THE ANTIMICROBIAL ACTIVITY OF BACTERIOCIN FROM *PSEUDOMONAS FLUORESCENS* AGAINST PATHOGENIC BACTERIA

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ABSTRACT

In this study four isolates of *Pseudomonas fluorescens* were tested for bacteriocin production by the agar block method. Clinical bacterial isolates like; *Aeromonas hydrophilia*, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Salmonella typhi, Staphylococcus aureus, Streptococcus faecalis* and *Vibrio cholerae(enaba)* were used as indicator isolates. All the isolates were positive as produce with a wide range effect on gram positive and negative bacterial growth, with diameter (8-21) mm.The results showed that *Pseudomonas fluorescens* P1 inhibit the bacterial growth of the tested isolates with a range of inhibition zone (8-18) mm. except *Vibrio cholerae(enaba)* while P2 inhibit the bacterial growth of the tested isolates and the range of inhibition zone was (7-21) mm . except *Vibrio cholerae(enaba)* and *Streptococcus faecalis* .On the other hand P3 inhibit the growth of the isolates with a zone of inhibition between (10-16) mm. except *Streptococcus faecalis* and *Klebsiella pneumoniae* .The isolate P4 inhibited the growth of all the tested isolates with a range of inhibition zone between (8-19) mm. *Escherichia coli* was the most affected bacteria by bacteriocin of *P. fluorescens,* followed by *Salmonella typhi* , *Staphylococcus aureus* , *Streptococcus faecalis*, *Klebsiella pneumoniae* , *Vibrio cholerae(enaba)* and *Pseudomonas aeruginosa* .

Key word: *pseudomonas fluorescens* ,antimicrobial activity,pathogenic bacteria

INTRODUCTION

The addition of substantial amounts of antibiotics and chemotherapeutics remains the method of choice for disease control in many parts of the aquaculture industry. Increased concern about antibiotic-resistant microorganisms has led to several alternative suggestions for disease prevention, including the use of nonpathogenic bacteria as probiotic biocontrol agents [1,2] . Microbial biological control agents, such as the rhizosphere bacterium *Pseudomonas fluorescens* Pf-5, represent alternatives to synthetic chemicals for combating plant disease in agriculture. An important aspect of plant disease suppression by rhizosphere bacteria is the production of low-molecular-weight metabolites with antibiotic properties against certain plant pathogens (reviewed in references *P. fluorescens* Pf-5 produces an array of secondary metabolites that inhibit plant pathogens, including pyoluteorin, pyrrolnitrin, 2,4-diacetylphloroglucinol, and hydrogen cyanide [3,4,5].*Pseudomonas fluorescens* strain AH2 was used against the fish-pathogenic bacterium *Vibrio anguillarum* as probiotics in fish farming [6] and strain *Pseudomonas fluorescens* F113 produces the Rhizobium small bacteriocin, which is used as a biocontrol strain against plant pathogenic bacteria [7].

The aim of this study was to detect the bacteriocin production from local isolates of *P. fluorescens* and its effect on pathogenic bacteria because there is a few studies on this subject .

MATERIALS AND METHODS

Bacterial isolates: four isolates of *P. fluorescens* were collected from wound cultures from central public laboratory in Baghdad and identified by bacteriological and biochemical tests[8,9].These isolates named as producing isolates of bacteriocin.

Indicator isolates: clinical bacterial isolates like; *Aeromonas hydrophilia, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Streptococcus faecalis* and *Vibrio cholerae(enaba)* were used as indicator isolates.These isolates were brought from central public laboratory .

Screening of bacteriocin production: *P. fluorescens* isolates were evaluated for

antimicrobial activity against Gram negative and positive bacterial isolates by the agar block method [10]. 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 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°.Bacteriocin activity was evaluated by measuring of the resulting inhibition zones for indicator isolates growth.

RESULTS AND DISCUSSION

All the four isolates of *P. fluorescens* produce bacteriocin with a wide range effect on gram positive and negative bacterial growth, with diameter (8-21) mm. as shown in table 1.

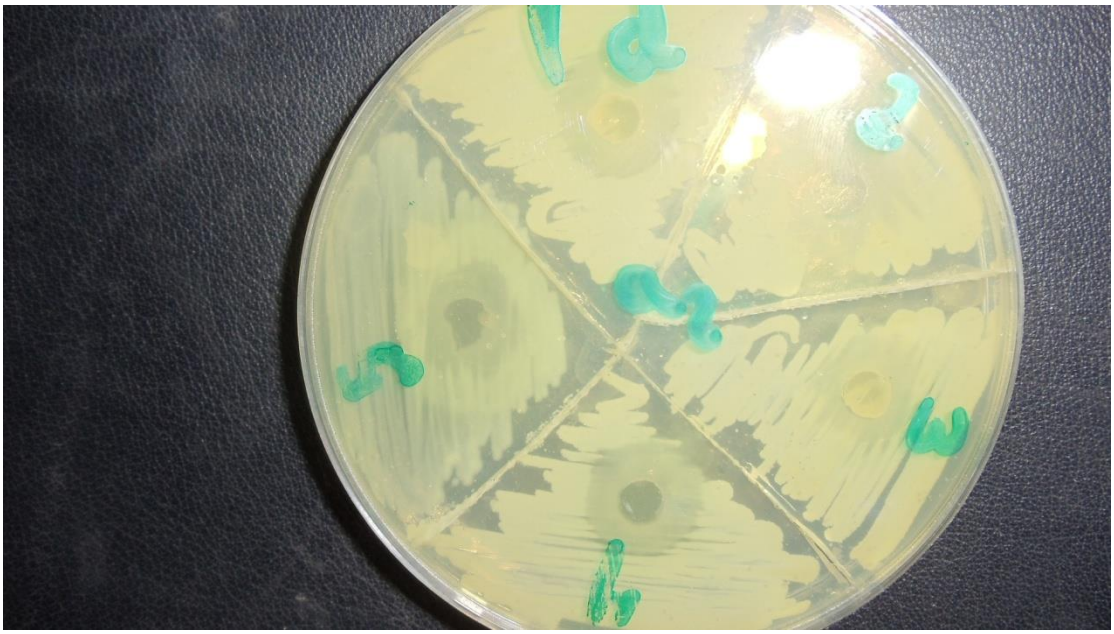
Table 1:Zones of inhibition (mm.)Producing by Four isolates of *Pseudomonas fluorescens*

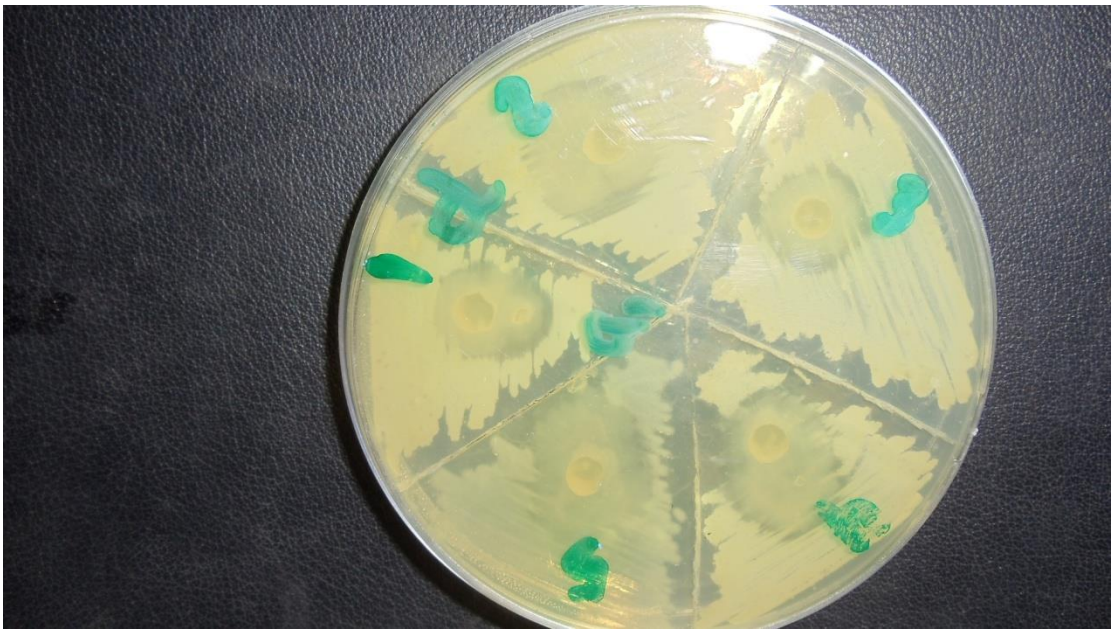
Indicator isolates	**Zones of inhibition (mm) Producing by Four isolates of *Pseudomonas fluorescens***				
	P1	**P2**	**P3**	**P4**	**Range**
Aeromonas hydrophilia	11-18	12-13	10-13	10-15	10-18
Escherichia coli	16-18	12-21	14-16	14-15	12-21
Klebsiella pneumoniae	9-12	8-16	N	11	8-16
Pseudomonas aeruginosa	8-9	7-10	0-13	0-8	7-10
Salmonella typhi	11-12	13-15	12-15	13-19	11-19
Staphylococcus aureus	12-18	10-14	12-14	11-15	10-18
Streptococcus faecalis	9-15	n	N	15-18	9-18
Vibrio cholerae(enaba)	N	n	11-13	8-14	8-14

P1= *P. fluorescens* 1, P2= *P. fluorescens* 2, P3= *P. fluorescens* 3 and P4= *P.fluorescens* 4.

n= no inhibition zone.

As shown in table 1 *Pseudomonas fluorescens* P1 inhibit the bacterial growth of the tested isolates except *Vibrio cholerae(enaba)* with a range of inhibition zone (8-18) mm.while P2 inhibit the bacterial growth of the tested isolates except *Vibrio cholerae(enaba)* and *Streptococcus faecalis* and the range of inhibition zone was (7-21) mm .On the other hand P3 inhibit the growth of the isolates except *Streptococcus faecalis* and *Klebsiella pneumoniae* with a zone of inhibition between (10-16) mm.





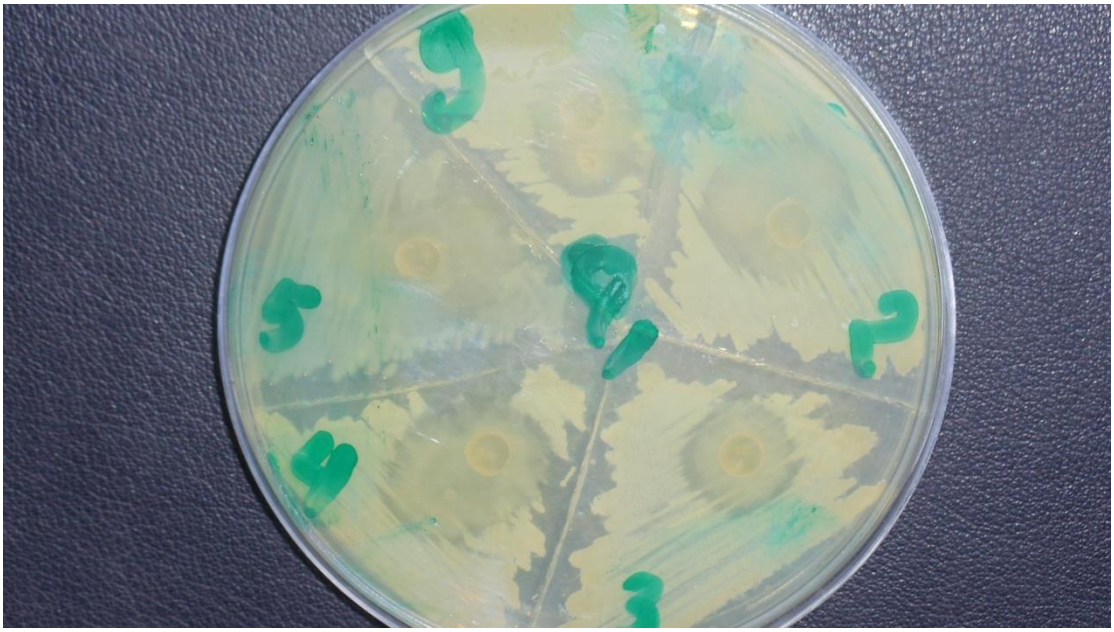


Figure 1, 2 and 3 :Inhibition bacterial growth zones by bacteriocin of *P. fluorescens* isolates .

The isolate P4 inhibited the growth of all the tested isolates with a range of inhibition zone between (8-19) mm. the inhibition zones are showed in figure 1,2 and 3.

The results in (Table 1) revealed that bacteria *Escherichia coli* was the most affected bacteria by bacteriocin of *P. fluorescens* which produce a range of inhibition zone between(12-21)mm. followed by *Salmonella typhi* (11-19)mm. , *Staphylococcus aureus* (10-18)mm. , *Streptococcus faecalis* (9-18)mm. , *Klebsiella pneumoniae* (8-16)mm. , *Vibrio cholerae(enaba)* (8-14)mm. and *Pseudomonas aeruginosa* (7-10)mm.

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 local isolates P1,P2,P3 and P4 inhibited the gram positive negative bacterial isolates like; *Staphylococcus aureus* and *Streptococcus faecalis* and gram negative bacteria like *Aeromonas hydrophilia , Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa , Salmonella typhi* and *Vibrio*

cholerae(enaba) .The results in this study agree with the results of [11] which mentioned that *P. fluorescens* inhibited the growth of methicillin resistant *Staphylococcus aureus* and *Salmonella Enteritidis* and with the study that used *P. fluorescens* as probiotics against the fish-pathogenic bacterium *Vibrio anguillarum* in fish farming[6].

REFERENCES

Austin, B.; Stuckey, P. A.; Robertson, I., and D. R. W. Griffith,(1995). A probiotic strain of *Vibrio alginolyticus* effective in reducing diseases caused by *Aeromonas salmonicida*, *Vibrio anguillarum* and *Vibrio ordalii*. J. Fish Dis., 18:93-96.

Bly, J. E.; Quiniou, L., A. Lawson, and L. W. Clem. (1997). Inhibition of *Saprolegnia* pathogenic for fish by *Pseudomonas fluorescens*. J. Fish Dis., 20**:**35-40.

Marion Brodhagen;Marcella D.; Henkels, and Joyce, E. Loper(2004). Positive Autoregulation and Signaling Properties of Pyoluteorin, an Antibiotic Produced by the Biological Control Organism *Pseudomonas fluorescens* Pf-5. Appl. and Environ. Microb.,70:(3): 1758-1766.

Haas, D., C. Blumer, and C. Keel. (2000),Biocontrol ability of fluorescent pseudomonads genetically dissected: importance of positive feedback regulation. Curr. Opin. Biotechnol.,11:290-297.

Haas, D., and C. Keel. , (2003)Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. Annu. Rev. Phytopathol., 41:117-153.

Lone, G.; Jette, M.; Bettina, S.; Ingrid, H. and Torben, F., (1999). Inhibition of *Vibrio anguillarum* by *Pseudomonas fluorescens* AH2, a Possible Probiotic Treatment of Fish . Appl. and Environ. Microb.,65(3): 969-973.

Laue, B. E. ; Yan, J. ; Siriram, C. ; Jacob, S.; Stewart, G., (2000). The biocontrol strain Pseudomonas fluorescens F113 produces the Rhizobium small bacteriocin, N-(3-hydroxy-7-cis-tetradecenoyl) homoserine lactone, via HdtS, a putative novel N-acylhomoserine lactone synthase. Microb.,146 (10): 2469-2480.

Collee,J.G.;Frazer,A.G.;Marmion,B.P.and Simmon,A., (1999).Mackie and Mccartneg :Practical Medical Microbiology 14th ed.Churchul livingstone.Newyork

Forbes,B.A.Sahm.D.F.and Weissfeld,A. S. (2007).,Baily and Scotts diagnostic Microbiology .12th ed.Mosby

Line ,J.E.;Svetoch,E.A. ; Erslaanov, B.V. , and perelygin ,V.V.,(2008) Isolation and purification Enteriocin E-760 with broad antimicrobial activity against Gram - positive and Gram –negative bacteria .Anti. Agents and Chemoth .,52(3) :1094-1100.

Parrk, S., and Itoh, K.,(2003) Screening of bacteriocin-producing bacteria from various sources. Bioscience and Microflora., 22 (2): 57-60 .

الفعالية التثبيطية لبكتريا *PSEUDOMONAS FLUORESCENS* على نمو البكتريا المرضيه

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الخلاصة

اختبرت قابلية اربع عزلات محلية من بكتريا ال *Pseudomonas f luorescens* على انتاج البكتريوسين بطريقة قطع الأكار agar block و استخدمت العزلات السريرية *Aeromonas hydrophilia* و *Escherichiacoli coli* و *Klebsiella pneumoniae* و *Pseudomonas aeruginosa, Salmonella typhi* و *Staphylococcus aureus* و *Streptococcus faecalis* و *Vibrio cholerae (enaba* كعزلات دالة حساسة .كانت العزلات الأربعة منتجة للبكتريوسين وبمدى تثبيط واسع (8-12 مليمتر) لنمو البكتريا الموجبة والسالبة لصبغة كرام .اظهرت النتائج ان العزلة P1 *Pseudomonas fluorescens* قد ثبطت النمو البكتيري بمدى (8-18 ملمتر) عدا بكتريا *Vibrio cholerae* .بينما العزلة P2 *Pseudomonas fluorescens* انتجت تثبيطا للعزلات الحساسة بمدى تراوح بين (7-21 ملميتر) *Vibrio cholerae* و *Streptococcus faecalis* .اما العزلة P3 فكان تاثيرها التثبيطي لنمو العزلات الحساسة بين (10-16 ملمتر) عدا *Streptococcus faecalis* و *Klebsiella pneumoniae* .كما اظهرت العزلة الرابعة تاثيرا تثبيطيا للنمو تراوح بين (8-16 ملمتر) . كانت بكتريا ال *Escherichia coli* هي الأكثر تاثرا بالبكتريوسين المنتج من *Pseudomonas fluorescens* تبعتها بكتريا *Salmonella Staphylococcus aureus* و *Streptococcus faecalis* و *Klebsiella pneumoniae* و *Vibrio cholerae(enaba)* ثم *Pseudomonas aeruginosa* على التوالي