CORPUS PUBLISHERS

Environmental Sciences and Ecology: Current Research (ESECR)

Volume 3 Issue 6, 2022

Article Information

Received date : August 22, 2022

Published date: September 05, 2022

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Keywords

Antibiotics; Fungus; Fungal pellet; Reactive oxygen species (ROS); Hybrid Plasma

Research Article

Prospecting for New Antibiotics: Extracting and Cultivating Fungi from Globally Sourced Beans

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Abstract

Introduction: One of the greatest health threats to society is the emergence of antibiotic resistant bacteria. There are many on-going research efforts to find new antibiotics that can overcome bacterial resistance. Our research has uncovered a novel method for discovering new anti-biotics and streamlining current prospecting methods. We discovered that beans sourced from various geographic locations contain residual fungus that can be cultivated and screened for anti-biotic properties. In the present report we sourced beans from global locations and cultivated fungal pellets from beans by confining them in a new form of Non-Thermal Plasma (NTP); Hybrid-Plasma (HP).

Methods: The agar plate method was used to evaluate fungal growth for antibacterial properties. The fungus from each of the fungal pellets was collected and sub-cultured in a glass tube with liquid growth media. Bacterial lawns of Staphlococcus. aureus and Eschericia coli grown on tryptic soy agar media plates were prepared and the fungal growth enrichment added to the plate to test for areas of growth inhibition.

Results: Testing confirmed measurable zones of inhibition in the agar plates that contained Staphcoccus aureus for 3 of the 4 beans tested. There was no effect on the plates with Eschericia coli.

Conclusion: These new methods for producing fungi from various geographic locations is more efficient for discovering and developing of new antibiotics.

Introduction

One of the greatest problems today in the treatment of infectious diseases is the emergence of antibiotic resistant bacteria [1]. The search for new antibiotics has become imperative as disease-causing bacteria are continuing to become increasingly resistant to current drugs. The Centers for Disease Control and Prevention estimates that each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics, at least 23,000 people die as a direct result of these infections [2]. It is well known that fungi from soil remains one of the most important resources for the discovery of new antibiotics [3], particularly from the areas surrounding the roots of plants, i.e., the rhizosphere. To extract the fungi from soil and test for antibacterial properties, various cumbersome chemical processes are required. A recent study identified 52 residual fungal species on mung beans from various locations in Pakistan [4]. Using these findings, we grew mung beans hydroponically [5]. Fungus was processed directly from the water surrounding the rhizosphere, thereby bypassing the complex prosseses of soil extraction now employed in the production of potential antibiotics. In the present report we have shown that beans from various geographic locations confined in our newly described Hybrid-plasma [6,7], induces development of fungus that enveloped the bean producing a fungal pellet. The fungus from the pellet was then used for antibiotic testing.

Methods

Five different beans: Mung beans, great northern beans, black beans and lima beans from various geographical locations were selected for these experiments. The bean types were sorted into separate petri dishes for confined exposure to HP. Within 7-10 days the beans developed a coating of fungus (Figure 1).

Figure 1: Beans confined in HP developed residual fungal growth.

The agar plate method was used to evaluate fungal pellets for antibacterial properties. The fungus from each of the fungal pellets was collected and sub cultured in a glass tube with liquid growth media (Sabouraud Dextrose Broth) at 30-35^{oC} for 72 hours. Bacterial lawns of Staphlococcus aureus and Eschericia coli grown on tryptic soy agar media plates were prepared and the fungal growth enrichment added to the plate to test for areas of growth inhibition. Plating for each challenge was performed in triplicate. After incubation was concluded 6 plates were labeled (two plates per bean type) and separated into S. aureus and E. coli and inoculated with 107CFU/mL 100µL of the respective cultured fungal pellet solution.

Results

The following table shows the zones of inhibition data from the bean fungi on two standard forms of test bacteria. A variety of store-bought beans were tested and several showed antibiotic properties particularly against Staphylococcus aureus. Fungal pellets produced from Lima, Great Northern and Black bean all showed various degrees of inhibition when tested in triplicate. In these tests none of the fungal extracts showed inhibition zones in E. coli plates (Figure 2A, 2B).

Figure 2A: is an example of the zones of inhibition exhibited by the fungus found on the lima beans on the S. aureus culture.

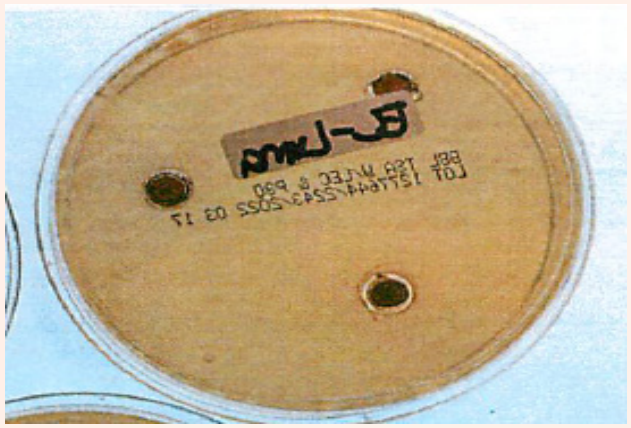

Figure 2B: In contrast no zones of inhibition were observed on all of the beans tested against the E Coli culture.

Table 1: Evaluation of antibiotic efficacy by testing effects of fungal extract against Staphylococcus aureus and Escherichia coli.

	Fungal Effect by Bean Type			
	Zone Size (cm)			
Bean Type	Staphylococcus aureus			Escherichia coli
Mung Beans Thailand	No Zone Observed			No Zone Observed
Mung Beans Middle East	No Zone Observed			No Zone Observed
Lima Beans USA	16.86	23.05	19.84	No Zone Observed
Great Northern Beans USA	20.66	21.08	20.98	No Zone Observed
Black Beans USA	17.85	17.42	14.52	No Zone Observed

Discussion

Major findings

Using a newly discovered form of non-thermal plasma, Hybrid-Plasma (HP),we exposed a variety of store bought beans to an HP environment. Within 7-10 days the beans were covered with fungus. The fungal pellets were subjected to a process which used the extracts to be tested against Staphylococcus aureus and Escherichia coli. Only three bean extracts showed zones of inhibition when applied to cultures of Staphylococcus aureus but none were effective vs. Escherichia Coli cultures.

Background

Currently, prospecting for new antibiotics requires cumbersome soil sampling methods to identify new fungi from which antibiotics can be produced. These methods that derived anti-microbial compounds from soil were pioneered by Selman Waksman [9] in the 1930's. Waksman started a systematic study of microbes as producers of antimicrobials. He defined an antibiotic as "a compound made by a microbe to destroy other microbes". He was instrumental in identifying soil-dwelling filamentous actinomycetes as prolific producers of antimicrobial compounds. Furthermore, he discovered numerous antibiotics made by soil-dwelling actinomycetes, including streptomycin, the first agent active against tuberculosis. Natural product antibiotic discovery peaked in the mid-1950s. Since then, a gradual decline in antibiotic discovery and development has occurred. Fewer new antibiotics have resulted in the evolution of drug resistance in many human pathogens and has led to the current antibiotic resistance problem. The search for new antibiotics and the quest for novel cost effective production methods are slowly continuing. In a previous study by Qureshi [4], in which fungi were extracted from different varieties of Mung bean found throughout Pakistan, found 54 fungal isolates from seeds in several provinces of Pakistan that had antifungal activity against plant pathogens. Finding that Pakistani beans were contaminated with a variety of fungi represents a new source for potential antibiotics. Our experiments showed that seeds from other worldwide geographic locations contaminated with local fungi can be a source for new antibiotics using our novel cultivation methods with Hybrid plasma.

Future studies

Filamentous fungi have an extensive metabolism and produce a wealth of bioactive compounds. Many of their secondary metabolites contribute substantially to the pathogenicity of fungi. Secondary metabolites may provide abiotic and biotic properties. Novel approaches will be of great value for identifying and engineering new secondary metabolites for biotechnological and pharmaceutical applications [10]. They can not only act as serious pathogens themselves but can also produce a cornucopia of highly beneficial drugs and antibiotics. This unique ability of such fascinating filamentous fungal species thereby makes them of key interest in further

biotechnological and pharmaceutical research. To improve on these effective but obsolete methods, we have developed a new way to quickly and efficiently cultivate fungi from worldwide locations in the search for new antibiotics without leaving the laboratory.

Conclusion

In the present report we sourced beans from global locations and cultivated residual fungi on those beans by confining them in a new form of Non-Thermal Plasma (NTP), Hybrid Plasma (HP). Beans that are confined in HP develop a coating of fungus (fungal pellets). These pellets were tested for antibiotic properties against Staphylococcus aureus and Escherichia coli by measuring the zone of inhibition around the pellet's fungal extract. Testing confirmed measurable zones of inhibition around the fungal pellets and provided evidence that we have described another effective and efficient method for discovery of potential new antibiotics.

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