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Cyanobacteria-Fungi: Friends to Waste Water Treatment

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Abstract

In nature, many relationships are found amongst various species, which inhabit and find a way of living together in this ecosystem. The aquatic *cyanobacterium* species have been known to have certain mutualistic interactions with the non-aquatic fungal species, but not all such interactions have been ventured in depth. The *cyanobacterium* species *Scytonema* was extracted from a polluted lake while the fungal species *Aspergillus flavus* was isolated from rotten apples and pears. These have originated in different environments and were exposed to different media. They were co-cultured for 45 days, as well as cultured separately in BG11 medium, cyanobacteria spent medium, fungal spent medium and domestic primary effluent collected from a local open drainage. The observation portrayed their mutualistic relationship to one another and their efficiency in treating wastewater. Such a relationship has led to a decrease in the percentage of biomass and thus in-turn, helping us analyzes its effect in treating wastewater systems.

Keywords: *Scytonema-Aspergillus* relationship • Cyanobacterium • Filamentous fungi • Wastewater treatment

Introduction

Microorganisms have significantly influenced our environment through their life cycles, which impact the dynamics of surrounding organisms. The interactions among these microbes have resulted in an array of advantages, such as the synthesis of vital enzymes and the breakdown of non-biodegradable waste materials. *Cyanobacteria* (*Scytonema*, *Nostoc*, *Anabaena*) and fungus (*Aspergillus* species) are found in different habitats, demonstrating the ecological diversity of microorganisms.

The latest studies [1] emphasize the intricate nature of interactions between algae and fungi, showcasing their promise in the advancement of eco-friendly strategies for treating wastewater. These interactions play a vital role in the biological treatment of wastewater, as mutualistic connections and biomass generation make important contributions. Furthermore, research [2] highlights the diverse capacities of microorganisms, namely their ability to break down chitin and to prevent oxidation. These abilities are particularly important in the interactions between bacteria and fungi.

Although the importance of algal-fungal interactions is widely acknowledged, a thorough examination of several sub-species is still necessary. An unknown interac- tion exists between Scytonema and Aspergillus [3-5]. Although there is separate research available on these species, the main objective here is to comprehend their ecological interactions and their contribution to biomass production. The existence of cytotoxins [6-10], anti-fungal enzymes [11,12], etc. introduces additional levels of complexity and uncertainty to this interrelationship, emphasizing the necessity for further investigation[13].

To enhance our understanding of environmentally friendly wastewater treatment utilizing living organisms, it is crucial to conduct a thorough investigation into the relationship between Scytonema and Aspergillus [14].

The objective is to examine the dynamics of their interactions by subjecting them to precisely controlled conditions, including parameters such as pH, temperature, and light exposure, whether these microbes demonstrate mutualistic interactions or engage in competitive connections [15,16].

Gaining a thorough comprehension of this complex subject not only enhances our understanding of interactions among microorganisms but also offers vital insights into their influence on the growth of living organisms[17,18]. These insights have the potential to completely transform the process of treating wastewater, leading to the development of more advanced and environmentally sustainable solutions to protect the environment [19].

Methods

The *cyanobacterium* chosen for this investigation was acquired from stagnant wastewater of HMT Lake, HMT nagar, Nacharam, Hyderabad, Telangana, India(Figure 1). All the media was purchased from Himedia, India. The collection consisted of many species, including *Nostoc*, *Anabaena*, and colonies of *Scytonema* found among the isolated algae. *Scytonema* was sub-cultured utilizing Bristol agar medium to obtain pure colonies. Meanwhile, fungal species were extracted from decomposed sections of apples and pears, spread onto PDA plates, and placed in an incubator for 2-3 days. Different fungal species were detected. Amongst them, Aspergillus flavus was selected and sub-cultured on PDA plates to get pure cultures. The *A. flavus* and *Scytonema* were cultured separately in BG11 medium using particular conditions: static growth, with a 10:14 hr light/dark cycle, and an average temperature of 25.6 ℃. Additionally, the experiment involved using filter-sterilized air that was enriched with 1% (v/v) CO_2 to supply inorganic carbon. Under these conditions, all cultures were consistently preserved, with glasswares and media being sterilized using autoclave.

Cyanobacteria isolation

Cyanobacterial cultures were obtained from a stagnant lake containing mixed cultures of microorganisms. The algal flocks were mostly separated and cultivated on Bristol medium, which consisted of 25 g/L $NaNO_3$, 2.5 g/L $CaCl_2 \cdot 2H_2O$, 7.5 g/L $MgSO_4 \cdot 7H_2O$, 7.5 g/L K_2HPO_4, 17.5 g/L KH_2PO_4, and 2.5 g/L NaCl solution. Deionized water was introduced to obtain a volume of 1 liter. This medium was used to carry out the initial serial dilution process to decrease the concentration of algal colonies. The cyanobacterial culture concentration thus achieved through dilution [20-23] was sub-cultured to obtain pure strains of Scytonema. This was subsequently utilized for further experimentation in the study. [6,13] After culturing cyanobacteria for 19 days in the medium, the cyanobacterial colonies were filtered out and the remaining medium was used as a spent medium.

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Aspergillus flavus isolation

To separate *Aspergillus* strains, decomposed sections of apples and pears were spread onto PDA plates and placed in an incubator at a temperature of 25.6 ℃, while keeping them in darkness. The observed colonies consisted of black mycelium, white-green mycelium, and white mycelium (Figure 2). The white cottony growth colonies were transferred on new PDA plates and kept in an incubator for 19 days (Figures 3 and 4). On the fourth day, we confirmed the successful sub-culturing of pure cultures. On the 19th day, fungal spores were isolated by rinsing with deionized water, and passing through a sterile glass wool filter. The spore cultures were subsequently placed in deionized water and incubated under comparable circumstances. [24] After culturing fungal colonies for 19 days in the medium, the colonies were filtered out and the remaining medium was used as a spent medium.

Co-culturing *Cyanobacteria* and fungi

The *Scytonema* strain was sub-cultured in BG11 medium to produce inoculum. The culturing was done in static growth, with an illumination intensity of 100 µmol/m2/s and a light/dark cycle of 10:14 hours. The temperature was maintained at 28 ± 1°C. Inorganic carbon was added through the addition of filter-sterilized air that was enhanced with 1% (v/v) CO_2. The BG11 medium composition consisted of 1.5 g/L $NaNO_3$, 40 mg/L K_2HPO_4, 75 mg/L $MgSO_4$ $7H_2O$, 36 mg/L $CaCl_2{\cdot}2H_2O$, 6 mg/L citric acid, 6 mg/L ferric ammonium citrate, 1 mg/L EDTA-Na_2, 20 mg/L Na_2CO_3, and 1 mg/L A5. Where A5, in turn, was a trace metal solution containing 2.86 g/L H_3BO_3, 1.86 g/L $MnCl_24H_2O$, 0.22 g/L $ZnSO_47H_2O$, 0.39 g/L $Na_2MO_42H_2O$, 0.08 g/L $CuSO_4$ $5H_2O$, and 0.05 g/L Co $(NO_3)26H_2O$ [25,26].

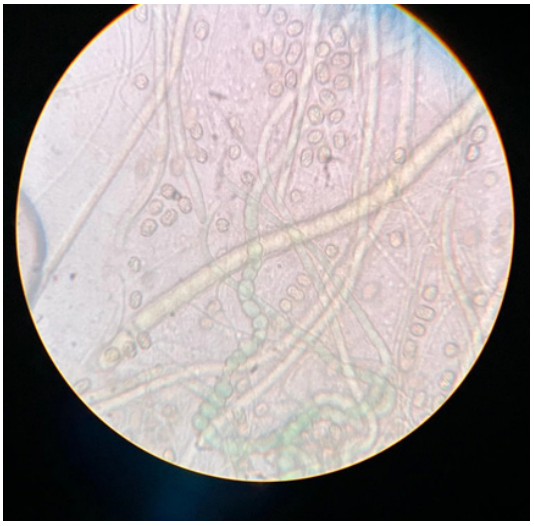

Figure 1. Mixed culture of cyanobacteria obtained from HMT Lake, HMT nagar, Nacharam, Hyderabad, Telangana, India 500076.

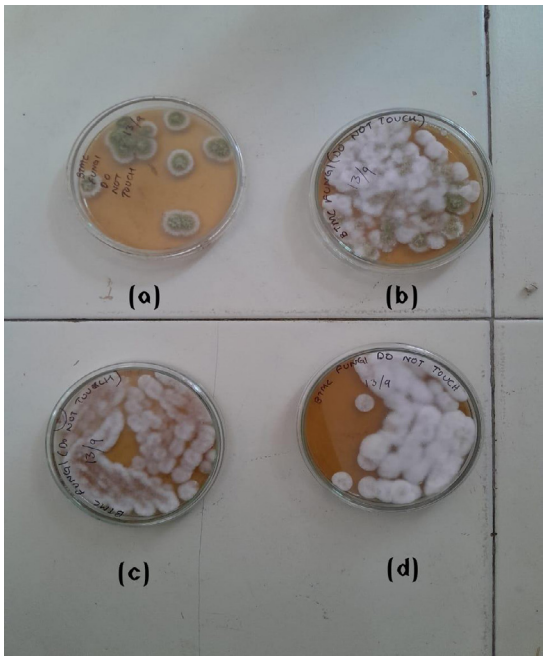

Figure 2. Culturing fungal species isolated from rotten apple and rotten pear. **a)** and **d)** have successfully been observed to have pure colonies, while **b)** and **c)** have shown to have mixed cultures. **a)** and **d)** have been used for further sub-culturing.

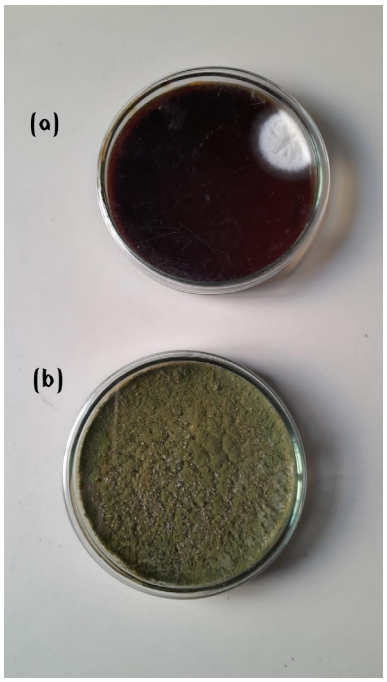

Figure 3. Subculturing pure strains of *Aspergillus* culture on PDA medium. The petri dish **b)** with green colonies, i.e, *Aspergillus flavus*, has been used for the experiment.

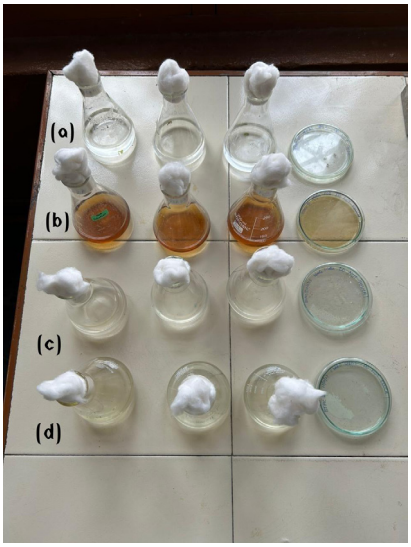

Figure 4. *Cyanobacteria* and fungi co-culture and individual cultures grown in **a)** BG11 medium, **b)** Fungal spent medium, **c)** *Cyanobacteria* spent medium and **d)** domestic waste water.

Results

Co-culture of *Cyanobacteria (Scytonema)* and fungus *(Aspergillus flavus)*

The interaction among cyanobacteria (*Scytonema*) and fungus (*A. flavus*) were cultured in BG-11 medium. [20-27] When these species were cultured

separately in BG-11, no significant growth was detected for *Aspergillus*, while *Scytonema* had significant growth. When *Scytonema* was cultured with the *A. flavus*, the green clumps became prominent during incubation (Figure 5). Hyphae of *A. flavus* with *Scytonema* cells beneath them were observed under microscope [20]. This observation indicates that *A. flavus* grows in the presence of *Scytonema* but cannot propagate alone in the BG-11 medium. Further, their coexistence points to a potential mutualistic relationship contributing to forming a synthetic lichen (Figure 6) [28,29].

Fungal growth in spent medium of *Scytonema*

The nutrition source for fungal growth was assessed in the *Scytonema* spent medium. There were two conditions implied: BG-11 medium and *Scytonema* spent medium (Figure 7). In the *Scytonema* spent medium, a noticeable growth of fungi was observed while there was no growth in the BG-11 medium. These results imply that *Scytonema* spent medium impart vital nutrients sufficient for fungal growth [30,31].

Scytonema growth in fungal spent medium

Scytonema growth was evaluated in the Fungal spent medium for the nutritional requirements. The two conditions were implied: BG-11 medium and Fungal spent medium (Figure 8) [32]. *Scytonema* demonstrated comparatively more growth in the Fungal spent medium than in the BG-11 medium. This results in the presence of adequate nutrients in the Fungal spent medium [33,34].

Co-culture in domestic primary effluent for wastewater treatment

Cyanobacteria-fungus co-culture for wastewater treatment was applied by culturing the microorganisms in domestic primary effluent [1,25]. A decrease in the Optical Density (O.D) values at 620 nm was initially observed and was stable later. This occurrence indicates possible wastewater treatment with microorganisms utilizing the organic content present in the primary effluent for their growth. The stabilization of O.D values infers an equilibrium point in the treatment process (Figures 9-11) [35].

Discussion

The co-culturing of Cyanobacteria and Fungi disclosed a symbiotic relationship among each other. Their collaborative presence in different mediums showed green clump formation suggesting a mutualistic association with a potential synthetic lichen formation. The Cyanobacteria-Fungi cultures grown in BG-11 media had an accelerated rate of Optical Density (O.D) values which was evident with the overall increase in the size of their colonies and biomass. In the case of primary effluent collected from a local drain, a decrease in optical density values was observed over a period of time in the co-culture. This was due to the fact that the nutrients present in the water were utilized by the cyanobacteria-fungi co-culture and thus, their mutualistic relationship proved to be efficient in treating domestic primary effluent. Relative interactions between Cyanobacteria and Fungi coincidences with previous studies [24]. The spore density and light conditions have an effect on nitrogen fixation and algal growth. This allineates with our study, drawing attention to the significance of factors influencing such interactions for possible benefits in a wide range of applications. The capability of Cyanobacteria to transform carbon dioxide into useful chemicals and fuels would be a latent environmental benefit. The Cyanobacteria-Fungi relationship ensures for sustainable practices in agriculture, industry and wastewater treatment.

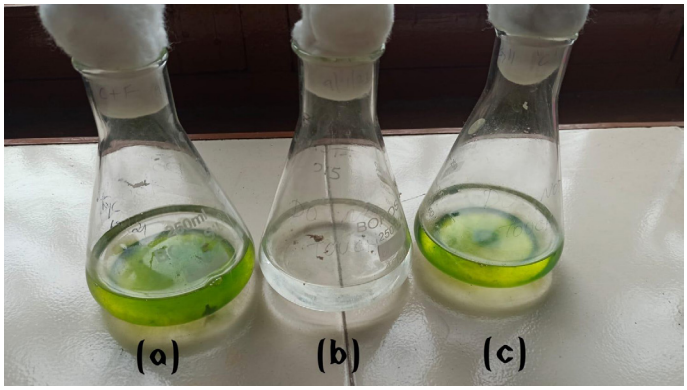

Figure 5. Observed growth in BG-11 medium for **a)** Cyanobacteria-fungi co-culture, **b)** Fungi individual culture and **c)** *Cyanobacteria* individual culture.

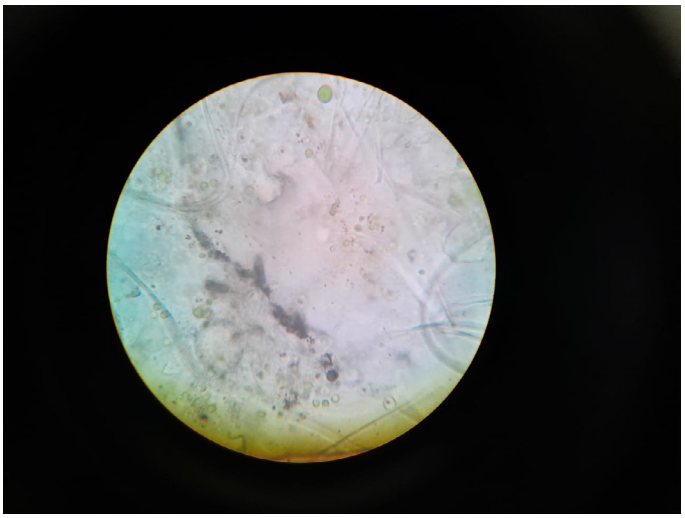

Figure 6. Visible associations of clumps of a *Aspergillus* spores with *Scytonema*.

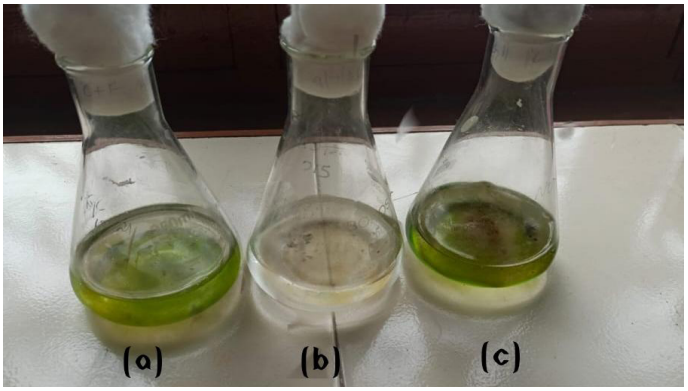

Figure 7. Observations made on cyanobacteria BG-11 spent medium for **a)** *Cyanobacteria* individual culture, **b)** Fungi individual culture and **c)** Cyanobacteria-fungi co-culture.

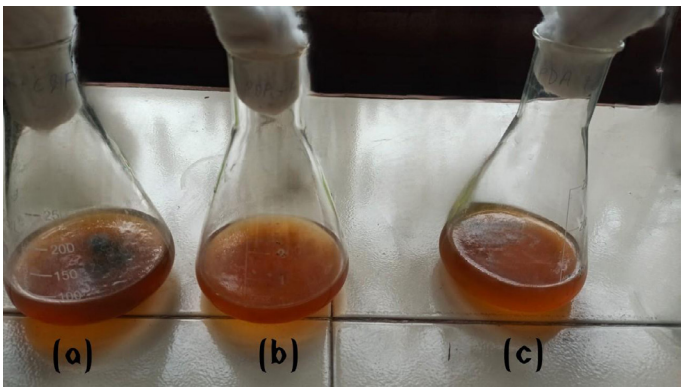

Figure 8. Observations made on fungal spent medium (PDA) for **a)** Cyanobacteria-fungi co-culture, **b)** Fungi individual culture and **c)** Cyanobacteria individual culture.

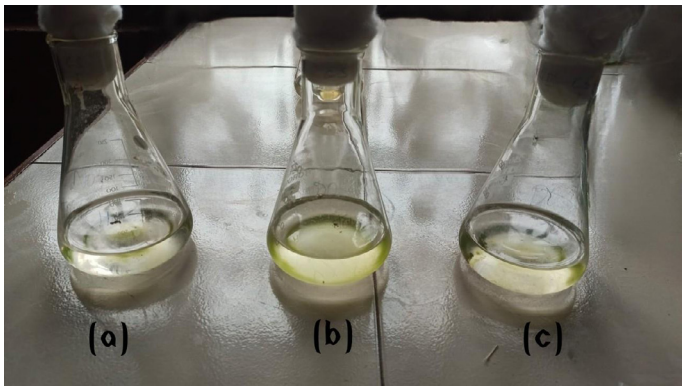

Figure 9. Observations made on domestic waste water collected from an open drainage in Mallapur, Medchal, Hyderabad, Telangana, India. 500076; **a)** Cyanobacteria-Fungi co-culture, **b)** Fungi individual culture and **c)** *Cyanobacteria* individual culture.

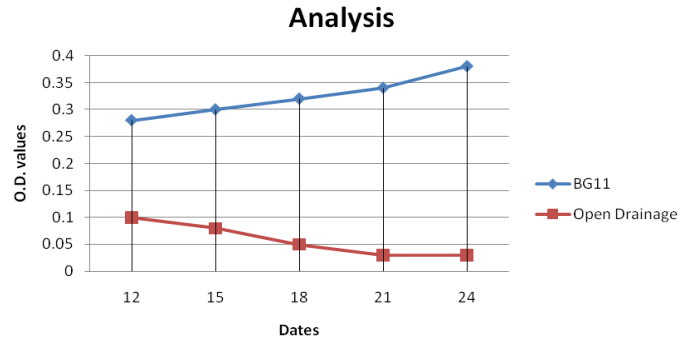

Figure 10. Graphical representation showcasing the increase in O.D values for cyanobacteria and fungi co-culture in BG-11 medium (blue) and decrease in the O.D value of domestic waste water that had the co-culture of cyanobacteria and fungi.

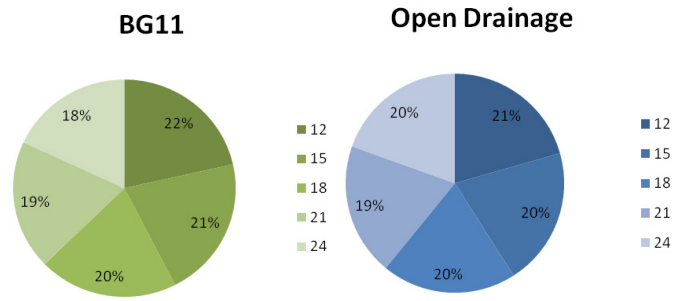

Figure 11. Percentage of transmittance in the case of BG11 medium and domestic wastewater as calculated by colorimeter.

Conclusion

In conclusion, these results provide insights into the mutualistic relationship between Cyanobacteria (*Scytonema*) and Fungi (*Aspergillus flavus*) along with probable applications in nutrient recycling and wastewater treatment. The divergent assemblage of secondary metabolites induced by cyanobacteria includes complexity to the interactions and unfolds new approaches for future research. Comprehending the specific compounds involved and their mechanisms can pave the way for sustainable applications in agriculture, medicine, industry and environmental management.

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Declaration

Ethical approval

Not applicable.

Data availability

The data is publicly available at https://doi.org/10.6084/m9.figshare.25329913.v1

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Author Contribution

Bandi Lasya, Shubhangi Kanjilal carried out literature reviews. Kasala Akshitha Reddy, Chandra Deepthi drafted the manuscript and performed the statistical analysis. Poosa Padmalatha offered guidance and planned the procedure. All contributing authors performed the experiment equally. All authors read and finalized the final manuscript.

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