Bioremediation of *Sargassum* using Antifungal Bacteria

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Keywords: *Sargassum*, bioremediation, *Fov4*, co-inoculation, antifungal

Background

Sargassum is a floating brown alga that thrives in low-nutrient waters, sustained by fish excretion, upwelling, and nitrogen fixation (NOAA, 2024). While it historically served as a critical habitat for various marine species, its chemical composition has changed since the 1980s, and it is now composed of toxic compounds that pose significant risks to coastal ecosystems (Milledge and Harvey et al. 2016) (NOAA, 2024). These shifts have led to an increase in *Sargassum* inundation events (SIEs)—when large amounts of algae wash ashore—that hinder aquatic ecosystems dependent on sunlight and oxygen.

Anthropogenic influences have increased nitrogen in coastal waters by 35% and decreased phosphorus by 42% since the 1980s, making phosphorus the new limiting nutrient (EPA, 2023). The increased nitrogen, driven by agricultural runoff, atmospheric deposition, and river discharges, expanded the Great Atlantic Sargassum Belt—delivering massive waves of algae to once-pristine shores (EPA, 2023).

Sargassum accumulations provide essential habitats in the open ocean but create issues when they wash into coastal areas. Dense mats of algae block sunlight needed for the photosynthesis, growth, and reproduction of organisms such as seagrasses and corals. Without sunlight, seagrasses and corals die, causing declines in oxygen production and food availability for other marine species (NOAA, 2024). Additionally, *Sargassum* blocks nutrients and light from reaching coral reefs, leading to the loss of symbiotic algae and coral bleaching. As the algae decomposes, it releases hydrogen sulfide and ammonia into the ocean, resulting in low pH, poor water quality, and elevated water temperatures (EPA, 2023). This can alter the ecosystem's balance, often favoring invasive species that further impede biodiversity. Specifically, *Sargassum* buildup affects endangered sea turtles by obstructing nesting sites, preventing hatchlings from reaching the ocean, and shifting sex ratios due to temperature changes caused by blocked sunlight (EPA 2023).

Additionally, there is a substantial economic burden surrounding *Sargassum* management on beaches. Annual *Sargassum* removal costs are estimated at $2.9 million in Texas, $45 million in Miami-Dade County, and $120 million across the Caribbean. These growing costs emphasize the need for a sustainable solution (Kelly, 2023).

Purpose and Objectives

While *Sargassum's* biofuel potential has been explored in previous studies, this project focuses on bioremediation. Specifically, this research examines whether *Sargassum* can be utilized as a bioamendment to promote the growth of bacteria with antifungal properties. By developing a *Sargassum*-based medium combined with potato dextrose agar (PDA), the experiment aims to cultivate bacteria effective against *Fusarium oxysporum* race 4—a highly destructive fungal pathogen in cotton production. This pathogen imposes significant economic losses, costing the agriculture sector millions in crop damage. Thus, this multidisciplinary project seeks to transform an environmental issue into an opportunity—bridging conservation, microbiology, and agriculture.

Methods

Preliminary tests on bacterial strains' antifungal activity were conducted using dual culture with *Fusarium oxysporum* race 4 (*Fov4*) on potato dextrose agar (PDA) media (Singh et al., 2019). Strains A and B exhibited antifungal activity against *Fov4*, with strain A demonstrating more effective inhibition, while strain C showed minimal antifungal activity. These initial activity tests were performed without measuring the optical density (OD) or colony-forming units (CFU) of the bacteria. To further prepare for testing, bacterial colonies from each strain were incubated in duplicate from stock on 200 ml nutrient broth (NB), nutrient agar (NA), and PDA plates using the four-quadrant streak method. Plates were then placed in a 28°C incubator. The 16S rRNA region of each bacterial strain was amplified through PCR using 27f/1492r primers (Weisburg et al., 1991), and DNA content was quantified using NanoDrop following gel electrophoresis. After extracting gel DNA for the 16S rRNA region, nucleotide sequences were confirmed through sequencing data analysis (Sanger sequencing via a third-party institution), which allowed for the amplification of marker genes and primers specific to each bacterium for detailed phylogenetic analysis. Finally, *Sargassum* was added to PDA media containing bacteria and *Fov4* to compare its antifungal activity with that of the control (existing media without *Sargassum*).

Results

The results indicate that while both A and B show strong antifungal properties, strain C does not exhibit any antifungal activity, as there was no inhibition zones observed in the assays. The bacterial growth was highest in the 0.1% seaweed media for both strains, suggesting that lower concentrations of seaweed may encourage bacterial proliferation. However, a significant decrease in bacterial growth occurred at higher concentrations (0.5% and 1%), indicating that these concentrations may inhibit growth.

Overall, a declining trend was observed between seaweed concentration and inhibition zones against bacteria and *Fov4*. This suggests that while lower concentrations of seaweed can promote growth, higher concentrations can have a negative impact. Interestingly, the higher seaweed concentrations (0.5% and 1%) led to bleaching effects on both *Fov4* and C, indicating potential phytotoxicity. This unexpected role of *Sargassum* in antifungal bacteria growth warrants further investigation into the mechanisms behind these interactions.

Conclusion and Future Directions

PDA media inoculated with *Sargassum* negatively impacts antifungal bacterial growth and induces bleaching effects. Subsequent experiments will explore the effects of higher *Sargassum* concentrations in ISP2 media on bacterial activity. Future research will also explore the effects of different seaweed species on fungal and bacterial interactions to identify the specific compounds that enhance or inhibit microbial growth. After identifying the specific compounds, the research could examine potential synergistic effects between *Sargassum* and other organic amendments, which could enhance the development of effective biocontrol strategies against fungal pathogens in agriculture (drug and fungicide development).

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