INVESTIGATION OF THE SYNERGISTIC EFFECT OF SELECT CLOVE OIL AND AMPHOTERICIN B ON Fungal Biofilm of Rhizopus oryzae

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Introduction

Previous antifungal research involving R. oryzae has focused on planktonic cells alone and not on biofilm formation and treatment. This is problematic due to the increased antifungal resistance seen when treating fungal biofilm compared to planktonic cells. The species R. oryzae is known to cause up to 80% of all Mucormycosis infections. Amp B is the current line of treatment for this disease, but it is known to have many negative side effects such as fever, shaking, nausea and severe liver and kidney toxicity. The basis of the experiment is to investigate the antifungal effects of select phytochemical compounds and Amphotericin B (Amp B) against fungal biofilm produced from the species R. oryzae. By using Amp B and phytochemicals synergistically, less Amp B would be needed to inhibit fungal growth than when using each antifungal agent alone. This research has important clinical application that could potentially affect the lives of those suffering from this rare, opportunistic disease.

Hypothesis

Phytochemical compounds can be used in synergism with Amphotericin B to lower the Minimum Inhibitory Concentration (MIC) and treat Mucormycosis infections.

Methodology

![Methodology Diagram]

Results

Single Treatment of Phytochemical Oil and Amp B

![Graph showing the concentration of Amp B and Oils]

Synergistic effect of Amp B and clove oil. Oil concentration decreases as you go from left to right and Amp B concentration decreases as you go from top to bottom showing the greatest amount of inhibition at higher concentrations.

Future Work

Nasidya corymbifera is the second most common species found in Mucormycosis infections. A. corymbifera has different properties and could be tested to lower the MIC of Amp B.

Breaking down the biofilm could be accomplished using low frequency ultrasound (LFUS). LFUS could be used in synergism with Phytochemical compounds and Amp B to treat Mucormycosis infections.

Conclusions/Discussion

Procedural Changes

2% RPMI was determined to be more efficient than 1% RPMI as media for biofilm growth. Depending on the time allowed to grow, hyphae would form. This was a problem because during the aspiration process, the hyphae would be removed and the biofilm would be lost as well. Initial growth of 24 hours in either 2% or 1% RPMI resulted in the formation of hyphae. A period of 12 hours proved to be the best duration of time for biofilm formation.

The combination of 2% RPM and an incubation period of 12 hours while shaking at 75 rpm at 37°C resulted in strong biofilm.

Conclusions

- Lower concentrations of phytochemical oils showed a similar effect on biofilm inhibition as Amphotericin B. Even without combination therapy, the oils show antifungal activity.
- On average the disruption of biofilm was increased by adding any of the four Phytochemical oils (Clove, Oregano, Cinnamon, Lemongrass) with the greatest differences occurring at a low concentration of Amp B.
- It is possible to reduce the amount of antifungal needed to treat infections by using Phytochemical oils.
- Using phytochemical oils in conjunction with Amp B, it may be possible to reduce toxicity, patient mortality and the minimum inhibitory concentration of Amphotericin B.

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Chemical Structures