PROTEOMIC ANALYSIS OF DRUG RESISTANT HIGH BIOFILM FORMING CLINICAL ISOLATES OF CANDIDA TROPICALIS ON TREATMENT WITH AQUEOUS GARLIC EXTRACT

Project Reference No.: *45S_BE_3986*

College ***:*** *Ramaiah Institute of Technology, Bengaluru*
Branch ***:*** *Department of Biotechnology*
Guide(s) ***:*** *Dr. Bindu S*
Student(S) ***:*** *Ms. Simrah Suhail Khan*
Ms. Janvi Tirlapur
Ms. Vaishnavi Murugan
Ms. Shreeya Kumaresan

Keywords:

Candida Tropicalis, Proteomics, Garlic Extract.

Introduction:

Candida tropicalis is one of the major candidemia agents, associated with the highest mortality rate. It has been identified as a powerful biofilm builder, outperforming C. albicans in most experiments. (Zuza-Alves et al., 2017)

The biofilm lifestyle of yeast cells is known to be more resistant to antifungal drugs and to escape host immunological responses. (Chatrath et al., 2019)

Several recent studies have revealed that C. tropicalis has become resistant to antifungal medicines already on the market, including azole derivatives, amphotericin B, and echinocandins. (Choi et al., 2016)

The inability to eradicate germs in the biofilm mode of growth and the rising resistance of microbes to antimicrobial medications has stimulated research on natural antimicrobial agents. (Saharkhiz et al., 2021)

A study was performed to evaluate the antifungal activity of aqueous garlic extract with fluconazole against common clinical isolates of the Candida species. The strongest activity of garlic extract was seen against C. tropicalis (MIC=0.78mg/ml). (Jafari et al., 2015).

Objectives:

These objectives are ongoing.

1. Candida tropicalis clinical isolates growth and treatment with aqueous garlic extract.
2. Sample preparation and fractionation for mass spectrometry.
3. Liquid chromatography and mass spectrometric analysis.
4. Data processing and analysis for identification of proteins.

Methodology:

Candida tropicalis clinical isolates growth and treatment with aqueous garlic extract:

Candida tropicalis clinical isolates, 1 high and 1 low biofilm formers will be used for this study. The cells will be cultured in tryptic soy broth (TSB) at 37°C with shaking (120 RPM) for 8– 10 hours.

Preparation of aqueous garlic extract:

The aqueous garlic extracts will be prepared with a modification of (Dahiya and Purkayastha 2012) by crushing 20 g of sample in 10 ml of sterile water in a pestle and mortar followed by centrifugation at 10000 rpm for 10min at 4°C. The supernatant will be filtered using a Whatman filter paper and the whole aqueous extract will be used for further study.

Sample preparation fractionation for mass spectrometry:

The cell pellets will be disrupted with sand, centrifuged at 10,000 g for 15 min and the clear supernatant will be collected. Protein estimation for cell lysate will be performed using Bradford method.

In-gel digestion and in-solution digestion:

For both total proteome and secreted fraction, the protein will be resolved on a 10% SDS-PAGE gel and stained with colloidal Coomassie blue. In-gel digestion was performed for these protein gel bands as described by Balakrishnan et al., 2014.
Whole cell lysate samples will be be subjected to in-solution digestion.

Liquid chromatography and mass spectrometry:

LCMS will be performed on the samples to characterize the proteins.

Data processing and analysis:

Database searches for peptide and protein identification The MS/MS data acquired will be searched against two databases:

1. C. tropicalis protein database.
2. six-frame translated genome.

The whole genome and protein databases will be downloaded from the resources of Broad Institute. The searches will be performed using SEQUEST and MASCOT through Proteome Discoverer (Version 1.4) software suite. Only those peptide-spectrum matches (PSMs) that qualify a 1% false discovery rate will be considered as authentic identifications.

Results And Conclusion:

We are doing a comparative proteomic analysis of the effect of aqueous garlic extract on high and low biofilm forming clinical isolates of Candida tropicalis. Aqueous garlic extract has an antifungal effect on Candida tropicalis. The antifungal effect of 2 mg/mL of aqueous garlic extract is higher than 10 mcg of fluconazole.

Since the protein concentration of the treated samples is less than the protein concentration of the control samples, we know that aqueous garlic extract has an inhibitory effect on the protein expression of Candida tropicalis.

To characterise the proteins that are affected by aqueous garlic extract, we will be performing LC-MS on whole cell lysate samples. We will also perform SDS-PAGE and perform LC-MS on excised and trypsinized protein bands.

Scope For Future Work:

The infections of Candida tropicalis are often associated with biofilm formation. Treatment of HBF clinical isolates of C. tropicalis with aqueous garlic extract could address the issue of increasing resistance to the drug. The proteomic analysis of the effect of aqueous garlic extract on C. tropicalis isolates could elucidate the mechanism of action and aid in the development of an alternate antifungal therapy.