

EVALUATION OF STRUCTURAL AND BIOCHEMICAL ALTERATIONS IN *ASPERGILLUS TERREUS* BY THE ACTION OF ANTIFUNGAL ANTIBIOTIC COMPOUND FROM *STREPTOMYCES* SP. JF714876

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*Email: gmvidyasagar@rediffmail.com**ABSTRACT**

Antifungal compound obtained by *Streptomyces* sp. JF714876 was examined for its effect on morphological and biochemical alteration in *Aspergillus terreus*. Microscopic observation revealed swelling of hyphae with deformation and distortion in mycelial structure in presence of moderate concentration of antifungal compound. At high concentration, the compound exhibited fungicidal action. Antifungal treated *Aspergillus terreus* showed changes in its biochemical content such as, protein, carbohydrates, peroxidase, catalase and amylase as compared to untreated.

Key words: *Aspergillus terreus*, Antifungal compound, *Streptomyces* sp. JF714876, Structural changes.

INTRODUCTION

Actinomycetes are gram positive bacteria produces secondary metabolite like, antibiotic, enzymes etc. 75% of commercially and medically available antibiotic and 60% antibiotic developed for agricultural use were isolated from *Streptomyces*¹. The *Streptomyces* species inhibit many fungal species such as, *Phytophthora capsici*², *Phytophthora cinnamomi*³, *F. oxysporum*, *Botrytis cinerea*, and *Monilinia laxa*⁴, *Sclerotium rolfsii* and *C. gloeosporioides*⁵. The extracellular metabolites of *Streptomyces* strains caused hyphal swelling, distortion and cytoplasm aggregation in the *C. acutatum* and *C. gloeosporioides*⁶. Sariah⁷ and Rahman et al.⁸ reported that the fungal mycelial malformation might be due to the antibiotic metabolites produced by the bacteria, which can penetrate and cause protoplasmic dissolution and disintegration.

Excessive morbidity and mortality in the immune compromised host is increased because of invasive Aspergilliosis caused by *Aspergillus* species^{9, 10}. Invasive aspergilliosis is mainly caused by *A. fumigatus*, whereas infections caused by *A. fumigatus* such as *A. flavus*, *A. niger*, *A. terreus*, and *A. nidulans* are on the rise. As per the epidemiologic studies in India, fungal keratitis is caused by the filamentous fungi *Fusarium* and *Aspergillus*¹¹. Different antibiotic targets the fungi at different sites. New drugs with activity against *Aspergillus* spp. include Voriconazole and Caspofungin. Azoles such as, voriconazole act primarily by inhibition of 14-demethylase¹², whereas echinocandins such as, caspofungin inhibits the synthesis of beta-1, 3-glucan¹³. Flucytosine inhibits fungal RNA and DNA synthesis¹⁴, and Amphotericin B binds to ergosterol¹⁵. As per the available records Sulfamethoxazole, Sulfamethoxazole-trimethoprim¹⁶, Sulfamethoxazole- caspofungin¹⁷, flucytosine in combination with Amphotericin B¹⁸, caspofungin with flucytosine and the triple combination of caspofungin and flucytosine with Amphotericin B¹⁹ act synergistically against *Aspergillus* sp. infection. Fungus is eukaryotes like mammalian cells and hence compound that inhibits the protein, RNA, DNA biosynthesis in fungi can also affect equally to the human being. Those antibiotics which targets the cell wall of fungi, do not affect the mammalian cells because of structural differences. In the present context, attempt was made to study the alteration in structure and its biochemical content of *Aspergillus terreus* by treating it with antibiotic of *Streptomyces* sp. JF714876.

MATERIAL AND METHOD**Structural changes in the antifungal compound treated *A. terreus***

An antifungal compound was obtained by the ethyl acetate solvent extraction and purification from the *Streptomyces* sp. JF714876. *Aspergillus terreus* was sub cultured on PDA plates prior to the experiments. To examine the morphological changes fungus was inoculated in PDA plate containing antifungal antibiotic with concentrations 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml and incubated for four days. After incubation the square samples (10 by 10 mm) of *A. terreus* grown in presence of antifungal compound at concentrations 10, 20, 30, 40 and 50 µg/ml were cut off from plates and mounted in cotton blue and examined microscopically for morphological changes

Biochemical changes in the treated *A. terreus*

A loopful of fungal cells were seeded in to 50 ml of potato dextrose broth in Erlenmeyer's flask and grown at 35°C for 24 h on shaker. This culture was used as an inoculum for experiment. 1 ml of culture mycelia was inoculated in to 100ml of fresh potato dextrose broth with 30µg/ml of antifungal compound. The flask without antibiotic served as a control. Both flasks were incubated at 35°C for four days with constant shaking. After incubation the content from flask was centrifuged at 10,000 rpm for 10min. Supernatant was used for the quantitative estimation of carbohydrate, protein, amylase, peroxidase and catalase following the method given by Morris²⁰, Lowry et al²¹, Bernfeld²² and Sadasivum and Manikum²³, respectively. Difference in the biochemical content between treated and untreated fungus were recorded.

RESULT AND DISCUSSION

The microscopic investigation revealed swelling of hyphae with changes in morphology of mycelial structure by treating the fungus with moderate concentration (10µg/ml) of antifungal compound. Further examination proved that increase in the concentration of compound above 10µg/ml to 30µg/ml leads to the distortion and deformation of mycelial structure. The distortion in structure of mycelia may be because of change in fungal membrane permeability where loss of cytoplasm leads to the flattened mycelium. These deformed structures were unable to form conidiophores and conidia. The highest concentration of compound above 30µg/ml was fungicidal, where growth of fungal mycelium completely inhibited. These results indicate that the antifungal compound is mainly targeting the fungal cell wall.

Along with the structural alteration in the antifungal compound treated *A. terreus*, biochemical content also changes. The protein

and carbohydrate contents in the treated *A. terreus* were decreased from 38mg/ml to 6mg/ml and 1.3mg/ml to 0.4mg/ml, might be because of the inhibition of protein and carbohydrate synthesis. Enzyme activity of peroxidase, catalase and amylase was also decreased from 0.083 μ m/ml/min to 0.07 μ m/ml/min, 1.11 μ m/ml/min to 0.86 μ m/ml/min and 0.0011unit/ml/min to 0.00057 unit/ml/min, respectively. This result indicates that the antifungal compound is either affecting the synthesis of the biochemically useful metabolite or degrades the metabolite present in the fungi.

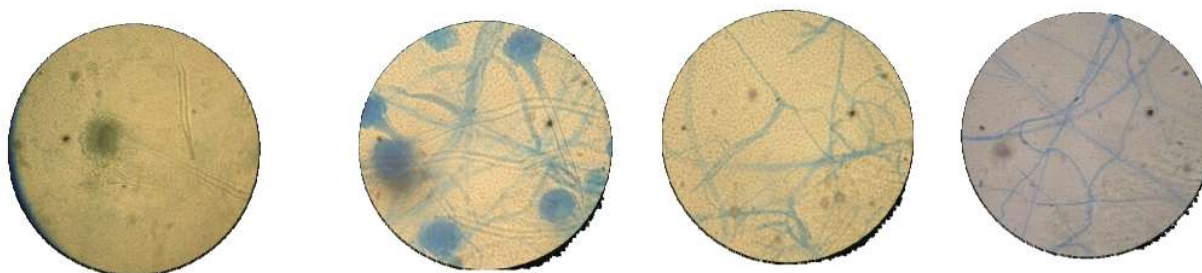
DISCUSSION

Antifungal compound act differently on fungi and leads to the complete fungal inhibition. In the present investigation, highest concentration of antifungal metabolite showed complete fungal inhibition²⁴, and moderate concentration of compound act on fungal wall leads to hyphal swelling and morphological change in fungal

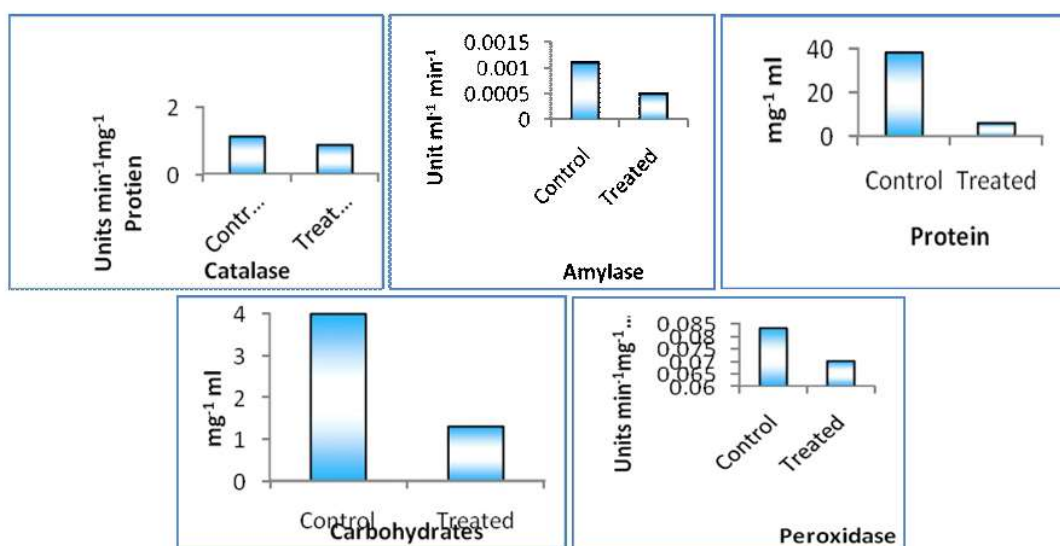
mycelia^{5,25}. There are reports on structural changes such as, increase in the thickness of fungi^{26, 27}, hyphae abnormally grown, stunted, shortened and highly branched with bipolar and vesicular tip swollen germ tube^{28, 29}, swelling of hyphae with abnormal deposition of chitin³⁰, decrease in the hyphal length and fungal metabolic activity¹⁷, increased hyphal branching, tip splitting, and multiple branches from single compartments, in addition curved hyphae³¹ by treating it with antifungal compound. Change in the morphology of *Candida albicans* cell was observed by treating it with Micafungin³². Antifungal substances from *Pseudomonas aeruginosa* act on asexual sporulation of fungi which affect and inhibit formation of conidiophores and conidia³³. Similar results were obtained when 10 μ g/ml to 30 μ g/ml of compound was added to the culture of *Aspergillus terreus*³⁴.



Fig 1- *Aspergillus terreus* treated with 10 μ g/ml (a) and without (b) antibiotic



Triocular microphotograph of untreated and antifungal compound treated *A. terreus* hyphal cells incubated at 30°C without (a) and with antifungal compound at the concentration of 10 μ g/ml (b), 20 μ g/ml (c) and 30 μ g/ml (d). After incubation for 3 days, samples were stained with cotton blue and observations were made.



CONCLUSION

It can be concluded that the antifungal compound extracted from *Streptomyces* sp. JF714876 is effective against fungi showed slight

to moderate change in morphology of fungal mycelium. At higher concentration, the compound leads to complete distortion and inhibition of fungal growth. This indicates that the compound might

be directly targeting the cell wall of fungi. There is also decrease in the amount of biochemical content as compared to control fungi. Hence, the compound can be used as an effective antifungal compound in the treatment of fungal diseases.

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