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Original Article

Antibacterial activity of *Pseudomonas fluorescens* isolated from Rhizosphere soil

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Abstract

A proteobacterium was isolated from rhizosphere soil and it was identified using morphological, cultural and biochemical characteristics as *Pseudomonas fluoresecens*. *Pseudomonas fluorescens* possess a variety of promising properties which make it a better biocontrol agent. The objectives of the present study was to isolate and screen the antibacterial activity of *Pseudomonas fluorescens* against ten target bacterial pathogens of health significance like *Staphylococcus aureus, Streptococcus mutans, Bacillus subtilis, Aeromonas hydrophila, Vibrio cholerae, Escherichia coli, Klebsiella pneumoniae, Shigella sonnei, Salmonella typhi* and *Serratia marcescens* by *in vitro* techniques. The result indicated that strains of *Pseudomonas fluorescens* presented a significant value against *Salmonella typhi, Streptococcus mutans, Bacillus subtilis, Shigella sonnei* and no activity against *Staphylococcus aureus, Aeromonas hydrophila, Vibrio cholerae, Escherichia coli, Klebsiella pneumoniae* and *Serratia marcescens.*
Key words: *Pseudomonas fluorescens*; Bacterial pathogens; Antibacterial activity; Rhizosphere soil.

Introduction

Nature has been a source of medicinal agents for thousands of years. An impressive number of modern drugs have been isolated from microorganisms, mainly based on their use in traditional medicine. In the past century, however, an increasing role has been played by microorganisms in the production of antibiotics and other drugs (Fenical,1993). The importance of bacteria and fungi as sources of valuable bioactive metabolites is very well established for more than half a century. As a result, over 120 of the most important medicines (penicillins, cyclosporin A, adriamycine, etc.) in use today are obtained from microorganisms (Alanis, 2005).

Microorganisms have been the study of importance in recent years because of the production of novel metabolites, which exhibits antibacterial, antiviral, anti tumour as well as anticoagulant properties. Most of the current antimicrobial drugs are the derivatives of the earlier generation and microbial resistance against them further intensify the need for new drug discovery. Acceptable options available are the metabolites of plants or animal origin, which are biocompatible, biodegradable and non-toxic in nature. These metabolites are widely studied and are produced by various groups of microorganisms like *Pseudomonas* (Chain and Mellows, 1997) and *Streptomyces* (Shanshoury *et al*., 1996) which are studied for their secondary metabolites.

The genus *Pseudomonas* has been heterogenous since Migula first named it in 1894 (Migula,1894). He designated and described the species associated with the genus in 1895 (Migula, 1895). *Pseudomonas* is Gram-negative, strictly aerobic, polarly flagellated rods. They are aggressive colonizers of the rhizosphere of various crop plants, and have a broad spectrum antagonistic activity plant pathogens, such as antibiosis (the production of inhibitory compounds) (Cartwright *et al*., 1995; Rasales *et al*., 1995), siderophores production (iron-sequestering compounds) (Winkelmann and Drechsel, 1997) and nutrition or site competition (Bull *et al*., 1991). Some species of *Pseudomonas* can also produce levels of HCN that are toxic to certain pathogenic microorganisms (David and O'Gara, 1994). *Pseudomonas fluorescens* is an antibiotic producer and has a wide spectrum of

antimicrobial activity against *Salmonella typhimurium* (Laine *et al*.,1996), *Bacillus subtilis*, *Proteus vulgaris* and *Candida albicans* (Trujillo *et al*., 2007).

These characteristics make *Pseudomonas* species good candidates for used as seed inoculants, root dips for biological control of soil-borne plant pathogen and also as antibacterial agents. It also has got broad spectrum activity against *Staphylococcus aureus*, *Escherichia coli* and *Aeromonas hydrophila* (Vachee *et al*., 1997; Czerwonka *et al*., 1997). The purpose of this study was to examine the antimicrobial activity of *Pseudomonas fluorescens* against ten target bacterial pathogens of health significance such as *Staphylococcus aureus*, *Streptococcus pyogenes var mutans*, *Bacillus subtilis*, *Aeromonas hydrophila*, *Vibrio cholerae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella sonnei*, *Salmonella typhi* and *Serratia marcescens* by *in vitro* techniques.

Materials and Methods

Isolation of Pathogenic bacteria

The various target bacterial pathogens of health significance were obtained from Kings Institute, Chennai. The pathogenic bacterial cultures like *Staphylococcus aureus*, *Streptococcus mutans*, *Bacillus subtilis*, *Aeromonas hydrophila*, *Vibrio cholerae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella sonnei*, *Salmonella typhi* and *Serratia marcescens* were maintained by growing in Nutrient agar medium and stored as Glycerol stock at 4° C. Strains were propagated twice before use in experiments.

Isolation of Pseudomonas fluorescens from Rhizosphere soil

1gm of rhizosphere soil sample was suspended in 99 ml of sterile distilled water. Samples were serially diluted and 0.1 ml of sample was spreaded on nutrient agar plates and incubated at 37°C for 24 hrs. Colonies were counted and the results were expressed as CFU/ml. The isolated bacteria was confirmed by morphological (staining and motility), cultural (Nutrient agar, Cetrimide agar), biochemical tests (IMIC test, triple sugar iron test, nitrate reduction test, catalase test, oxidase test, starch hydrolysis, casein hydrolysis, lipid hydrolysis, gelatin liquefaction and carbohydrate tests).

Screening of Antibacterial Activity of *Pseudomonas fluorescens* isolated from rhizosphere soil by Agar well diffusion method.

Antibacterial activity of *Pseudomonas fluorescens* isolated from rhizosphere soil was tested against ten target bacterial pathogens of health significance like *Staphylococcus aureus*, *Streptococcus pyogenes var mutans*, *Bacillus subtilis*, *Aeromonas hydrophila*, *Vibrio cholerae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella sonnei*, *Salmonella typhi* and *Serratia marcescens* by *in vitro* techniques using Muller Hinton agar plates (MHA) at 37 °C for 24 hrs.

A fresh colony of potential antibacterial *Pseudomonas fluorescens* isolated from rhizosphere soil was inoculated in nutrient broth and incubated at 37 °C for 24 hrs. After swabbing the target pathogenic bacteria of health significance on the sterile Muller Hinton agar plates, wells of 6mm were punched for agar well diffusion assay method. Different concentration (20µl,50µl,75µl, 100µl) of overnight free culture broth (log phase) of potential antibacterial *Pseudomonas fluorescens* was centrifuged at 10,000rpm for 20min and poured in to the well of Muller Hinton agar plates. Then the plates were incubated at 37°C for 24 hrs, where upon inhibitory activity was observed as a zone of clearing around the wells. The diameter of the clearing zones was measured in mm using the ruler scale. The experiment was done in triplicate for each pathogenic bacterium.

Results

In the present study, soil samples were plated on nutrient agar. From soil samples, $1.3x10^7$ CFU/ml bacteria could be isolated. 54 strains were isolated and in that 5 strains were selected. Among these strains, one potential bacterial strain *Pseudomonas fluorescens* was selected and identified based on morphological, cultural and biochemical characteristics (Table-1) including carbohydrate fermentation tests and it showed activity against all the ten target bacterial pathogens of health significance (Table- 2, Fig. 1 A & B) and the experiment was done in triplicate for each pathogenic bacteria. Measurement of inhibition zones (expressed in mm) as observed in agar well diffusion assay method (Table-2).

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The culture filtrate showed antibacterial activity against only four pathogenic bacteria such as *Salmonella typhi*, *Streptococcus mutans*, *Bacillus subtilis*, *Shigella sonnei*. Maximum inhibition was found against *Salmonella typhi* (15.333 ± 0.577) and *Streptococcus mutans* (14.0 ± 1.000) from 20µl of *Pseudomonas fluorescens* onwards and no antibacterial activity against *Staphylococcus aureus*, *Aeromonas hydrophila*, *Vibrio cholerae*, *Escherichia coli*, *Klebsiella pneumoniae* and *Serratia marcescens.*

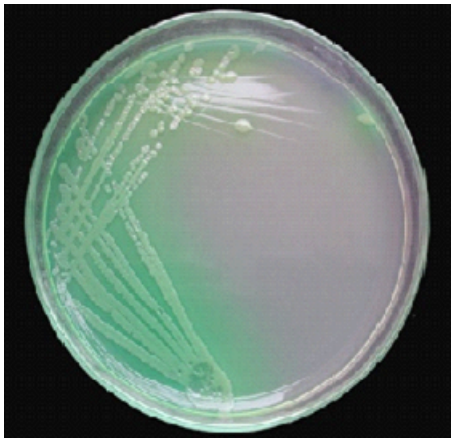

Fig.1A: Pure Culture of *Pseudomonas fluorescens* on Cetrimide agar under normal light

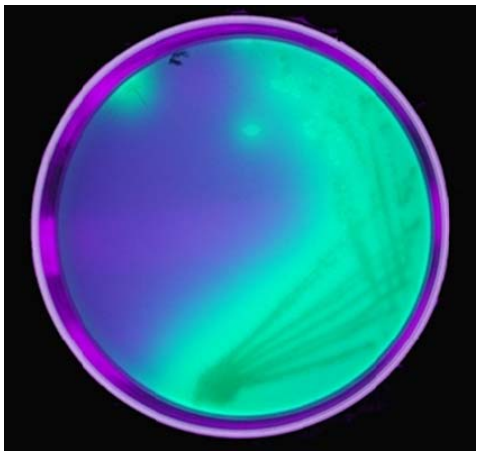

Fig.1B: Pure Culture of *Pseudomonas fluorescens* on Cetrimide agar under U.V light

Discussion

Bacteria - bacterial pathogen interactions are having an increased interest in the area of biocontrol. Many of the antibacterial interactions involved *Pseudomonas sp.* The genus *Pseudomonas* actively solubilizes phosphate *in vitro* and produces several antibiotics with high specificity against several microorganisms (Trujillo *et al.*, 2007) like *B. subtilis* and *P. vulgaris.*

Table-1: Morphological, cultural and biochemical characteristics of *Pseudomonas fluorescens*

Microbial isolate	Variables	Characteristics
Colony and Cell Morphology	Colony size	Large
	Surface	Irregular
	Opacity	Opaque
	Color	Yellow green
	Motility	Motile
	Cell shape	Rod
	Cell size	Small
	Gram's staining	Gram negative
Biochemical characteristics	Citrate	Positive
	Indole	Negative
	MR	Negative
	VP	Negative
	Oxidase	Positive
	Catalase	Positive
	TSI	Positive
	Nitrate reduction	Positive
	Gelatin liquefaction	Positive
	Starch hydrolysis	Negative
Carbohydrate fermentation test	Lactose	*Negative*
	Xylose	*Positive*
	Maltose	*Negative*
	Insulin	*Positive*
	Furctose	*Negative*
	Dextrose	*Positive*
	Galactose	*Positive*
	Trehalose	*Negative*
	Melibiose	*Negative*
	Sucrose	Negative
	L-arabinose	Positive
	Mannose	Positive
	Ruffinose	Negative
	Glycerol	Positive
	Salicin	Negative
	Glucosamine	Negative
	Dulcitol	Negative
	Inositol	Negative
	Sorbitol	Negative
	Mannitol	Negative
	Actonitol	Negative
	Ribose	Positive
	Cellobiose	Negative
	Melezitose	Negative
	Xylitol	Negative
	ONPG	Negative
	Esculin	Positive
	D-arabinose	Positive
	Malonite	Positive

In the present study, *Pseudomonas fluorescens* bacterial strains were isolated from rhizosphere soil and tested for its antibacterial ability against pathogenic bacteria. Undoubtedly the present study along with other studies reviewed through literature indicated that complicated interactions occur between microbe and microbes.

Table – 2: Inhibitory activity of *Pseudomonas fluorescens* against pathogenic bacteria.

S.No	Pathogens tested	Zone of Inhibition (mm)			
		20 µl	50 µl	75 µl	100 µl
1	*Aeromonas hydrophila*	-	-	-	-
2	*Serratia marcescens*	-	-	-	-
3	*Bacillus subtilis*	-	7.7 ± 0.58	10.7 ± 0.58	15.3 ± 0.58
4	*Staphylococcus aureus*	-	-	-	-
5	*Shigella sonnei*	-	-	-	15.3 ± 0.58
6	*Escherichia coli*	-	-	-	-
7	*Vibrio cholera*	-	-	-	-
8	*Salmonella typhi*	15.3 ± 0.58	17.3 ± 0.58	19.7 ± 0.58	20.7 ± 0.58
9	*Klebsiella pneumonia*	-	-	-	-
10	*Streptococcus mutans*	14 ± 1.0	15.7 ± 0.58	18 ± 2.000	19.7±0.58

Values are means ± SD of three sets of experiments in each set

The *Pseudomonas fluorescens* strain isolated from the study seemed to be highly potential in controlling bacterial pathogens of health significance. Further study in this strain may result in development of a potential biocide.

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