Screening of pathogenic strains of *Pseudomonas aeruginosa* in wound infections using a mushroom myco-model as a host - a novel approach

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ABSTRACT

Introduction and Aim: The detection of pathogenic strains of *Pseudomonas aeruginosa* is usually carried out by antimicrobial susceptibility testing, molecular characterisation, selective isolation, 16S rRNA sequencing, immunological assays and through other methods. This study aimed to use mushroom as a host model for detecting pathogenic strains of *P. aeruginosa* in wound infection.

Materials and Methods: The strains of *P. aeruginosa* from wound infection were isolated. The pathogenic, non-pathogenic and ATCC strains of *P. aeruginosa* as well as *Escherichia coli* were injected respectively into the cap region of the mushroom and incubated for the production of lesion. The collagen was extracted from mushroom by salt precipitation method and pure collagen was purchased commercially. The collagen degradation by all the above organisms was detected by performing UV spectrophotometric analysis. The organisms were allowed to grow on medium containing extracted collagen and pure collagen.

Results: The lesions were found at the site of the injected region on mushroom cap when injected with pathogenic strain of *P. aeruginosa* while the rest of the strains failed to produce lesion. The collagen was degraded when infected with the pathogenic strain and the rest of the strains failed to degrade collagen. This is in accordance with the OD value. The medium containing collagen also showed growth only for pathogenic strain of *P. aeruginosa*.

Conclusion: Thus, the study proved that mushroom can be used as a host model to differentiate the pathogenic and non-pathogenic strains of *P. aeruginosa* from wound infections.

Keywords: Collagen; wound infection; mushroom; mycomodel; *Pseudomonas aeruginosa*.

INTRODUCTION

A large number of detection methods have been developed utilizing the optical, electrochemical, biochemical and physical properties of pathogenic bacteria. The predominant techniques currently used to identify microbial pathogens rely upon conventional clinical microbiology monitoring techniques. Usually, immunological and molecular methods are followed for screening of pathogenic strains. It is reported that animals, plants, insects can be used as host models. Hence, in this present study, an innovative approach is tried out using mushroom as a host model to screen the pathogenic strains of *Pseudomonas aeruginosa* in wound infections by injection method and UV spectrophotometric analysis.

Collagen is the major protein component of human skin. Collagenase is an enzyme produced by *P. aeruginosa*, which penetrates human skin by utilizing collagen (1). It breaks down the peptide bond and liberates hydroxyproline from collagen (2). Collagen acts as a source for the pathogenic bacteria to grow and survive in a wound. Since mushroom contains collagen, it can be used as a source to identify pathogenic *P. aeruginosa*.

Hence, in the present study, mushroom is used as model to determine the pathogenic strains of *P. aeruginosa.* Collagenase acts as the key factor in detecting the pathogenic strains of *P. aeruginosa* by collagen degradation. Based on degradation of collagen due to injection of pathogenic isolate of *P. aeruginosa* lesions are produced by the organism on the mushroom, which can be used as an identification system. Lesions are directly related to the pathogenicity of the strain (3). It has been found that the size of lesions is directly related to the measure of pathogenicity (4).

MATERIALS AND METHODS

The samples were collected from wound infections at the Government Hospital of Tambaram Sanatorium, Chennai and the strains of *P. aeruginosa* were isolated by standard biochemical procedures.

Injection method

The button mushrooms (*Agaricus bisporus)* were purchased commercially and thoroughly checked to be lesion free with fresh cap regions. Mushrooms were injected at the cap region respectively with 75µl of pathogenic, non-pathogenic and ATCC strains of *P. aeruginosa* (ATCC-27853) as well as *Escherichia coli*. A mushroom injected with sterile saline was used as negative control. The injection procedure was carried out using microinjection needle by saline soaking method and moist chamber method.

The injected mushrooms were soaked in a glass beaker with 10 ml of sterile saline (Saline soaking method) and the injected mushrooms were placed in a beaker provided with a moist chamber (Moist chamber method). The beakers were covered tightly and incubated at 37°C for 24 hours. The method of injection was carried out at an angle of 30° as depicted in the fig. 1.

Fig.1: Injection method: Injection of bacteria on the cap region of the mushroom

Collagen extraction from mushroom

The collagen protein was extracted from mushroom by salt precipitation method with the following steps like drying, grinding, extraction and purification followed by ninhydrin test.

Drying the mushroom

The mushrooms were dried to remove their moisture content by spreading it on a tray. The tray was placed in hot air oven for 4 hours at 80°C for further drying then the mushrooms were sun dried for 2-3 days.

Grinding the mushroom

The dried mushrooms were powdered finely using a mixer. The mushroom powder was used for the collagen extraction. The collagen extraction was carried out using salt precipitation. The extraction was carried out in neutral salt solutions by the gradual addition of sodium chloride.

Extraction and purification of collagen

The sodium chloride solution (0.45M) was prepared with a pH of 7.5 with stirring for 24 hours. The test tubes were filled with 3ml sample and the salt solution was added gradually along with regular stirring. After 24 hours the precipitated protein was subjected for purification. In the process of purification, all the added salts must be removed from the collagen. The precipitate was mixed with a buffer containing SDS, Tris-HCl and phenol for 3minutes. The precipitate that comes out of it will contain salt less concentrated collagen.

Ninhydrin test

A small quantity of extracted compound was allowed to react with 2% Ninhydrin. Ninhydrin solution was prepared by dissolving 0.8g of Ninhydrin in 10ml of acetone or ethanol. Ninhydrin solution was allowed to react with the extracted product. After 5 minutes the tubes were checked for any colour change. The negative control maintained was non collagen protein while positive control was pure collagen. Pure collagen was commercially purchased.

UV spectrophotometric analysis

Both extracted and pure collagen was used separately as sources for UV spectrophotometric analysis. The test tubes were provided with minimal media of collagen and sodium chloride. The different tubes were inoculated with pathogenic, non-pathogenic and ATCC strains of *P. aeruginosa* as well as *E. coli*. The test tubes were incubated at 37°C and the samples were collected for every hour until 5 hours. The readings were noted under UV spectrophotometry at a wavelength of 280nm. The trials were carried out in triplicates.

Medium containing collagen

Pure collagen medium and extracted collagen medium was prepared. Pure collagen medium constituted pure collagen powder, sodium chloride and agar powder while extracted collagen medium constituted with extracted collagen powder, sodium chloride and agar powder. The pathogenic, non-pathogenic and ATCC strains of *Pseudomonas aeruginosa* as well as *Escherichia coli* were streaked and incubated at 37° C for 24 hours.

Statistical analysis

One-way ANOVA was carried out for the triplicate values of UV spectrophotometric screening procedure using MS-Excel.

RESULTS

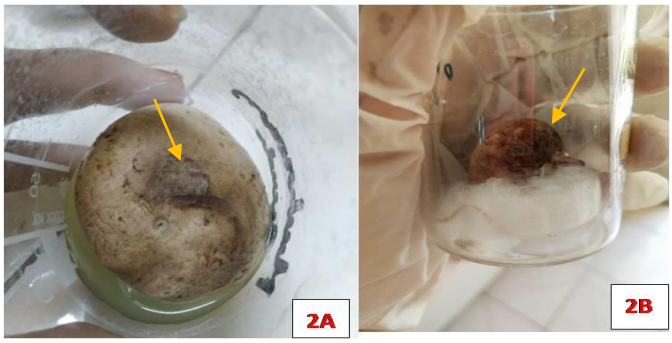

Fig. 2A & 2B: Positive reaction: Lesion was observed on mushroom at the site of injected area when injected with pathogenic strains of *P. aeruginosa.*

Fig. 3A & 3B: Negative reaction: No Lesion was observed on mushroom at the site of injection when injected with non-pathogenic strains of *P. aeruginosa*

Injection method

The strains isolated were found to be *Pseudomonas aeruginosa*. The injection method resulted in lesions at the site of injection only when injected with pathogenic strains of *Pseudomonas aeruginosa* (Fig. 2A & 2B) while the rest of the strains showed no lesion (Fig. 3A & 3B).

Collagen extraction from mushroom

The extracted collagen was confirmed by ninhydrin test showing positive result due to presence of hydroxyproline.

UV Spectrophotometric analysis

The OD values showed rapid decrease in case of pathogenic strains of *P. aeruginosa* while for the rest of the strains they remain unchanged in both the extracted and pure collagen (Fig. 4).

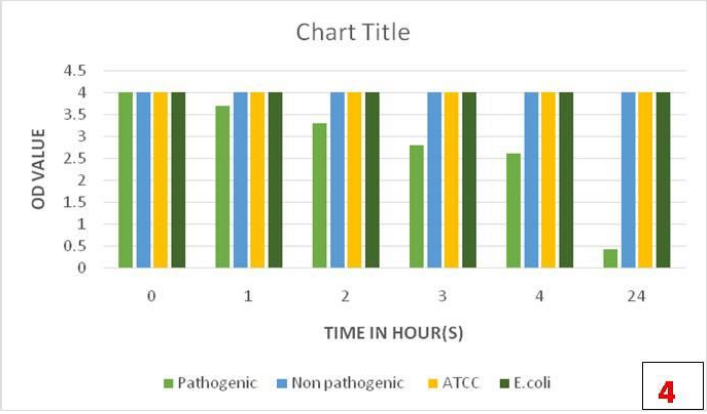

Fig. 4: Collagen degradation: The collagen levels decreased in case of pathogenic strains of *P. aeruginosa* while for rest of the strains they remain unchanged.

Medium containing collagen

Green coloured colonies were observed on the medium with collagen when inoculated with pathogenic strains while the rest of the strains failed to grow on the medium containing collagen.

The results were obtained due to the production of collagenase enzyme by the pathogenic strains of *P. aeruginosa* present in the wound infection which degrades the collagen of the mushroom while the non-pathogenic strains fail to produce collagenase enzyme.

Statistical analysis

In accordance with the one-way ANOVA, there was significant difference at α=0.05 between the OD

values of pathogenic isolates of *P. aeruginosa* and ATCC strain of *P. aeruginosa*. The null hypothesis was accepted since the critical value was smaller than that of F value.

DISCUSSION

The present study focussed on using mushroom as a host model to screen pathogenic isolates of *P. aeruginosa* in wound infection. It is very difficult to handle laboratory animals for pathogenic identification hence mushroom can be used as a host model. Presence of collagen in mushroom has been reported (5). This method would be cost effective than other screening procedures.

In the past few years, a number of different invertebrate host model systems have been described. Experiments with plants, insects, protozoa, nematodes and slime moulds have recently come to the forefront in the study of pathogenic microorganisms (6). Among the alternative experimental models, plants were economic screening tool to identify putative virulence determinants (7).

A recent study suggested the use of plant *(Lactuca sativa)* as a model for screening the pathogenic isolates of *P. aeruginosa* from clinical samples in which the pathogenicity level varies from strain to strain (4). A similar work was carried out in the present study-using mushroom as a host model.

Inoculation procedure was performed using mushrooms in which 15 mushrooms were inoculated by dispensing a drop of appropriate *Staphylococcus aureus* cell suspension on the cap for enterotoxin production (8). With the above reference, the inoculation procedure was carried out in this study on the cap region of mushroom.

Thus, the present study showed that mushroom can be used as a host model to screen pathogenic isolates of *P. aeruginosa* present in wound infections. Future studies are warranted to screen other wound infecting pathogens capable of producing collagenase enzyme.

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