Detection of The Antibacterial Activity of Bacteriocin from Local Isolates of *Pseudomonas fluorescens* Against Gram Positive Bacteria

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Abstract

The antibacterial activity of local isolates of *Pseudomonas fluorescens* (P1and P2) were tested against some pathogenic bacteria, *Staphylococcus aureus, S. epidermidis, Streptococcus faecalis, Streptococcus faecium* isolated from stool urine and wounds by modified agar block method at 37^0C for 24 hr. The isolates of *P. fluorescens* were positive as producer of bacteriocin with a wide inhibition range on growth of gram positive pathogenic bacteria. P1 inhibited the bacterial growth of *S. aureus* isolates with a range of inhibition zone (12-20) mm. while P2 inhibited the bacterial growth with a range of inhibition zone was (13-22) mm .The range of inhibition zones of *S.epidermidis* by P1 were (10-18) mm. while the range by P2 was (9-20) mm**.** The isolate P1 inhibited the growth of all the tested isolates of *S. faecalis* with a range of inhibition zone between (12-19) mm. The range of inhibition zones of *S. faecalis* by P2 were (10-18) mm. The bacterial growth of *S. faecium* isolates were inhibited by P1 with a range of (15-19) mm. and (16-19) mm. with P2. Agar block method was suitable and easy for screening of bacteriocin production from this bacterium.

Key words*: Pseudomonas fluorescens, antibacterial activity, bacteriocin, pathogenic bacteria*

الخلاصة :

أختبرت الفعالية الضد بكتيرية لبكتريوسين العزلات المحلية لبكتريا *Pseudomonas fluorescens* P1 و P2 ضد البكتريا الموجبة لصبغة غرام *Staphylococcus aureus* و *S. epidermidis* و *Streptococcus faecalis* و *Streptococcus faecium* المعزولة من الخروج و الأدرار و الجروح باستخدام الطريقة المحورة لقالب الأكار بدرجة حرارة 37 درجة مئوية لمدة 24 ساعة. كانت عزلات *P. fluorescens* موجبة لأنتاج البكتريوسين المثبط للنمو البكتيري وبمدى واسع. فالعزلة P1 ثبطت نمو عزلات *S .aureus* بمدى (12-20) ملمتر بينما P2 فقد ثبطت بمدى (13-22) ملمتر. كان مدى ثبيط النمو لعزلات *S.epidermidis* بواسطة P1 (10-18) ملمتر و P2 (9-20) ملمتر.

أظهرت النتائج ان P1 ثبطت نمو *S. faecalis* بواقع (12-19) ملمتر وP2 (10-18) ملمتر، أما عزلات *S. faecium* فقد كان مدى التثبيط لنموها هو (15-19) ملمتر بواسطة P1 و (16-19) ملمتر بواسطة P2. كانت طريقة قالب الأكار طريقة مناسبة وسهلة للتحري عن البكتريوسين لهذه البكتريا.

Introduction:

Bacteria of the genus *Pseudomonas fluorescens* are gram negative aerobic rods with size of cells from 2 to 3 μm. They are usually occurred in the wild, in the waste water and pure water and in the intestinal tract of man and animals, which live as saprophytes. A healthy individual has in his digestive tract; these microorganisms are present and are not dangerous for him. They occur in contaminated environment with colonization, but no signs of disease[1]. This bacterium is a good environmental bioreporters and as a model organism for understanding bacterial colonization and transport, cells immobilization strategies, and the kinetics of cellular bioluminescent emission. *P. fluorescens* HK44 has the extensive range of applications in the monitoring of bioremediation processes and biosensing of environmental pollution [2]. Mupirocin (pseudomonic acid A), an antibiotic produced by *P. fluorescens*, showed a high level of activity against staphylococci and streptococci and against certain gram-negative bacteria, including *Haemophilus influenzae* and *Neisseria gonorrhoeae*, but was much less active against most gram-

negative bacilli and anaerobes. Nearly all clinical isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis*, including multiple resistant strains, were susceptible to mupirocin. There was no cross-resistance between mupirocin and clinically available antibiotics, and the selection of resistant [3]. *P. fluorescens* HV37a also inhibited growth of the fungus *Pythium ultimum* on potato dextrose agar PDA [4]. *P. fluorescens* strain AH2 was used against the fish-pathogenic bacterium *Vibrio anguillarum* as probiotics in fish farming [5]. The bacterial strain MM-B16, which showed strong antifungal and antioomycete activity against some plant pathogens, was isolated from a mountain forest soil in Korea [6]. Few studies mentioned the role of this bacterium in human infections ,an outbreak of *P. fluorescens* in bacteremia among oncology patients, one in the oncology ward andthree in the chemotherapy room was recorded in 1997 [7].

Bacteriocins are proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s) [8]. Bacteriocins are of interest in medicine because they are made by non-pathogenic bacteria that normally colonize the human body. Loss of these harmless bacteria following antibiotic use may allow opportunistic pathogenic bacteria to invade the human body. Bacteriocins have also been suggested as a cancer treatment. They have shown distinct promise as a diagnostic agent for some cancers, but their status as a form of therapy remains experimental and outside the main thread of cancer research. Partly this is due to questions about their mechanism of action and the presumption that anti-bacterial agents have no obvious connection to killing mammalian tumor cells [9,10,11]. Bacteriocins were tested as AIDS drugs (around 1990) but not progressed beyond in-vitro tests on cell lines [12].

The aim of this study was to test the production of bacteriocin from local isolates of *P. fluorescens* from clinical isolates and its effect on some Gram positive pathogenic bacteria because there is only one study in Iraq on environmental isolates

Materials and Methods:

Bacterial isolates:

Two isolates of *P. fluorescens* (P 1 and P2) were collected from wound cultures from central public laboratory in Baghdad and identified by bacteriological and biochemical tests [13,14]. These isolates named as producing isolates of bacteriocin.

Indicator isolates:

Clinical bacterial isolates from stool, urine and wound infections like; methicillin resistant *Staphylococcus aureus* (MRSA), methicillin resistant *S. epidermidis* (MRSE), vancomycin resistant *Streptococcus faecalis* and *Streptococcus faecium* (VRSF) were used as indicator isolates. These isolates were identified by bacteriological and biochemical tests according to [13,14].

Screening of bacteriocin production:

P.fluorescens isolates were tested for antibacterial activity against Gram positive bacterial isolates by the agar block method [15]. Approximately 10^7 CFU of each isolate of *P. fluorescens* was individually suspended in normal saline, cultured on the surface of nutrient agar, and incubated for 24 hr. at 37°C. 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 37° C. Bacteriocin activity was evaluated by measuring of the resulting inhibition zones for indicator isolates growth.

Results:

The two isolates of *P. fluorescens* (P1 and P2) produced bacteriocin with a wide range effect on growth of gram positive bacteria as shown in table-1.

As shown in table-1**,** P1 inhibited the bacterial growth of *S. aureus* isolates with a range of inhibition zone (12-20) mm. while P2 inhibited the bacterial growth with a range of inhibition zone (13-22) mm. The range of inhibition zones of *S. epidermidis* by P1 were (10-18) while the range by P2 was (9-20) mm. as in table-1.

The isolate P1 inhibited the growth of all the tested isolates of *S. faecalis* with a range of inhibition zone between (12-19) mm. The range of inhibition zones of *S. faecalis* by P2 were (10-18) mm. as showed in table-2. The bacterial growth of *S. faecium* isolates were inhibited by P1 with a range of (15-19) mm. and (16-19) mm. with P2.

The results in table-1 revealed that bacteria *S.aureus* was the most affected bacteria by bacteriocin of *P. fluorescens* which produce inhibition zone between 12-22) mm. followed by *S.epidermidis* (9-20) mm. then *S.faecium* (16-19) mm. and *S. faecalis* (10-19) mm.

Table -1: Inhibition zones (mm.) producing by *P. fluorescens* 1 and 2.

Indicator isolates	**Zones of inhibition (mm) Producing by *P. fluorescens***	
	P1	**P2**
***S .aureus* 1**	20	16
***S. aureus* 2**	16	13
***S. aureus* 3**	12	22
***S. aureus* 4**	16	18
Range of inhibition zones	12-20	13-22
***S.epidermidis* 1**	18	13
***S.epidermidis* 2**	16	10
S.epidermidis 3	15	9
S.epidermidis 4	10	20
Range of inhibition zones	10-18	9-20

Table 2: Inhibition zones (mm.) producing by *P. fluorescens* 1 and 2

Indicator isolates	**Zones of inhibition (mm) Producing by *P. fluorescens***	
	P1	**P2**
***Streptococcus faecalis* 1**	15	10
***S. faecalis* 2**	19	15
***S. faecalis* 3**	15	17
***S. faecalis* 4**	12	18
Range of inhibition zones	12-19	10-18
***S.faecium* 1**	19	16
***S.faecium* 2**	18	19
S. faecium 3	15	18
S.faecium 4	19	19
Range of inhibition zones	15-19	16-19

Discussion:

As shown in table 1 and 2 *P. fluorescens* inhibited the bacterial growth of pathogenic bacteria and these results in this study agree with the results of the agar spot method; the substance of *P. fluorescens* isolates from soil inhibit the growth of *K. pneumoniae* and *S. aureus* isolates at 37ºC for 24hr [16], because most of the studies mentioned that the activity of this bacterium on plant bacteria and fungi. The local isolates of *P. fluorescens* succeed in growth and production of bacteriocin on nutrient agar which is considered as a simple medium.

Agar block method was suitable for screening of bacteriocin production from this bacterium because all the producing isolated were able to produce bacteriocin and inhibit the growth of indicator isolates in this study. The results in this study agree with the results of[17] in Iraq which mentioned that *P. fluorescens* inhibited the growth of methicillin resistant *Staphylococcus aureus* and *Salmonella Enteritidis*. Bacteriocins are one class of antimicrobials, they have received increasing attention because of the high levels of bacteriocin diversity observed and the use of bacteriocins as preservatives in the food industry and as antibiotics in the human health industry. They are generally high-molecular-weight protein antibiotics that kill closely related strains or species.In this study bacteriocin of *Pseudomonas fluorescens* inhibeted the gram positive bacterial growth and this explained according to the mechanism that the bacteriocin gains entry into the target cell by recognizing specific cell surface receptors and then kills the cell by forming ion-permeable channels in the cytoplasmic membrane, by nonspecific degradation of cellular DNA, by inhibition of protein synthesis through the specific cleavage of 16s rRNA, or by cell lysis resulting from inhibition of peptidoglycan synthesis and this is the reason of inhibition or killing.

Frequently, the bacteriocin is released from the cell through the action of a lysis protein, although other export mechanisms may be involved [18]. More recently, interest in bacteriocins as a replacement for traditional antibiotics has increased. A good example was Nisin which has been used to inhibit plaque-producing bacteria [19]. Also Colicinogenic *E. coli* have been examined for inhibition of *Shigella sonnei* infection of the conjunctivae [20].

Colonization of bacteriocin-producing strains of *H. influenza* (in the rat nasopharyngeal region) and *S. mutans* (in the human oral cavity) has been investigated. Each of these studies suggests that bacteriocins may play a significant role in the control of bacterial infections[21,22].

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