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RESEARCH ARTICLE

INVESTIGATION OF ANTAGONISTIC ACTION OF *PSEUDOMONAS FLURESCENS* BESIDE HUMAN SKIN PATHOGENS

***,[1]Elamathi and [2]Mohamed Tariq, N. P. M.**

[1]Department of Microbiology, Kamban College of Arts and Science for Women, Thiruvannamalai
[2]Department of Biotechnology, Islamiah College, Vaniyambadi

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ABSTRACT

Isolate Pseudomoans fluoresces for studying the multiple antagonistic activity. The five different rhizosphere soil samples were collected and inoculated into selective media and their morphology was observed. Human skin swabs were collected for the isolation of five different pathogens such as Staphylococcus aureus, Streptococcus, Bacillus subtilis, Salmonella, Clostridium. The morphology characteristic of human skin pathogens causing microorganisms identified by performing Gram's staining, capsule staining, motility test. The biochemical characteristics of five bacterial human pathogens causing micro organisms were studied. Pseudomonas fluorescens skin infections caused by various pathogens can be prevented.

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INTRODUCTION

Pseudomonas species is a common bacteria which can cause disease in animals and humans. It is found in soil, water, skin flora and mostly man made environment throughout the world. It thrives not only in normal atmosphere but also with little oxygen and artificial environment. It uses a wide range of organic materials for food in animals. The symptoms of such infection are generalized inflammation and sepsis in lungs, urinary track and kidney results can be fatal. Because it thrives on most surfaces, this bacteria is also found in medical equipment including cathodes, causing cross infections in hospitals and clinicals. *Pseudomonas* secretes a variety of pigments including pyocyanin (blue – green), Pyoverdin (Yellow – green) Pyorubin (red brown). *Pseudomonas* is a gram negative, aerobic rod shaped bacteria with unipolar motility. It is an opportunistic human pathogen and also plant pathogen. The genus *Pseudomonas* is one of the most diverse Gram negative bacterial genera isolated from sources ranging from plants to soils and water of this genus are straight or slightly curved rods, motile by means of polar flagella. Antagonistic action was also observed for *Pseudomonas* species in the rhizoshpere has been recognizes as major factor in the suppression of many phytopathogens.

***Corresponding author: Elamathi**
Department of Microbiology, Kamban College of Arts and Science for Women, Thiruvannamalai

Bacteria of the genus *Pseudomonas* comprise a large group of the active biocontrol strains as a result of their general ability to produce a diverse array of potent antifungal metabolites. *Pseudomonas fluorescens* have received considerable attention as potential biocontrol agent of a number of soil borne pathogens. *Pseudomonas fluorescens* from soil, to check its antagonistic activity and effect of its secondary metabolites on human pathogens by invitro techniques.

Common skin infection include cellulitis, erysipealas, impetigo, folliculitis and furuncles and carbuncle. Cellulitis is an infection of the dermis and subcutaneous tissue that has poorly demarcated borders and is usually caused by *Streptococcus* or *Staphylococcus* species. Erysipelas is a superficial form of cellutis with sharply demarcated borders and is caused almost exlusively by *Streptococcus*. Impetigo is also caused by *Streptococcus* or *Staphylococcus* and can lead to lifting of the stratum corneum resulting in the commonly seen bullous effect. Folliculitis is an inflammation of the hair follicles. When the infection is bacterial rather than mechanical in nature, it is most commonly caused by Staphylococcus if the infection of the follicle is deeper and involves more follicles, it moves into the furncle and carbuncle stages and usually required incision and drainage. All of these infections are typically diagnosed by clinical presentation and treated empirically. If antibiotics are required, one that is active against gram – positive organisms such as penicillins,

cephalosporins, macrollides, or fluoroquinolones hould be choosen. Bacterial skin infections are the 28th most common diagnosis is hospitalized patients.

REVIEW OF LITERATURE

In recent times, there has been a renewed interest in the search of plant growth promoting rhizobacteria (PGPR) for sustainable crop production. Rice is an economically important food crop, which is subjected to infection by a host of fungal, viral and bacterial pathogens. In this study, an attempt was made to isolates *Pseudomonas* sps a potent PGPR in the rhizophere. Augmentation of such PGPR including, *Pseudomonas* in the rice ecosystems will ensure a healthy micro climate for rice. (Prakash Nathan *et al*., 2011).

All but nine of the inhibiting strains were found to inhibit the growth of 38 psychorotrophic *Staphylococcus putrefaciens* strains isolated from spoiling fish and fish products. Siderophore containing *Pseudomonas* culture supernatant inhibited growth of *Staphylococcus puterfaciens* as did the addition of the chelators (ethyl enediamine dinydroxy phenylacetic acid (EDDHA). In particular, *Pseudomonas* strains from newly caught and spoiled Nile perch (tales niloticus) inhibited *Staphylococcs putrefaciens*. This suggests than microbial interaction (e.g competition or antagonism) may influence the selection of a micro flora for some chilled food products. (Longe Gram, 1993).

Pseudomonas fluorescens CHAO colonizes plant roots, produces several secondary metabolites in stationary growth phase and suppresses a number of plant diseases, including thielavcopsis bascicola-induced black root rot of takacco. We discovered that mutations in a *Pseuodomonas* gene named gacA (for global antibiotic and cyanide control) pleiotropically block the production of the Secondary metabolites 2,4 diactyl, phloroglucinol (phl), HCN and pyluteorin. The gacA mutants of strain CHAO have a drastually reduced ability to suppress black root rot of the gacA gene is directly followed by a uvrc gene. Double gacA-uvrc mutations render *Pseudomonas fluorescens* by a uvrc gene specifies a trans – active 24 – KDA protein. Sequence data indicate that the GACA protein is a response regulator in the fixJ / Degu family of 2 component regulator of secondary metabolism in *Pseudomonas fluorescens*. (Jacques lavile *et al*., 1992)

Each *Pseudomonas fluorescens* strain successfully colonized alfalfa at adequate densities for biocontrol activity. Results showed that *Pseudomonas fluorescens* strains provide 10-13% increase in the number of established plants relative to the control, an intermediate compared to the fungicide treatment. Therefore, results this study demonstrated that the three *Pseudomonas fluorescens* provided effective control against soil-born pathogens and suggest a potential use in the development of a commercial inoculants to be applied for control of legume seedling diseases. (Leticia Quagliotto *et al*., 2009)

The production, isolation and characterization of an antibiotic substance from cultures of *Pseudomonas fluorescens* 2-79 (NRRL B-15132) is described. *Pseudomonas fluorescens* 2-79 originally was isolated from the roots of wheat and is suppressive to the wheat root disease take – all caused by *Gaeumannomyces graminis var*, *tritici.* The antibiotic was isolated form potato glucose broth cultures of strain 2-79 by solvent extraction. It was purified by silica gel column chromatography and was a greenish yellow, needle – shaped crystal with a melting point of 242 degrees C(Decomposition). It was soluble in methylene chloride, chloroform, acetone, 2 N sodium hydroxide, and 2 N hydrochloric acid and was insoluble in water. The antibiotic showed excellent activity against several species of fungi, including the wheat pathogens *Gaeumnnomyces graminis var. tritici, Rhizoctonia solani,* and *Pythium aristosporum* and it any have a role in suppression of take – all in vivo by strain 2-79.(Gurusiddaiah *et al*., 1986)

In this study, *Pseudomonas* species were isolated from the rhizospheres of two plant hosts rice and maize. This analysis showed that both plant varieties selected for two distinct populations of *Pseudomonas.* There was a significant difference between isolates from rice and maize rhizosphere in terms of biological control against *Bacillus subtillis.* (Phueksa Lawongsa *et al*., 1984)

Thirty isolates of *Pseudomonas fluorescens* obtained from rice rhizosphere were tested for antifungal activity against *Magnaporthe grisea*. *Dreschelaria oryzae*, *Rhizoctonia solani and sarocladium oxyzae* that are know to attack rice plants. One *Pseudomonas fluorescens* isolate (*Pseudomonas fluorescens* 003) effectively inhibited the mycelia growth in all there fungi in dual cultures test (62-85%). The antifungal compound were extracted with equal voulume of ethyl acetate. The antifungal compounds from *Pseudomonas fluorescens* at 5% completely inhibited the pathogens. One compound with Rf 0.35 on TLC completely inhibited the mycelia growth of all test fungi at 0.5%. This compound showed melting point at 168-173c. The proton nmr and C^3 nmr confirmed its idenfity as 2,4 diacetyl phloroglucinol (DAPG). (Reddy *et al*., 2007)

Antagonistic effects of *Pseudomonas fluorescens*, *Pseudomonas aeruginosa,Pseudomonas putida* were studied against 12 strains of *Aeromonas hydrophila* (Ah1-Ah12).

Four different fractions of cellular components (i.e whole cell product, heat killed whole cell product, intra cellular product and exta cellular product) of all *Pseudomonas species* were equally effective in reducing growth of *Aeromonas hydrophila* strain as measured by the zone of inhibition in an invitro sensitivity test and have potential action against *Aeromonas hydrophila* infection in fishes. (Basanta Kumar Das *et al*., 2005)

MATERIALS AND METHODS

Collection of human pathogen from skin swabs

The specimen for culture is obtained as skin swab there were totally 10 skin swabs were collected and all where used to isolate and identify organisms that caused skin infection. All the 10 samples were screened for gram's staining and biochemical test to identify the bacteria causing skin infection.

Preparation of bacterial culture from skin swabs

24 hours old culture of selected bacteria was mixed in nutrient broth and turbidity was observed after 24 hours of incubation.

Determination of inhibitory effect of *Pseudomonas fluorescens*

AGAR WELL DIFFUSION METHOD

- Muller-hinton agar was sterilized and then poured in petriplates and allowed to solidify.
- By lawn culture, the culture was swabbed on medium using sterile cotton swab.
- A well was made on the centre of the medium.
- Then the *Pseudomonas fluorescents* was inoculated in the well using micro pipette at the different concentration of µg/ml.
- The plates were then incubated at 37^0 C for 24 hours.
- After incubation the diameter of zone of incubation was observed.

Antibacterial activity test using disc diffusion method

- Whatman no:1 filter paper disc (5mm) were prepared. The discs were sterilized by autoclave at 121^0 C.
- After sterilization the discs were dipped into 10µl of the centrifuge culture of *Pseudomonas fluorescens* and allowed to dry at room temperature.
- The discs were placed on muller- hinton agar plates which were seeded with respective test organisms.
- The plates were incubated in an upright position at 37^0C for 24 hours.
- The diameter of inhibition zones formed was measured in mm and the results were recorded.

RESULTS AND DISCUSSION

The rhizosphere soil samples were collected and serially diluted, with distilled water. From 10^{-4}, 10^{-5} and 10^{-6} dilution factor, 1ml was transferred to 3 nutrient agar plates respectively. It forms ubiquitous colonies on nutrient agar plate. The colonies formed on nutrient agar was transferred into cetrimide agar.

Table: Inhibitory activity of *Pseudomonas fluorescens* against human pathogens (agar well diffusion method)

S.No	Pathogens tested	Zone of Inhibitin (MM)				
		10µL	20 µL	50 µL	75 µL	100 µL
1	*Bacillus subtills*	16	18	19	20	22
2	*Staphylococcus aureus*	15	17	19	20	21
3	*Clostridium*	-	-	-	-	-
4	*Streptococcus*	18	20	21	21	22
5	*Salmonella*	21	22	25	25	26

On cetrimide agar, *Pseudomonas fluorescens* produced yellowish green coloured, smooth, opaque, shiny, large, convex colonies. In Gram's staining, *Pseudomonas fluorescens* showed gram negative rods. In capsule staining, it showed capsulated bacilli. Its motility was demonstrated by performing hanging drop preparation method. It showed swaming motility.

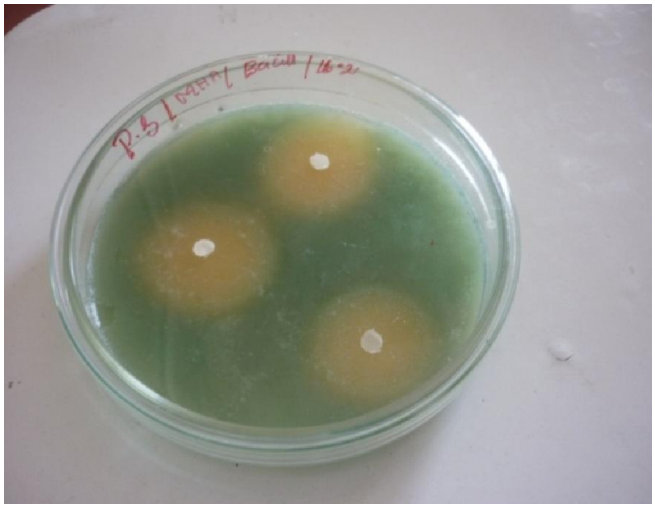

Fig. 1. Antagonastic action of *Pseudomonas fluorescens* beside bacillus subtilis (agar well diffusion method)

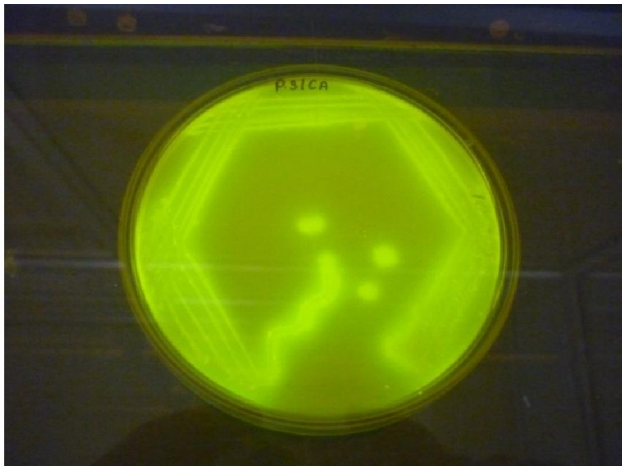

Fig.2. Isolation of *Pseudomonas fluorescens* on cetrimide agar plate

The skin infection samples were collected and inoculated into nutrient agar plates. On nutrient agar skin swab produce smooth, opaque, shiny, large, convese colonies. Morphological characteristics of human skin pathogens were identified by gram staining. They showed gram positive cocci in chain, gram positive rods. Gram negative rods etc. In capsule staining, out of five skin pathogens two were capsule and 3 were non capsulatated. In motility test, out of five skin pathogens, only one gram negative rod, showed motility other four organism are non – motile. *Staphylococcus aureus* showed golden yellow, opaque, round shiny, spherical cocci, grape like clusters.

I may be identified as staphylococcus aureus and also compared with standared strain of staphylococcus aureus. *Streptococcus* showed circular are small, low convex disis, spherical or ovoid in shape, smaller are arranged in chains. It may be identified as streptococcus and also compared with standard strainof streptococcus. *Bacillus subtilis* showed round, dull, opaque, irregular larger colonies. It may be

identified as bacillus subtilis and also compared with standared strain of Bacillus subtilis. *Salmonella* showed black colour colonies, circular, low convex, smooth, shiny colonies. It may be identified as salmonell and also compared with standard strain of salmonella. *Clostridium* showed straight or slighty curved rod and frequently swollen at both ends. It may be identified as clostridium and also compared with standard strain of clostridium. The antagonistic activity of *Pseudomonas fluorescens* was determined by measuring the zone of inhibition observed on muller hinton agar. The result showed in Table V. The diameter of zone of inhibition for *Staphylococcus aureus* was 21 mM. The diameter of zone of inhibition for Bacillus subtilis was 22mM. The diametes of zone of inhibition for *Salmonella* was 26 mM. The diameter of zone of inhibition for *Streptococcus* was 22 mM. The antagonistic activity of *Pseudomonas fluorescen*s in higher for *Salmonella*. The antagonistic activity of *Pseudomonas fluorescens* is lower for *Staphylococcus* aureus. The overall results of antoganistic action of *Pseudomonas fluorescens* show in Table.

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