteria Gram-positive bacteria were used as test organisms [9]. The test was performed in 96-well flat polystyrene plates. 10µl of test extracts (final concentration of 250 µg/ml) were added to 80 µl of lysogeny broth (LB broth) followed by addition of 10 µl of bacterial culture suspension (log phase), then the plates were incubated overnight at 37°C. After incubation, the positive antibacterial effect of the tested compound was observed as clearance in the wells, while for compounds that didn’t affect the bacteria, the growth media appeared opaque in wells, The control is the

pathogen without any treatment. The absorbance was measured after about 20 h at OD600 in a Spectrostar Nano Microplate Reader (BMG LABTECH GmbH, Allmendgrun, Germany).

Coculturing Study

Fungal and bacterial strains were individually cultured under controlled laboratory conditions.

Cocultures were established, and growth patterns were monitored throughout the experiment [(10)].

Results and discussion

Sample collection.

Three marine samples have been collected from different locations in Egypt including Hurghada, Ras sedr, Ain Sokhna. Each sample is denoted by a specific code (HG, RS, AS) corresponding to its origin, indicating sea water in Hurghada, sediments in Ras Sedr, and sediments in Ain Sokhna. The data invites exploration into the unique characteristics of each marine environment. The sea water sample from Hurghada (HG) prompts analysis of coastal water dynamics, including salinity and microbial presence. The sediment samples from Ras Sedr (RS) and Ain Sokhna (AS) provide an opportunity to delve into substrate composition, potential pollutants, and ecological conditions specific to each location **Table 1.**

Table (1): Number of Samples and Its Location

No. of sample	Code	Location
1	HG	Sea water Hurgada
2	RS	Ras Sedr sediments
3	AS	Ain Sokhna sediment

Isolation of fungi and bacteria

The results of the isolation of Streptomyces and fungi from three distinct marine samples, namely Sea water Hurgada, Ras Sedr sediments, and Ain Sokhna sediment, are presented in Tables 2 and 3.

Table 2 illustrates the distribution and percentage of Streptomyces spp. isolated from the specified marine localities. The highest number of isolates was obtained from Ras Sedr sediments, accounting for 15 isolates, followed by Sea water Hurgada with 11 isolates and Ain Sokhna sediment with 10 isolates. The total number of Streptomyces isolates across all three locations amounts to 36.

Table 3, on the other hand, outlines the distribution and percentage of fungal isolates from the same marine localities. The highest number of fungal isolates was recorded in Ras Sedr sediments, totaling

12 isolates, followed by Sea water Hurgada with 9 isolates and Ain Sokhna sediment with 7 isolates. The cumulative number of fungal isolates across all three locations sums up to 28.

Table 2. *Streptomyces* spp. isolated from different marine localities.

No. of sample	Location	Isolates account
1	Sea water Hurgada	11
2	Ras Sedr sediments	15
3	Ain Sokhna sediment	10
Total isolate		36

Fermentation, extraction, and selection of the most potent_bacteria and fungi based on their activity.

All isolates (fungi and one bacteria) have been cultivated on ISP2 media and extraction was carried out to the crude extract. Based on the biological activity of the isolated fungi and bacteria, only two isolates one fungi and one bacterium have been selected based on their pronounced antimicrobial. the fungus coded as FGH2 and actinomycetes coded as AGH5.

Table 3. Fungal isolates isolated from different marine localities.

No. of sample	Location	Isolates account
1	Sea water Hurgada	9
2	Ras Sedr sediments	12
3	Ain Sokhna sediment	7
Total isolate		28

Coculturing study between fungi and bacteria

Coculturing fungi and actinomycetes stand at the forefront of microbial research, offering a promising avenue for exploring the synergistic interactions between these two diverse groups of microorganisms. Both fungi and actinomycetes are renowned for their prolific production of secondary metabolites, including antimicrobial compounds and bioactive molecules. When cocultured, these microorganisms can potentially exhibit synergistic effects in the production of secondary metabolites

that may surpass the capabilities of individual cultures. This collaborative behavior opens avenues for the discovery of novel compounds with enhanced bioactivity and potential applications in various fields [5].

The enhanced inhibitory activity observed in the coculture of fungi and actinomycetes, as illustrated in the presented Figure 1, underscores the potential benefits of this combined cultivation strategy. The synergy observed against various bacterial strains suggests that the cooperative action of these microorganisms may lead to the production of antimicrobial compounds with a broader spectrum or increased potency compared to individual extracts. This finding has significant implications for the development of novel antimicrobial agents, addressing the ongoing challenges of antibiotic resistance. A key advantage of coculturing fungi and actinomycetes lies in the complementary nature of their metabolic pathways. Fungi excel in breaking down complex organic matter, while actinomycetes are recognized for their diverse secondary metabolite production. In coculture, these complementary metabolic capabilities may result in a more efficient utilization of resources and the synthesis of unique compounds. This collaborative metabolic activity has the potential to yield valuable products for biotechnological applications, ranging from enzymes to bioactive compounds with pharmaceutical or agricultural relevance [11].

Studying the coculturing of fungi and actinomycetes not only holds promise for biotechnological advancements but also provides valuable insights into ecological interactions within microbial communities. Understanding how these microorganisms collaborate or compete in natural environments contributes to our knowledge of microbial ecology. Such insights can inform conservation efforts and strategies to manipulate microbial communities for desired outcomes, addressing environmental challenges and promoting sustainability. While the coculturing of fungi and actinomycetes presents exciting opportunities, it is not without its challenges. Optimizing growth conditions, managing potential competition for resources, and deciphering the molecular mechanisms underlying their interactions are crucial aspects that warrant further exploration. Future research efforts should focus on unraveling these complexities, ensuring the reproducibility and scalability of coculture systems for practical applications. As the field advances, the coculturing of fungi and actinomycetes holds the potential to unlock new opportunities for biotechnological innovations and deepen

our understanding of microbial dynamics in natural ecosystems.

Biological evaluation of actinomycetes, fungus, and coculturing

The presented **Figure 1** illustrates the inhibitory activity of Crude extracts of actinomycetes, Fungi, and a Coculture of Actinomycetes and Fungi, with inhibition percentages against four different bacterial strains: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Pseudomonas aeruginosa*.

In the Actinomycetes category, the inhibitory percentages vary across the bacterial strains, ranging from 60.39% for S. aureus to 84.78% for *P. aeruginosa*. This suggests that the Actinomycetes extract has a notable inhibitory effect, with a higher potency against *E. coli* and *P. aeruginosa* compared to *S. aureus* and *B. subtilis*.

The Fungi category also exhibits variability in inhibitory activity, with percentages ranging from 60.05% for *B. subtilis* to 82.3% for *E. coli*. Interestingly, the Fungi extract appears to be more effective against *E. coli* and *P. aeruginosa* compared to *S. aureus* and *B. subtilis*.

The most striking results are observed in the Coculture category, where a combination of Actinomycetes and Fungi extracts demonstrates enhanced inhibitory activity. The inhibitory percentages surpass those of individual extracts, indicating a potential synergistic effect between Actinomycetes and Fungi. Notably, the coculture exhibits the highest inhibition against *B. subtilis*, suggesting a particularly effective combination against this bacterial strain.

In conclusion, the table highlights the differential inhibitory activities of Crude extracts against various bacterial strains, emphasizing the potential benefits of combining Actinomycetes and Fungi for increased antimicrobial efficacy. Further research and exploration of the underlying mechanisms could provide valuable insights into developing novel and potent antimicrobial agents.

The provided Figure 2 illustrates the inhibitory potential of Crude extracts against two fungal strains, *Candida albicans* and *Aspergillus niger*. Three distinct categories are considered: Actinomycetes, Fungi, and a Coculture of Actinomycetes and Fungi, each displaying inhibition percentages for the specified fungal strains.

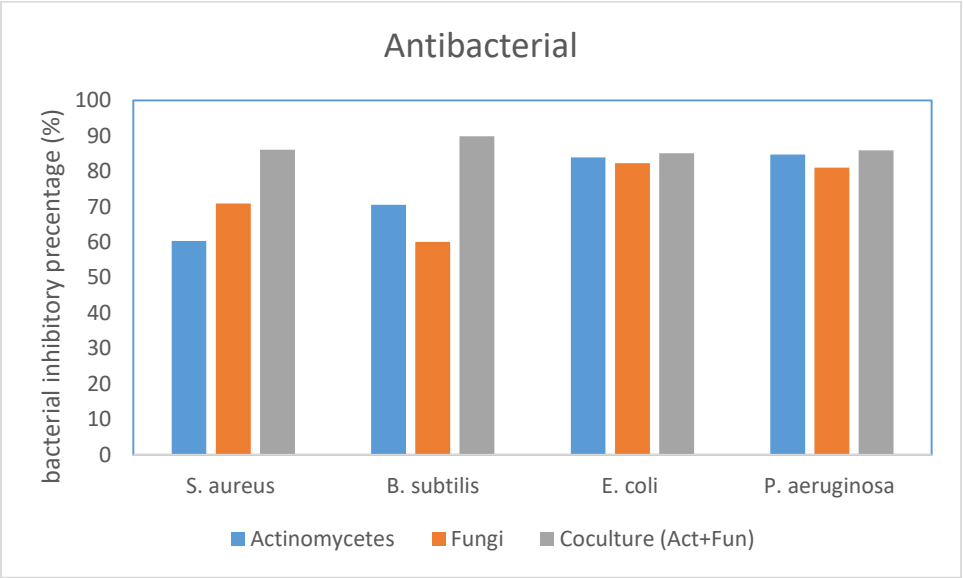

Fig.1. Antibacterial activity of actinomycetes, fungus, and coculturing

In the Actinomycetes category, the inhibitory activity is observed to be moderate, with C. albicans showing a percentage of 65.93% and *A. niger* registering 58.99%. The data implies that the Actinomycetes extract exhibits some antifungal properties, though *A. niger* appears to be less susceptible compared to *C. albicans.*

Conversely, the Fungi category reveals a varied inhibitory impact, with *A. niger* demonstrating a higher susceptibility (74.81%) compared to *C. albicans* (65.9%). This suggests that the Fungi extract is more potent against *A. niger* than *C. albicans*.

The most intriguing results emerge from the Coculture category, where the combined action of Actinomycetes and Fungi extracts results in a substantial increase in inhibitory activity. Against *C. albicans*, the percentage rises to 81.05%, indicating a synergistic effect. A similar pattern is observed for *A. niger*, with the Coculture registering an impressive 84.55% inhibition. This highlights the potential synergy between Actinomycetes and Fungi, suggesting that their combined action produces a more potent antifungal effect compared to individual extracts.

The data underscores the differential antifungal activities of Crude extracts against *C. albicans* and *A. niger*. Furthermore, the synergistic effect observed in the Coculture category emphasizes the potential for combining Actinomycetes and Fungi extracts to enhance overall antifungal efficacy. Further exploration and understanding of the underlying mechanisms could pave the way for developing novel antifungal agents.

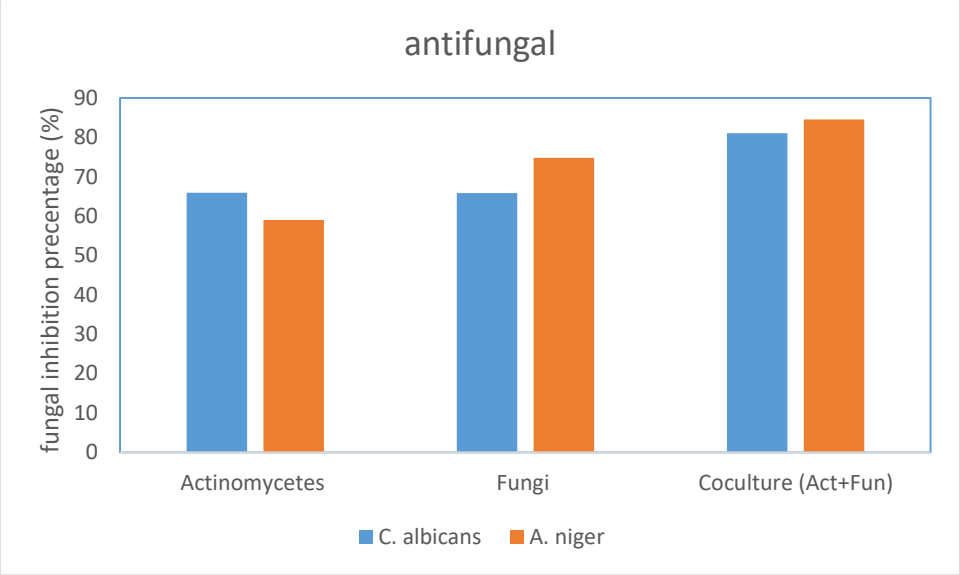

Fig.2. Antifungal activity of actinomycetes, fungus, and coculturing

Conclusion

In conclusion, our study decided that the fungal and actinomycetal metabolomes, both individually and in co-culture, has provided a rich tapestry of insights into the metabolic capabilities of these microorganisms. This cooperative interaction suggests a potential enhancement of biosynthetic pathways, leading to the production of metabolites with varied and potentially novel biological activities. The findings from this study not only contribute to our understanding of the metabolic diversity within these microorganisms but also highlight the significance of microbial co-culturing as a strategy for expanding the repertoire of bioactive compounds. Moving forward, further exploration into the functional roles of these identified metabolites and the underlying molecular mechanisms governing microbial interactions will undoubtedly pave the way for innovative applications in pharmaceuticals, agriculture, and biotechnology.

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