Research Article

Efficacy of the Bioactive Substance of *Pseudomonas Fluorescens* against Bacteria from Skin Infections

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ABSTRACT

Two isolates of *Pseudomonas fluorescens* were tested for production of bioactive substance as antibacterial agent against some pathogenic bacteria *(Klebsiella pneumoniae, Proteus mirabilis* and *Staphylococcus aureus)* isolated from skin infections (boils, impetigo, pus and wounds) which are also used as indicator isolates. The two isolates of *P. fluorescens* were positive as a producer of antibacterial agent. The clinical isolates *P. fluorescens* 1 and 2 were able to grow and inhibit the bacterial growth of *K. pneumoniae, P.mirabilis* and *S.aureus* by using nutrient agar. On TSA agar the diameter of inhibition zones for three genera was; *K. pneumonia* (10-16mm.), *P.mirabilis* (12-13mm.) and *S.aureus* (15-17 mm.) which increase than in using NA. In BHA the diameter of inhibition zone was (18-20 mm.) for *S.aureus,* (13-17 mm.) for *K.pneumoniae* and (13-17 mm.) for *P.mirabilis*. Agar block method was used for detection of antibacterial agent from *P. fluorescens*. The incubation temperature at 37°C was suitable for *P. fluorescens* from clinical specimens to produce its antibacterial activity.

Keywords: *Pseudomonas fluorescens,* antimicrobial agent, pathogenic bacteria.

INTRODUCTION

The over prescription of antibiotics and failure of patients to complete antibiotic treatment regimens have contributed to the emergence of bacterial multi-drug resistance (MDR). At the same time, the large costs involved in developing new drugs, exacerbated by a complicated drug approval and patent process[1]. This Rapid spread of resistant microbes affected the effectiveness of antimicrobials and created world-wide problem[2]. As a result, fighting MDR bacterial infections in patients is becoming increasingly difficult with treatment options becoming very limited[3].

Bacteria living in a competitive environment are able to secrete proteineous toxins, known as bacteriocins, which can kill closely related bacterial competitors while causing little harm to the bacteriocinogenic cells. These bacterial inhibitors are produced by all major groups of Bacteria[4].

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[5].

Pseudomonas fluorescens is group of common, nonpathogenic saprophytes that colonize soil, water and plant surface environments. It is a common gram negative, rod-shaped bacterium. *P. fluorescens* has simple nutritional requirements and grows well in mineral salts media supplemented with any of a large number of carbon sources[6].

P. fluorescens Pf-5 is one of the important producer of secondary metabolites that inhibit plant pathogens, including pyoluteorin, pyrrolnitrin,2,4-diacetylphloroglucinol, and hydrogen cyanide[7,8]. *P. fluorescens* SBW25 also was found to produce and secrete a ninhydrin-reactive compound with selective antimicrobial properties[9]. The aim for this study was to detect the bioactive substance as antibacterial agent from clinical specimens of *P. fluorescens* and its efficacy on pathogenic bacteria isolated from skin infections and wounds, because most of the studies detect the antibacterial substance from soil specimens.

MATERIALS AND METHODS

Bacterial isolates

Producing isolates: Two isolates of *P. fluorescens* were collected from human wound infections and identified by bacteriological and biochemical tests through using API-20E (Biomérieux)[10,11]. These isolates named as producing isolates of bioactive substances as antibacterial agent.

Indicator isolates: *Klebsiela pneumoniae* and *Proteus mirabilis* isolated from wounds and *Staphylococcus aureus* collected from pus, boils and impetigo as indicator isolates. The isolates were identified according to[10,11].

Detection of Antibacterial Activity (bioactive substance) *in Vitro* Assay

P. fluorescens isolates were evaluated for antimicrobial activity against Gram negative and positive bacterial isolates by the agar block method[12]. Approximately 10^7 CFU of each isolate of *P. fluorescens* was individually suspended in normal saline, cultured on the surface of nutrient agar (Difco), and incubated for 24 h. at 37C°. Agar blocks diameter (diameter, 5mm) containing growth were aseptically excised from the nutrient agar and placed upside down on the surface of Muller-Hinton agar

seeded with 0.1ml of ~10^7cells of indicator isolates. Plates were incubated for 24h.at 37C° for the clinical isolates[13]. Antibacterial activity was evaluated by measuring of the resulting inhibition zones for indicator isolates growth.

Detection of the antibacterial substance by three types of media

In addition of using nutrient agar for detection, two types of media were used: brain heart infusion agar and tryptic soy agar.

Approximately 10^7 CFU of each isolate of *P. fluorescens* was individually suspended in normal saline, cultured on the surface of medium: brain heart infusion agar and tryptic soy agar (Difco), and incubated for 24 h. at 37C°. Agar blocks diameter (diameter, 5mm) containing growth were aseptically excised from the medium and placed upside down on the surface of Muller-Hinton agar seeded with 0.1ml of ~10^7cells of indicator isolates. Plates were incubated for 24h. at 37C°. Antibacterial activity was evaluated by measuring of the resulting inhibition zones for indicator isolates growth.

RESULTS AND DISCUSSION

The isolates *P. fluorescens* 1 and 2 produced the antibacterial activity substance which inhibited the growth of the indicator isolates as in tables 1, 2 and 3, with a wide range effect on gram positive and negative bacterial growth.

As shown in table 1 *P. fluorescens* P1 and 2 inhibited the bacterial growth of *S. aureus* with a range (10-13) and (11-13) mm. respectably.

In table 2 the results showed antibacterial activity against *k.pneumoniae* with a range (9-12) mm. The results in table 3 showed antibacterial activity against *P.mirabilis* with a range (10-11) and (8-10) mm respectably.

Table 1: Antibacterial activity of *P. fluorescens* against *S. aureus* by using three types of media.

Indicator isolates	Zone of inhibition (mm)					
	P1			P2		
	NA	TSA	BHI	NA	TSA	BHI
***S. aureus* 1**	12	16	18	13	17	20
***S. aureus* 2**	11	16	18	12	16	19
***S. aureus* 3**	11	15	19	13	14	19
***S. aureus* 4**	13	17	19	12	16	18
***S. aureus* 5**	10	15	18	11	14	20
Range	10-13	15-17	18-19	11-13	14-17	18-20

P1= *P. fluorescens* 1, P 2= *P. fluorescens* 2, NA = Nutrient agar, TSA=Tryptic soy agar, BHI= Brain heart infusion agar.

Table 2: Antibacterial activity of *P. fluorescens* against *K.pneumoniae* by using three types of media.

Indicator isolates	Zone of inhibition (mm)					
	P1			P2		
	NA	TSA	BHI	NA	TSA	BHI
***K.pneumoniae* 1**	9	12	15	11	14	17
***K.pneumoniae* 2**	12	16	15	12	15	17
***K.pneumoniae* 3**	10	12	15	10	12	13
***K.pneumoniae* 4**	10	10	13	10	11	13
***K.pneumoniae* 5**	10	11	11	9	11	13
Range	9-12	10-16	11-15	9-12	11-15	13-17

P1= *P. fluorescens* 1, P 2= *P. fluorescens* 2, NA = Nutrient agar, TSA=Tryptic soy agar, BHI= Brain heart infusion agar.

Table 3: Antibacterial activity of *P. fluorescens* against *P. mirabilis* by using three types of media.

Indicator isolates	Zone of inhibition (mm)					
	P1			P2		
	NA	TSA	BHI	NA	TSA	BHI
***P. mirabilis* 1**	10	13	15	8	9	13
***P. mirabilis* 2**	10	12	15	10	10	13
***P. mirabilis* 3**	10	13	17	9	11	13
***P. mirabilis* 4**	11	13	14	9	13	12
***P. mirabilis* 5**	10	12	13	9	10	14
Range	10-11	12-13	13-17	8-10	9-13	12-14

P1= *P. fluorescens* 1, P 2= *P. fluorescens* 2, NA = Nutrient agar, TSA=Tryptic soy agar, BHI= Brain heart infusion agar.

The clinical isolates *P. fluorescens* 1 and 2 were able to grow and inhibit the bacterial growth of *K. pneumoniae, P.mirabilis* and *S.aureus* by using nutrient agar which is considered as a simple medium.

Three types of media were used to detect the production of antibacterial substance as shown in table 1, 2 and 3.

According to inhibition zones around the block by using TSA agar the diameter of inhibition zones in all three genera *K. pneumonia* (10-16), *P.mirabilis* (12-13) and *S.aureus* (15-17) increase than in using NA.

This may referred to components of this medium (Casein peptone and Soya peptone)[14].

In BHA the diameter of inhibition zone were (18-20) for *S.aureus,* (13-17) for *K.pneumoniae* and (13-17) for *P.mirabilis*, this may be referred to the protein components of this medium (Calf Brains, beef Heart, peptone, Dextrose) which stimulate and increase the production of antimicrobial agent[15].

The antibacterial activity of *P. fluorescens* 1 and 2 was more effective on *S.aureus* than *K. pneumoniae, P.mirabilis* and *S.aureus* and this may be referred to the difference between Gram negative and positive bacteria cell wall.

Agar block method was successful for detection of antibacterial agent from *P. fluorescens* because the cells will be in touch with the indicator cell and that may stimulate the cell for production.

The results in this study agreed with the study that used *P. fluorescens* as probiotics against the fish-pathogenic bacterium *Vibrio anguillarum* in fish farming[16] and another study which mentioned that *P. fluorescens* inhibited the growth of methicillin resistant *S. aureus* and *Salmonella Enteritidis*[17]. Also the culture filtrate of *P. fluorescens* showed antibacterial activity against pathogenic bacteria such as *Salmonella typhi, Streptococcus mutans* and *Bacillus subtilis*[18].

The incubation temperature at 37°C was suitable for *P. fluorescens* from clinical specimens to produce its antibacterial activity against the pathogenic bacteria like; *S. aureus*, *K .pneumoniae* and *P.mirabilis*, this result agreed with that mentioned by[13], while most the studies mentioned that the incubation temperature of *P. fluorescens* and indicator cells at 25°C.

CONCLUSION

The clinical isolates *P. fluorescens* were able to produce the antibacterial substance at 37°C and inhibit the bacterial growth of clinical isolates from skin infections. Agar block method was successful for detection of antibacterial agent from *P. fluorescens*.

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