Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.101, 2025

Isolation and Assessment of Antagonistic Microbial Interactions from Environmental Samples

Matilda Domeyo Ahadjie* Fauzia Nurudeen Cynthia Adinortey

Department of Molecular Biology and Biotechnology, University of Cape Coast, 4P85+5MJ, Cape Coast, Ghana.

* E-mail of the corresponding author: matildaahadjie48@gmail.com

Abstract

This study assessed the intricate patterns of antagonistic microbial interactions in the quest to help manage and promote human and animal health, and contribute to our understanding of complex microbial communities, like the human gut microbiome. Antagonistic microorganisms are known to possess different kinds of metabolites, antibiotics, etc., that can inhibit the growth of pathogens around them. The identification of antagonistic microorganisms from environmental samples holds significant potential for application in areas like agriculture, biotechnology, environmental bioremediation, and even healthcare. This study focused on the isolation of microbial species that exhibit antagonistic interactions, with a specific emphasis on their inhibitory effects on pathogenic organisms. Environmental samples were collected from environmental sources, particularly from air and soil sources, and subjected to serial dilution techniques. The resulting microbial isolates were cultured on selective media to foster antagonistic interactions. Various microbial strains were successfully isolated and identified. Through analyses, antagonistic interactions were observed among bacterial-fungal pairs. The findings reveal a rich diversity of antagonistic microorganisms, with potential applications ranging from agriculture, mining, and biopharmaceutical production.

Keywords: Antagonism, Antibiotics, Antimicrobial Interactions, Bacteriocins, Pathogens, Bioremediation, Microbial Strains.

DOI: 10.7176/ALST/101-07

Publication date: December 31st 2025

1. Introduction

Antagonism comes from the Greek word ‘antagonisetes’, which means opponent. In pathology, antagonism can be said to be the inhibition of growth or the suppression of an activity of a microorganism by another microorganism. Antagonistic microorganisms can be traced to the early 20th century when scientists first discovered the presence of microorganisms that can inhibit the growth of pathogenic microorganisms. In 1915, Frederick Twort and Felix d'Herelle independently discovered bacteriophages, viruses that infect and kill bacteria, and showed that they could be used to treat bacterial infections in animals. In the 1930s and 1940s, the discovery and development of antibiotics revolutionized medicine and agriculture, as antibiotics were found to be effective against a wide range of bacterial infections and could also be used as growth promoters in animals. An antibiotic is a kind of antimicrobial substance that is active against bacteria. According to the World Health Organization, antibiotics are said to be medicines used to prevent and treat bacterial infections. However, the overuse and misuse of antibiotics have led to the emergence of antibiotic-resistant bacteria, which have become a major public health issue (World Health Organization, 2014). Antibiotic resistance occurs when bacteria evolve and gain the ability to protect themselves from the effects of antibiotics. It occurs naturally and over time when an antibiotic is used for a while to fight a bacterial infection. In response to the problem of antibiotic resistance, researchers have turned their attention to antagonistic microorganisms as potential alternatives to antibiotics. In the 1970s, the first probiotic products were introduced to the market, consisting of live microorganisms that can confer health benefits to the host by colonizing the gut and inhibiting the growth of pathogenic bacteria (Gibson et al., 2017). Since then, there has been increasing interest in the use of antagonistic microorganisms for various applications, such as biocontrol of plant diseases, treatment of infections in humans and animals, and preservation of food. In recent years, advances in genomic and metagenomic technologies have enabled the discovery and characterization of new antagonistic microorganisms and their mechanisms of action, providing new opportunities for their use in different fields (Lamont et al., 2020).

Figure 1. Antimicrobial Interactions and Resistance (Source: WHO, 2014)

1.1 Antagonistic Microorganisms

Antagonistic microorganisms are microorganisms that possess the ability to suppress and inhibit the growth of other microorganisms, including pathogenic microorganisms, through various mechanisms such as competition for nutrients or production of antimicrobial compounds. There are several types of antagonistic microorganisms, namely, bacteria, fungi, and viruses. An example of antagonistic bacteria is Streptococcus pneumoniae, which produces a compound called pneumocin that is toxic to other bacteria in the same ecological niche. Pneumocin had proven to be effective against a range of gram-positive bacteria, including Staphylococcus coli. The production of pneumocin by S. pneumoniae is believed to play an important role in the colonization of the nasopharynx and the prevention of infection by other bacteria.

Another antagonistic bacterium is the bacterium Pseudomonas aeruginosa, which produces a compound called pyocyanin that inhibits the growth of other bacteria in the same environment. Pyocyanin has been shown to be effective against a range of gram-positive and gram-negative bacteria, including Staphylococcus aureus and Escherichia coli. In addition, P. aeruginosa can compete for iron, an essential nutrient for many bacteria, by producing siderophores that bind to iron and make it unavailable to other microbes.

The fungus Penicillium notatum is an example of an antagonistic fungus. It produces the antibiotic penicillin. Penicillin is effective against a wide range of bacteria, including many that cause serious infections such as Streptococcus pneumoniae and Staphylococcus aureus (Lax and Gilbert, 2014). Penicillin was the first antibiotic to be discovered and has been widely used to treat bacterial infections since its discovery in the early 20th century.

1.2 Mode of Action for Antagonistic Microorganisms

Antagonistic Microorganisms can be classified into three main categories based on their mode of action.

Mode of Action can be said to be the specific way or mechanism by which a substance produces an effect or interacts with other substances, in this case, microorganisms. The three categories of antagonistic microorganisms are: predation, antibiosis, and exclusive competition.

Competitive exclusion is when one microorganism competes with another for nutrients and resources. For instance, lactic acid bacteria present in the gut microbiome can prevent the colonization of pathogenic bacteria by producing lactic acid and other organic acids, which create an acidic environment that is unfavourable for the growth of pathogenic bacteria.

Antibiosis is the most common mechanism by which antagonistic microorganisms inhibit the growth of other microorganisms. Various antagonistic microorganisms, including bacteria, fungi, and actinomycetes. They produce various antimicrobial compounds, such as antibiotics, enzymes, and bacteriocins. Antibiotics are the most widely used antimicrobial compounds produced by bacteria and actinomycetes. Antibiotics can be classified into various categories based on their mechanism of action, including inhibition of cell wall synthesis, inhibition of protein synthesis, inhibition of nucleic acid synthesis, and inhibition of membrane function. For instance, Streptomyces species are known for producing various antibiotics, including streptomycin, tetracycline,

and erythromycin, which have significant clinical applications. Enzymes like lysozymes, produced by various bacteria and fungi, can degrade the cell wall of other bacteria, leading to their death. Bacteriocins are antimicrobial peptides produced by bacteria and are specific to other bacteria. Bacteriocins can be classified into various categories based on their structure and mechanism of action, including antibiotics, pediocin-like bacteriocins, and colicins. For example, lactic acid bacteria produce bacteriocins, which have potent antimicrobial activity against a wide range of pathogenic bacteria, including Listeria monocytogenes, Staphylococcus aureus, and Escherichia coli.

Predation is a less common mechanism, which antagonistic microorganisms control the growth of other microorganisms. Some microorganisms, including protozoa and fungi, have been reported to prey upon other microorganisms. For example, protozoa such as Paramecium caudatum and Tetrahymena pyriformis are known to consume bacteria, including pathogenic bacteria. Antagonistic microorganisms have numerous significances in numerous fields like agriculture, ecology, medicine, and the body.

In agriculture, the utilization of antagonistic microorganisms has gained considerable attention as an eco-friendly alternative to synthetic pesticides. Biocontrol agents derived from antagonistic microorganisms can effectively suppress plant pathogens and reduce the reliance on chemical treatments, leading to sustainable and environmentally friendly farming practices. For instance, Trichoderma species are well-known antagonistic fungi that can control various soil-borne pathogens. They produce enzymes capable of degrading pathogenic cell walls, while also inducing systemic resistance in plants (Vinale et al., 2008). Trichoderma-based bio-fungicides have been successfully employed to manage diseases caused by fungi, such as Fusarium and Rhizoctonia, in various crops. Antagonistic microorganisms have been shown to play a fundamental role in modulating the immune system. For instance, certain strains of probiotic bacteria, such as Lactobacillus and Bifidobacterium, have been shown to enhance the immune response by stimulating the production of cytokines and increasing the activity of natural killer cells and phagocytes (Isolauri et al., 2001). These interactions promote immune system homeostasis and help in the defense against pathogenic microorganisms.

Antagonistic microorganisms protect against pathogenic infections by competing for resources, producing antimicrobial compounds, and influencing the local microenvironment. One well-known example is the production of bacteriocins by lactic acid bacteria, which inhibit the growth of closely related pathogens (Cleveland et al., 2001). These antimicrobial substances act as natural antibiotics, preventing the colonization and proliferation of harmful microorganisms. Also, the human gut harbors a complex ecosystem of microorganisms collectively known as the gut microbiota.

1.3 Antagonistic Microbial Interactions, Ecological Significance, and Implications.

The interactions between microorganisms in natural environments are diverse and complex. One notable relationship and the focus of this study is the antagonistic interaction between bacteria and fungi. Bacteria and fungi engage in antagonistic interactions through a variety of mechanisms, including competition for resources, production of antimicrobial compounds, and interference with each other's growth and development. They often compete for the same nutrients and space in their environment, leading to a constant struggle for survival (Kinkel, 2014). This competition can limit the growth and establishment of one organism in favor of the other. Certain bacteria possess mycoparasitic abilities, meaning they can actively parasitize fungi by invading and colonizing fungal hyphae. This parasitic interaction can result in the degradation of fungal structures, nutrient exploitation, and eventually, the death of the fungus (Schmidt, 2011). Bacterial mycoparasites secrete enzymes, toxins, or siderophores that damage fungal cells and facilitate their colonization and survival. The antagonistic relationship between bacteria and fungi has significant ecological implications. It influences microbial community structure and dynamics, nutrient cycling, and ecosystem functioning. The competition for resources and the production of antimicrobial compounds by bacteria and fungi shape the composition and abundance of microbial populations in various habitats (Kinkel, 2014).

This antagonism can lead to the maintenance of microbial diversity and prevent the dominance of a single organism. For this reason, the study focused on the process of isolating microbial species that exhibit antagonistic interactions, with a specific emphasis on their inhibitory effects on pathogenic organisms. The identification of antagonistic microorganisms from environmental samples and the analysis of the antagonistic interactions hold significant potential for application in areas like agriculture, biotechnology, environmental bioremediation, and even healthcare.

2. Materials and Methods

2.1 Data Collection Technique

The data used for the study were primary data. There were two (2) main samples obtained for the study: the air sample and the soil sample. Air samples were obtained from the Science market, Science quadrangle, and Science taxi rank of the University of Cape Coast campus. The soil samples were also obtained from a dump site at the University of Cape Coast Botanical Garden, the dump site at the Marplins hostel, and an old dump site at the Science taxi rank, all on the university's campus.

Bacterial reference strains, Mannitol Salt Agar, and MacConkey Agar were obtained from the Bacteriology Department of the Noguchi Memorial Institute for Medical Research (NMIMR). Potato Dextrose Agar, Nutrient agar, Mueller-Hinton Agar, and all other chemicals and reagents were supplied by the laboratory of the Department of Molecular Biology and Biotechnology, University of Cape Coast.

2.2 Methods

2.2.1 Serial Dilution

- 1 ml of each of the soil samples was put in a test tube containing 9 ml of Phosphate Buffered Saline (PBS) and labeled according to the locations from which they were obtained.
- The solutions were vortexed to obtain an even distribution of soil particles.
- Three other test tubes are filled with 9 mL of phosphate buffer saline and labelled 10^{-1}, 10^{-2}, 10^{-3}, for each of the samples.
- 1ml of the solution labeled science was pipetted into the test tube labelled 10^{-1}, and the solution was vortexed as well.
- Then, 1ml of the solution in the tube labelled 10^{-1} was again pipetted into the test tube labelled 10^{-2}, and the solution was vortexed again.
- This same step was repeated for the test tube labelled 10^{-3}.
- This was done for all three samples.

2.2.2 Culturing of Organisms using Pour-plate Technique (Inoculation of Soil samples)

- 1ml of each of the solutions in the test tubes was pipetted onto the center of the Petri plates.
- Melted agar was then poured into the petri plates as well.
- The agar plate was then moved gently in a circular motion while keeping the plate flat in the flow hood to allow the inoculum to mix with the media, and it was allowed to solidify.
- The plates were incubated at room temperature for 72 - 96 hours.

2.2.3 Culturing of Organism using Open-plate Technique (Inoculation of Soil Samples)

- The air samples were obtained using the open-plate technique.
- Melted agar was poured into sterilized test tubes and allowed to solidify for a few minutes.
- The plates were then labeled with the names of the various sampling points
- The plates were safely transported to the locations.
- At each location, the plates were opened for about 15 minutes and then covered.
- It was then transferred back to the lab for incubation.
- The organisms were incubated for about 72 hours.

2.2.4 Morphological Examination for the Identification of Suspected Antagonistic Microorganisms

Morphological Examination was employed to determine the growth on the plates. Some plates showed growth in multiple organisms alongside suspected antagonistic interactions. Other plates showed some growth without any form of antagonistic interactions. The plates with antagonism were kept for confirmation using the Coculture method.

2.2.5 Co-culturing Method of Fungi and Bacterial Samples

The co-culture method involves growing a bacterium and a fungus on the same plate to observe if the fungi can inhibit the growth of the bacteria or the bacteria can inhibit the growth of the fungi. Here, PDA was the medium of choice because, without antibiotics, it supports the growth of both fungi and bacteria. The organisms of interest identified from the various plates were subcultured individually. For the fungi samples, *Aspergillus niger* and *Penicillium sp* were used, and they were grown for six days. For the bacteria, Bac. 1 and Bac. 2 were renamed to Bac A and Bac B, and the 5 reference strains obtained *were Klebsiella pneumonia, Escherichia coli, Salmonella typhii, Bacillus cereus, and Pseudomonas aeruginosa*.

The subculturing was done 24 hours before the co-culturing.

- On the day of the testing, bacterial suspensions were prepared for each organism.
- A distinct colony of bacteria was picked with an inoculation loop and placed into a test tube containing 5 mL PBS.
- The test tube was then vortexed to get an even mixture.
- The test tubes were divided into three parts using a marker from behind and labeled with the various organisms.
- Each bacterium was assigned two plates.
- One plate for *Aspergillus niger* agar discs and the other for *Penicillium sp*.

To create the agar discs, a cork borer was wrapped with an aluminum foil and autoclaved. After autoclaving, it was placed in the oven to dry. The cork borer was then heated using the spirit lamp, left to cool down, and then used to perforate the medium. The bacterial suspension was spread all over the surface of the media using a cotton swab. After this, three agar discs were placed upside down on the three divisions created. This was done for all bacteria plates; for the bacteria plate labeled with *Aspergillus, Aspergillus* agar discs were used, and for the bacteria plates labeled *Penicillium, Penicillium* agar discs were used.

3. Results and Discussion

3.1 Soil and Air Samples

Serial dilution was done to isolate potential antagonistic bacteria and fungi from the soil samples. Two types of Aspergillus species were obtained from the dump site at the University of Cape Coast Botanical Garden. Bacterial species grew on all the plates. The air samples also showed the presence of both fungal and bacterial isolates. Two of the bacterial isolates were renamed Bac. A and Bac. B is to be used for further analysis. It was interesting to note the presence of potential antagonistic microorganisms on one of the plates. The reference bacteria strains obtained, as well as Bac A and Bac B, were grown for 24 hours, and they all showed growth. Both Bac A and Bac B showed growth on MacConkey agar, Nutrient agar, and Mannitol Salt agar. The plates were incubated upright for 7 days. The plates were assessed on day 1, day 4, and day 7.

3.2 Serial Dilution and Culturing of Organisms

The figure below shows the mixed culture obtained from the serial dilution performed. The organisms of interest, i.e, *Aspergillus* and *Penicillium,* were subcultured to obtain a pure culture.

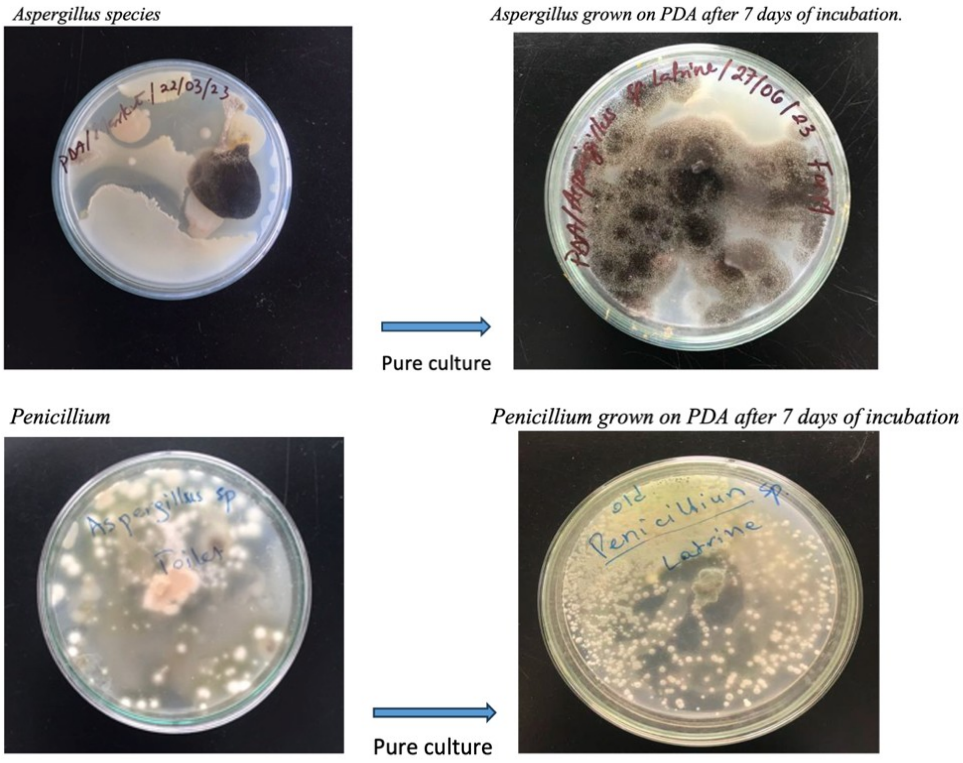
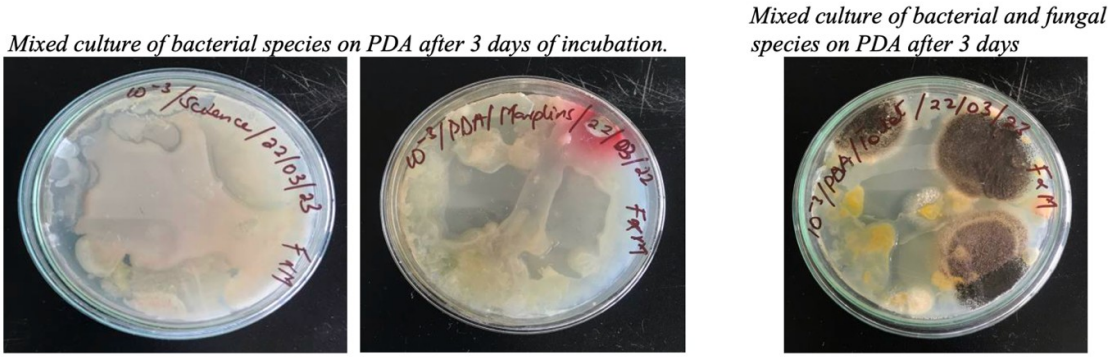


Figure 2. Serial Dilution and Culturing of Aspergillus and Penicillium

3.3 Co-culturing of Fungi and Bacterial Samples

Bac A and Bac B were isolated from the mixed culture above. They were cultured on three media: MacConkey agar, Nutrient agar, and Mannitol agar to confirm whether the bacteria are gram-positive or gram-negative, since the actual organisms are not known. From the figure below, Bac A showed more growth on the Mannitol Salt agar plate, so Bac A is a suspected Gram-positive bacterium. Bac B, on the other hand, showed massive growth on the MacConkey agar, so Bac B is suspected to be a Gram-negative bacterium.



3.3 Co-culturing of Fungi and Bacterial Samples

Bac A and Bac B were isolated from the mixed culture above. They were cultured on three media: MacConkey agar, Nutrient agar, and Mannitol agar to confirm whether the bacteria are gram-positive or gram-negative, since the actual organisms are not known.

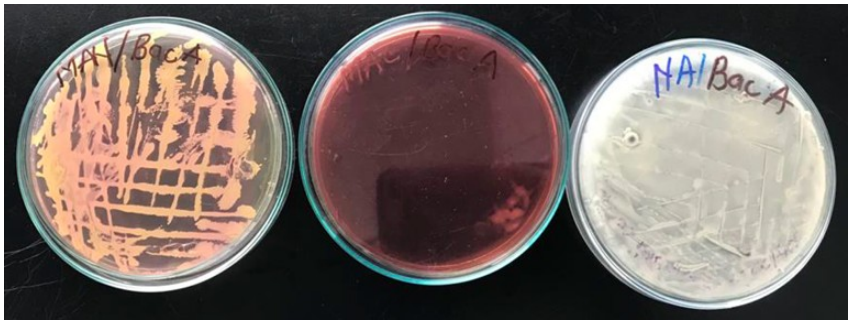


Figure 4. Bac A Cultured on MacConkey agar, Nutrient agar, and Mannitol Salt agar.

Figure 5. Bac B Grown on MacConkey agar, Nutrient agar, and Mannitol Salt agar.

From figure 4 and 5 above, Bac A showed more growth on the Mannitol Salt agar plate, so Bac A is a suspected Gram-positive bacterium. Bac B, on the other hand, showed massive growth on the MacConkey agar, so Bac B is suspected to be a Gram-negative bacterium.

3.3 Assessment and Evaluation

This is the morphological examination for the identification and confirmation of suspected antagonistic microorganism interactions using the co-culture method.

- *Day One*

On day 1, most of the plates were observed to have no activity or no growth. A few of the plates that contained *Aspergillus* showed the presence of mycelium, indicating the initiation of fungal growth. Some of these plates showed bacterial growth as well. Bacterial growths were observed in the plate containing Bac B as well as Bac A.

Figure 6. Mixed Culture of Bac A and Aspergillus showing the Presence of Mycelium, and Mixed Culture of Bac B and Aspergillus showing the Presence of Mycelium and Bacterial Growth.

- *Day Four*

On day 4, there was visible fungal growth on all the plates. There was also bacterial growth in most of the plates. On the plate containing Aspergillus and Klebsiella pneumonia, there were some clear zones, which indicated that the bacterium was inhibiting the growth of the Aspergillus in some parts of the plate. On the plate containing Aspergillus and Salmonella typhii, there was tremendous bacterial growth, which was suppressing the fungal growth and preventing the spread of the fungal spores. On the plate containing Bac A and Aspergillus, Bac A was observed to be struggling to grow, whereas Aspergillus was flourishing.

On the plate containing Bac B and Penicillium, the growth of Penicillium was observed to be suppressed by the growth of Bac B, as there were color changes only near the agar discs.

Similarly, on the plate containing Salmonella typhii and Penicillium. Salmonella was observed to inhibit the growth of Penicillium, and there was no color change on the plate.

On the plate containing Penicillium and Klebsiella pneumonia, Penicillium was observed to be inhibiting the growth of Klebsiella pneumonia, even though Klebsiella was still present on the plate.

On the plate containing Pseudomonas aeuriginosa and Penicillium, Pseudomonas was observed to be inhibiting the growth and spread of Penicillium.

On the rest of the plates, the bacteria did not survive at all, leaving the fungi on the plate to grow on its own.

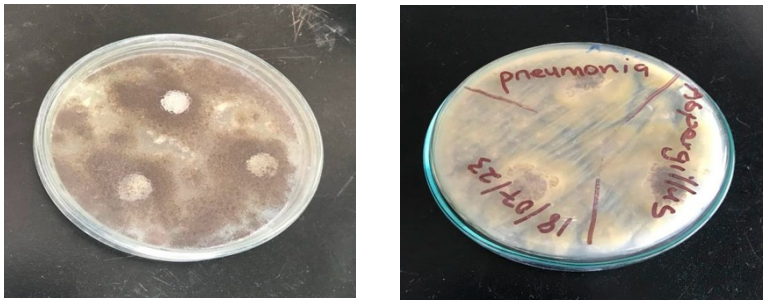


Figure 7. Front and Back Views of Mixed Culture of Klebsiella Pneumonia and Aspergillus on PDA.

Figure 8. Mixed Culture of Salmonella typhii and Penicillium on PDA.

- *Day Seven*

On day 7, most of the plates had excess fungal growth and little to no bacterial growth. Two plates were used as a control for the experiment. On the plate containing *Salmonella typhii* and *Aspergillus niger*, *Salmonella* was more prominent than *Aspergillus*; *Salmonella* was observed to inhibit the growth of *Aspergillus*.

On the plate containing *Aspergillus* and *Pseudomonas aeruginosa*, *Aspergillus* was again struggling to spread; even though there were no visible clear zones, there were patches on the plates.

On the plate containing *Klebsiella pneumonia* and *Aspergillus*, even though the *Aspergillus* was seen to grow better than *Klebsiella*, some patches were observed, indicating the presence of *Klebsiella* on the plate.

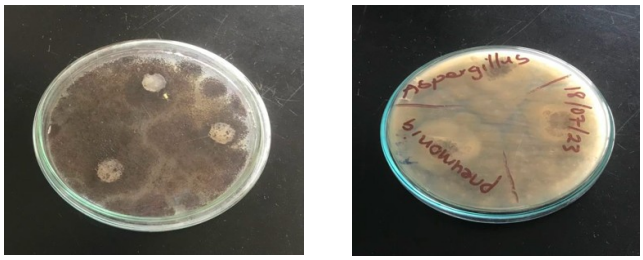


Figure 9. Front and Back Views of Mixed Culture of Aspergillus and Klebsiella Pneumonia on PDA

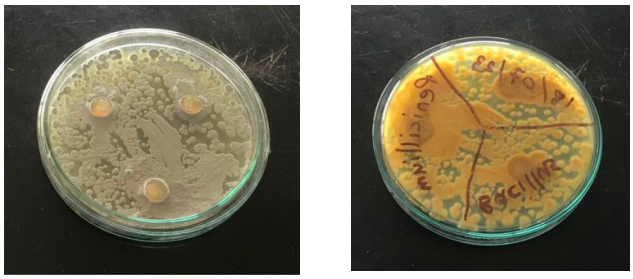


Figure 10. Front and Back Views of Mixed Culture of Penicillium and Bacillus Cereus on PDA

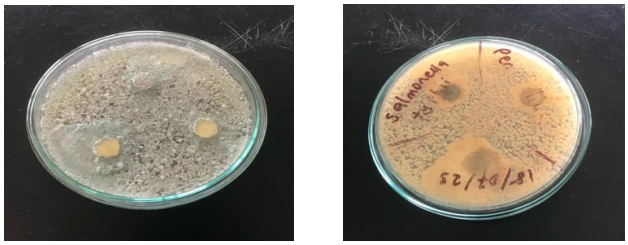


Figure 11. Front and Back Views of Mixed Culture of Penicillium and Salmonella Typhii on PDA

- *Control Experiment*

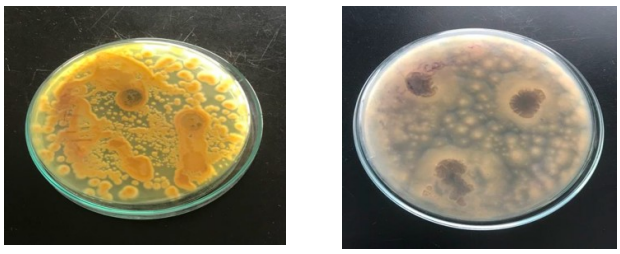


Figure 12. A Pure Culture of *Penicillium* and *Aspergillus niger* on PDA was Used as a Control Experiment

Then, on the plate containing Bac A and Penicillium, the Penicillium was observed to grow, and there was a

visible color change when the plate was turned upside down. The color looked greenish, which indicated the color of Penicillium. There was the same color change on the plate that had Bacillus cereus and Penicillium. However, on the plates containing Penicillium and Salmonella typhii and Penicillium and Klebsiella pneumonia, the case was different. There was no color change on the plate.

Figure 13 shows a heatmap summarizing the sequential color transformation observed in Aspergillus and Penicillium co-cultured with different bacterial species from Day 1 to Day 7. The progression from "no change" (gray) to black pigmentation in Aspergillus and to green and brown tones in Penicillium reflects the morphological evolution and varying antagonistic or cooperative interactions between the microorganisms. Plates containing Aspergillus generally showed dark pigmentation by Day 4, indicating rapid fungal colonization despite partial bacterial suppression.

Table 1: Co-culturing of Fungi and Bacterial Samples

Microorganisms and Microbe Pair on *Co-culturing Sample* plate	
Aspergillus	Penicillium
Aspergillus and Salmonella typii	Penicillium and Salmonella typhii
Aspergillus and *Klebsiella pneumonia*	Penicillium and Klebsiella pneumonia
Aspergillus and *Escherichia coli*	Penicillium and Bacillus cereus
Aspergillus and *Bacillus cereus*	Penicillium and *Escherichia coli*
Aspergillus and *Pseudomonas aeruginosa*.	Penicillium and Bac A
Aspergillus and BacA	Penicillium and Bac B
Aspergillus and BacB	Penicillium and Psedomonas aeruginosa

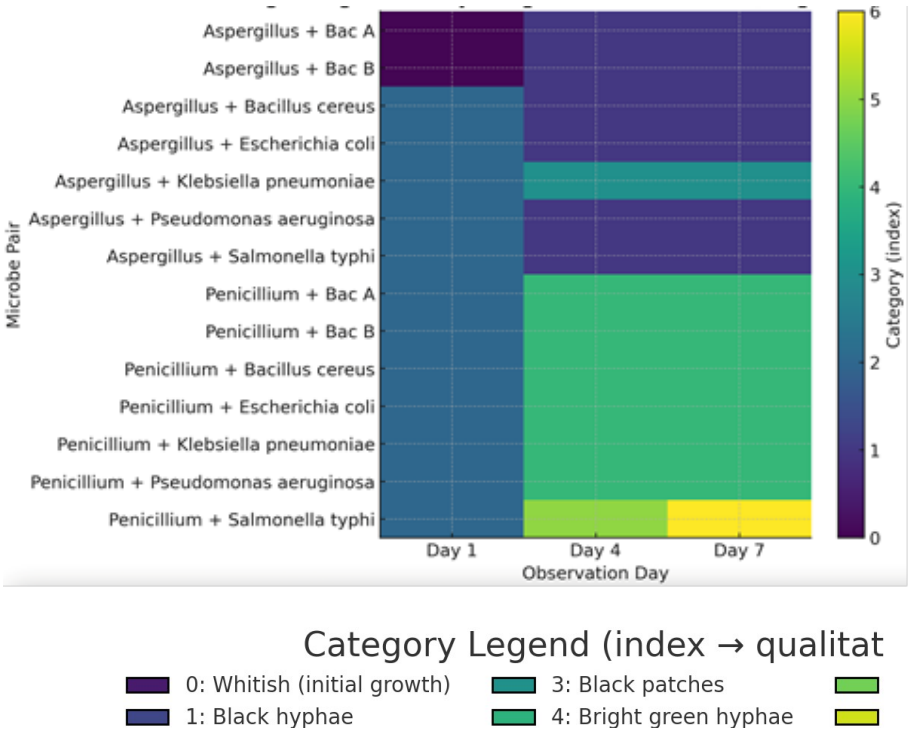


Figure 13. Color Progression Pattern Antagonistic Microorganism Interactions using the Co-Culture Method.

Conversely, Penicillium exhibited bright green coloration—especially with Bacillus cereus, Escherichia coli, and Klebsiella pneumoniae, suggesting active sporulation and metabolic activity.

The absence of color change in certain Penicillium combinations (e.g., with Salmonella typhii) supports the observed bacterial inhibition during morphological assessment.

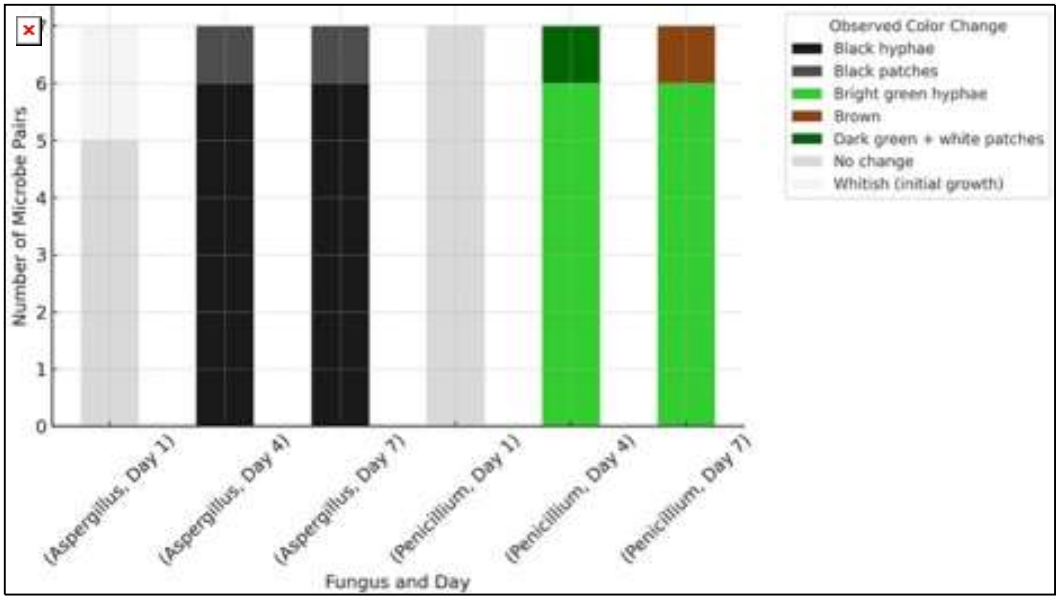


Figure 14. Pigment-Matched Stacked Bar Chart Showing Distribution of Observed Color Change.

Figure 14 depicts the proportion of color responses recorded for Aspergillus and Penicillium at each observation day. On Day 1, most plates displayed no visible color change, consistent with the incubation period before active growth. By Day 4, Aspergillus plates shifted predominantly to black or dark gray pigments, indicating strong hyphal expansion. By Day 7, Penicillium plates transitioned to bright green or occasionally brown, representing mature conidial formation.

The contrasting pigment intensities between both fungi emphasize the distinct growth kinetics and antagonistic dynamics: Aspergillus displayed rapid mycelial dominance, whereas Penicillium showed delayed but characteristic pigment development under selective bacterial stress.

These findings align with our morphological examination observations that inhibition and color change varied depending on the bacterial partner, highlighting the complexity of microbial competition in mixed cultures.

3.4 Discussion

The experimental results obtained from the isolation of antagonistic microorganisms from the environmental samples provide valuable insights into the intricate dynamics of microbial interactions. The observations made during this study shed light on the potential antagonistic properties of certain microorganisms and contribute to our understanding of their effects on other species within a given environment. The current study's observation of antagonistic interactions between Salmonella typhii and Aspergillus niger does not correspond with the findings of Balbontin et al (2014). With that study, there was a mutualistic relationship between Salmonella typhii and Aspergillus niger, whereas in this study, Salmonella typhii was seen inhibiting the growth of Aspergillus niger. Interestingly, the patches observed on plates containing Aspergillus and Pseudomonas aeruginosa resemble the observations made on the plate containing Salmonella typhii and Aspergillus niger, as well as Klebsiella pneumonia and Aspergillus.

The absence of visible clear zones on the plates highlights the complex nature of microbial interactions.

where inhibition might not always be indicated by distinct zones, which is in line with the findings of Aoki et al (2005).

The color change observed during the growth of Penicillium in the presence of Bacillus strains indicates metabolic interactions and potential metabolic cooperation. Contrastingly, the lack of color change on plates containing Penicillium and Salmonella typhii or Klebsiella pneumonia diverges from other published reports of fungal–bacterial co-culture instances where metabolic cooperation or pigment shifts are evident. Benoit et al.

(2015) and Sazanova et al. (2023). This divergence underscores the unique and context-specific nature of microbial interactions, highlighting that inhibition, neutral coexistence, or delayed interaction may result instead of clear cooperative pigment formation. This emphasizes the complexity of each microbial interaction.

The observed results from the isolation of antagonistic microorganisms provide valuable insights that align with and differ from existing research. The findings reflect the multifaceted nature of microbial interactions, where antagonism, inhibition, and metabolic cooperation can all play significant roles. Only the plates that had suspected antagonistic interactions were reported on.

4. Conclusion

The study undertaken to isolate and identify microorganisms from environmental samples has provided valuable insights into the intricate world of microbial interactions. Through meticulous analysis, there was a successful identification of a diverse range of microorganisms in the sampled environments. The investigation focused on detecting the presence of possible antagonistic microorganisms, and this revealed intriguing patterns of antagonistic interactions. To delve deeper into the antagonistic properties observed, the co-culture method was employed to confirm these interactions. This method helped simulate and observe direct interactions between microorganisms under controlled conditions. The outcomes of these Co-culture experiments provided compelling evidence of the antagonistic properties displayed by some microorganisms.

4.1 Policy Implementation and Recommendations

Based on the findings of this study, it is recommended that further studies and analysis should be done to provide a more comprehensive understanding of bacterial-fungal interactions, which can then lead to the production of bioactive compounds with potential pharmaceutical applications. Antibiotics like penicillin 39 are derived from fungal-bacterial interactions, and studying more interactions could lead to the discovery of novel drugs. In addition, this will contribute to our understanding of complex microbial communities, like the human gut microbiome. This knowledge can help manage and promote human and animal health.

References

World Health Organization. (2014). *Antimicrobial resistance: Global report on surveillance 2014.* World Health Organization. https://www.who.int/publications/i/item/9789241564748

World Health Organization. (2014). *Antibiotic resistance.* World Health Organization. https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance

Gibson, G., Hutkins, R., Sanders, M., et al. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, 14(8), 491–502.

Riley, M. A., & Wertz, J. E. (2002). Bacteriocins: Evolution, ecology, and application. *Annual Review of Microbiology*, 56, 117-137.

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Marra, R., Woo, S. L., & Lorito, M. (2008). Trichoderma–plant–pathogen interactions. *Soil Biology and Biochemistry*, 40(1), 1-10.

Kloepper, J. W., Ryu, C.-M., & Zhang, S. (2004). Induced systemic resistance and promotion of plant growth by Bacillus spp. *Phytopathology,* 94(11), 1259-1266.

Isolauri, E., Sütas, Y., Kankaanpää, P., Arvilommi, H., & Salminen, S. (2001). Probiotics: Effects on immunity. *The American Journal of Clinical Nutrition, 73(2 Suppl),* 444S-450S.

Cleveland, J., Montville, T. J., Nes, I. F., & Chikindas, M. L. (2001). Bacteriocins: Safe, natural antimicrobials for food preservation. *International Journal of Food Microbiology*, 71(1), 1-20.

Hill, C., Guarner, F., Reid, G., Gibson, GR, Merenstein, DJ, Pot, B., Morelli, L., Canani, RB, Flint, HJ, Salminen, S., Calder, PC, & Sanders, ME. (2014). Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews. Gastroenterology & Hepatology*, 11(8), 506-514.

Ramos, J. L., Duque, E., Gallegos, M. T., Godoy, P., Ramos-González, M. I., Rojas, A., Teran, W., & Segura, A. (2002). Mechanisms of solvent tolerance in gram-negative bacteria. *Annual Review of Microbiology*, 56, 743-768.

Schmidt, M. W., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., & Trumbore, S. E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature,* 478(7367), 49-56.

Meena, K. K., Sorty, A. M., Bitla, U. M., Choudhary, K., Gupta, P., Pareek, A., & Minhas, P. S. (2017). Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. *Frontiers in Plant Science*, 8, 172.

Chouhan, S., Sharma, K., & Guleria, S. (2017). Antimicrobial activity of some essential oils: present status and future perspectives. *Medicines*, 4(3), 58.

Lax, S., Smith, D. P., Hampton-Marcell, J., Owens, S. M., Handley, K. M., Scott, N. M., & Gilbert, J. A. (2014). Longitudinal analysis of microbial interaction between humans and the indoor environment. *Science,* 345(6200), 1048-1052.

Gut, A. M., Vasiljevic, T., Yeager, T., & Donkor, O. N. (2018). Salmonella infection - prevention and treatment by antibiotics and probiotic yeasts: a review. *Microbiology (Reading, England)*, 164(11), 1327–1344.

Snopková, K., Dufková, K., Chamrád, I., Lenobel, R., Čejková, D., Kosina, M., Hrala, M., Holá, V., Sedláček, I., & Šmajs, D. (2022). Pyocin-mediated antagonistic interactions in Pseudomonas spp. isolated on James Ross Island, Antarctica. *Environmental microbiology*, 24(3), 1294–1307.

El-Masry, S. S., El-Dougdoug, K. A., Othman, B. A., Mohamed, S. H., & S Sadik, A. (2021). Isolation and Characterization of Antagonistic Activity of Some Streptomycetes and their Specific Actinophages from Soil. *Pakistan journal of biological sciences*: PJBS, 24(4), 516–526.

Babich, O., Sukhikh, S., Dyshlyuk, L., Shishko, O., Milentyeva, I., Prosekov, A., Pavsky, V., Ivanova, S., & Dolganyuk, V. (2021). Evaluation of Biocompatibility and Antagonistic Properties of Microorganisms Isolated from Natural Sources for Obtaining Biofertilizers Using Microalgae Hydrolysate. *Microorganisms,* 9(8), 1667.

Balbontín R, Vlamakis H, Kolter R. Mutualistic interaction between Salmonella enterica and Aspergillus niger and its effects on Zea mays colonization. *Microbial Biotechnology*. 2014 Nov;7(6):589-600. https://doi: 10.1111/1751-7915.12182. PMID: 25351041; PMCID: PMC4265077.

Christaki, E., Marcou, M., & Tofarides, A. (2020). Antimicrobial Resistance in Bacteria: Mechanisms, Evolution, and Persistence. *Journal of molecular evolution*, *88*(1), 26–40. https://doi.org/10.1007/s00239-019-09914-3

Aoki, S. K., Pamma, R., Hernday, A. D., Bickham, J. E., Braaten, B. A., & Low, D. A. (2005). Contact-dependent inhibition of growth in Escherichia coli. *Science (New York, N.Y.)*, *309*(5738), 1245–1248. https://doi.org/10.1126/science.1115109

Benoit, I., van den Esker, M. H., Patyshakuliyeva, A., Mattern, D. J., Blei, F., Zhou, M., ... & Kovács, Á. T. (2015). Bacillus subtilis attachment to A spergillus niger hyphae results in mutually altered metabolism. *Environmental microbiology*, *17*(6), 2099-2113.

Sazanova, K. V., Zelenskaya, M. S., Izatulina, A. R., Korneev, A. V., Vlasov, D. Y., & Frank-Kamenetskaya, O. V. (2023). Carbonate and oxalate crystallization effected by the metabolism of fungi and bacteria in various trophic conditions: The case of Penicillium chrysogenum and Penicillium chrysogenum with Bacillus subtilis. *Crystals, 13*(1), 94. https://doi.org/10.3390/cryst13010094