AJPS, 2017, Vol. 17, No.1 **Date of acceptance:30-10-2016**

The inhibitory effect of Lactobacillus on siderophore production in Pseudomonas aeruginosa (in vitro).

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Abstract:

Probiotic are alive microorganisms presented in food and dietary supplements, probiotics beneficially affect the individual by improving the intestinal microbial balance properties . This study revealed anew pathway of siderophore attenuation in Pseudomonas aeruginosa, using Lactobacillus. Out of forty P. aeruginosa isolates , 20 isolates were siderophore produced , Cell free supernatant (CFS) and Cell free culture (CFC) of Lactobacillus the antimicrobial agent were able to produce zones of inhibition against P. aeruginosa growth in average (8-15 mm) and (9-15 mm) by CFS and CFC respectively .These antimicrobial agent also were able to attenuate the pathogenicity of P. aeruginosa by prevent its ability to siderophore production.

Key words: Pseudomonas , Siderophore , Lactobacillus.

مفاتيح الكلمات: دراسة المركبات الأيضية الثانوية والقيم الغذائية وقابلية مضادات الأكسدة للمر Commiphora myrrha

الخلاصة:

لمعززات الحيوية هي كائنات حيوية توجد في الغذاء والمكملات الغذائية والتي تؤثر على الفرد من خلال تحسين خصائص التوازن الميكروبي المعوي ،في هذه الدراسة تم تنفيذ مسلك جديد للتخفيض من انتاجية Pseudomonas aeruginosa للـ siderophore (حوامل الحديد) باستخدام بكتريا . Lactobacillus كانت 20 عزلة فقط منتجة لحوامل الحديد من بين 40 عزلة تعود لبكتريا .P. Aeruginosa واظهرت عوامل الـ CFS Cell free supernatant)) و Cell ((CFC free culture الضد ميكروبية قدرتها على تكوين مناطق تثبيط ضد نمو P. Aeruginosa بمعدل (8-15) ملم و (9-15) ملم على التوالي، استطاعت تلك العوامل الضد ميكروبية ايضا ان تخفف من إمراضية P. aeruginosa من خلال الغاء قدرتها على انتاج حوامل الحديد.

Introduction:

Pseudomonas aeruginosa is a ubiquitous found in various environments, such as, water or the rhizosphere.It produce a large array of colonization and virulence factors ,allowing it to infect in plants , insects, mammals, including humans, causing different types of infection (Cornelis and Dingemans , 2013; Saxena, et al., 2014) .

P. aeruginosa produce different virulence factors including exotoxin (Coggan and Walfgar 2012; Jimenez et al., 2012 ; Balasubramanian, 2013) and Siderophores (Sidrophores are low molecular weight iron chelating compounds produced in response to iron deprivation by Pseudomonas and excrete or binding and uptake of iron into their cells, (Srifueng fung et al.,2010) and other virulence factors (Balasubramanian ,2013) .

P. aeruginosa is able to switch its lifestyle from planktonic unicellular to a sessile form in biofilm (Mikkelsen et al., 2011) .For example , cooperative production of iron-scavenging siderophores achived by the P.aeruginosa .It is a key determinant of virulence and the links between cooperation and virulence in this bacterium have been extensively studied (reviewed in : Harrison ,2013). Probiotic is a word derived from Greek origon and it means (for life) , as opposed to antibiotic , which means , against life , (Jungersen

et al., 2014) They are living microorganisms, that confer beneficial effects to the host, when it is administered in adequate mounts (Denkova et al., 2013a) . Lactobacillus acidophilus is included in the composition of probiotics and probiotic foods (Kirtzalidou et al., 2011) ,to select bacterial strains to use in the production of probiotic functional food , a number of criteria needs to be considered .One of the important mechanisms is the ability to increase the natural defenses against enteropathogens, by either producing antimicrobial substances or competitive inhibition. Expulsion of these pathogens are acquired to meet the probiotic strains (Denkova et al., 2013b). Supernatant of most Lactobacillus bacteria contains several antimicrobials which kill pathogens (Aminnezhad et al., 2015) .The recent study is aiming to study details of the mechanisms , at the basis of switch to acute virulence with the production of sidrophores but, in this paper , our aim was to investigate the role and the influence of cultures and cell free supernatant of Lactobacillus, with probiotic potential on growth and virulence factor productivity of pathogenic P. aeruginosa isolates.

Materials and Methods:

Test pathogen

Forty P.aeruginosa isolates were obtained from high studies laboratory in college of science, Al-Mustansiriyah University, then identified again biochemical test.

Lactobacillus source

Lactobacillus isolate was obtained from Al- Mustansiriyah University, postgraduate studies laboratories (from dairy source) and then identified according to Forbes and Daniel, 2007.

Preparation of cell-free super-natant (CFS) of Lactobacillus:

A culture of MRS broth 10ml and Lactobacillus and then incubated at 37Cº for 24hr.After incubation, cell free supernatant was obtained by centrifuging the bacterial culture at 6000xg for 15 min followed by filtration of the supernatant through 0.2 mm pore size filter (Muhisn et al., 2015).

Siderophore production test:

To investigate the ability of P.aeruginosa for siderophore production, the medium M9 was prepared as follows:

(Na2HPO4 6 , KH2PO4 3 , NaCl 0.5 , NH4Cl 1 and agar 15) gm. Materials were collected 900 ml and D.W was add , pH adjusted at 7, sterilized for 15 m and cooled in 45Cº,then added the following solutions:

(Glucose solution 10 , MgSO4 solution 20 , CaCl2 solution 1 and Dipyridine solution 1)ml ,that previously prepared as follows:

Glucose solution prepared by (20gm glucose ,90ml D.W and completed to 100ml),sterilized by millipore filters (0.22mm),CaCl2 solution prepared by (0.3gm Cacl2 ,90 ml D.W and completed to 100ml), sterilized with autoclave for (10) min, Dipyridine solution Prepared by(0.005gm from Dipyridine material ,9ml D.W) and completed to (10ml), sterilized with millipore filters, MgSO4 solution prepared by (2.5gm MgSO4 ,90 ml D.W and completed to 100ml.) sterilized with autoclave for (10) min. After the addition of solutions, (0.1gm) Thymine was added to complete the volume to (1000ml). Then the solution were mixed and poured into dishes .This experiment was made according to Johnson et al., (1988).

Pseudomonas siderophore production test:

M9 medium was used to detect siderophore production. P.aeruginosa colonies were cultured (originally grown on brain – heart – lnfusion) on M9 medium .The plates was incubated at 37Cº for 24 hr.The existence of growth was the positive result for P.aeruginosa utilize the component of medium M9 and

siderophore production, no growth was the negative result .

The antibacterial activity of Lactobacillus against Pseudomonas:

Antibacterial activity of Lactobacillus was tested (against P. aeruginosa growth) using agar-well diffusion method. Lactobacillus CFS and CFC (Cell free culture: this is taken from broth (MRS) without centrifuge) that originally Lactobacillus grown in it), were antibacterial agents that filled the wells , with control(MRS without CFS), that means 3 wells were made in each plate, these plates incubated at 37Cº for 18- 24 hr.Inhibition zones were recorded on Muller-Hinton agar (Jebur, 2010).

Lactobacillus activity against P.aeruginosa siderophore production:

Was accomplished by culture media mixing (Al –Jassani. 2006). Anti-P.aeruginosa factors of Lactobacillus CFS and CFC were added to MRS medium, and then stored in vials a sterial MRS medium was added as a control. Three types of MRS were added with (10%), the addition operation was completed with courtesy on the vials walls to prevent the formation of bubbles . After mixing by vortex then left to be solid .Each plate was inoculated with siderophore producing P.aeruginosa isolate, that previously tested .Then plates were incubated at 37Cº for 24 hr.

Results and discussion:

Incidence of siderophore produced Pseudomonas isolates among the total isolates:

The present study showed that 20 isolates of P. aeruginosa were producing siderophore with percentage of 50%, Table (1).

Table (1): P. aeruginosa siderophore producing.

Number of isolate	Siderophore production	Number of isolate	Siderophore production
P.1	-	P.21	-
P.2	-	P.22	-
P.3	+	P.23	-
P.4	-	P.24	-
P.5	+	P.25	+
P.6	+	P.26	-
P.7	-	P.27	-
P.8	-	P.28	+
P.9	-	P.29	-
P.10	-	P.30	+
P.11	-	P.31	-
P.12	+	P.32	+
P.13	-	P.33	+
P.14	-	P.34	+
P.15	-	P.35	-
P.16	+	P.36	+
P.17	+	P.37	-
P.18	+	P.38	-
P.19	-	P.39	+
P.20	-	P.40	+

Total number 40 isolates

(-) = Non-producing isolate,

(+) = Producing-isolate.

Anti–siderophore produced P. aeruginosa growth activity of Lactobacillus CFS and CFC:

A total of 20 isolates that produced siderophore were examined to detect the anti – Pseudomonas growth activity of CFS and CFC of Lactobacillus. The result revealed that CFS and CFC inhibited 16 isolates; only 4 isolates were completely resistant to these Anti – P. aeruginosa – agent. Only 5 isolates (P.3,P.4,P.30,P.33,P.39)showed moderate sensitivity to CFS (with inhibition zone 10mm), the rest ,6 isolates (P.12,P.19,P.25,P.28,P.32,P.34,P.35) were sensitive to CFS with inhibition zone ranged (11-13) mm. Only 4 isolates gave low inhibition zone ranged (8-9) mm.

CFC of Lactobacillus not differ very much from CFS , but , it gave higher inhibition zone against (P.32, P.35) both with (15 mm) , and the low zone was (9mm) against (P.3, P.17) , So CFC cleared high activity with inhibition zones ranged (9-15) mm . Table (2) and figure (1).

Table (2): Anti- *Pseudomonas* growth activity of Lactobacillus CFS and CFC.

Number of isolate.	Inhibition zone (mm)	
	CFS ***Lactobacillus***	**CFC** ***Lactobacillus***
3	10	9
4	10	12
5	8	10
6	9	11
12	-	-
16	12	13
17	8	9
18	-	-
19	12	10
20	-	-
25	11	13
28	12	13
30	10	12
32	13	15
33	10	11
34	12	11
35	13	15
36	-	-
39	10	12
40	9	12

CFS: Cell free supernatant
CFC: Cell free culture

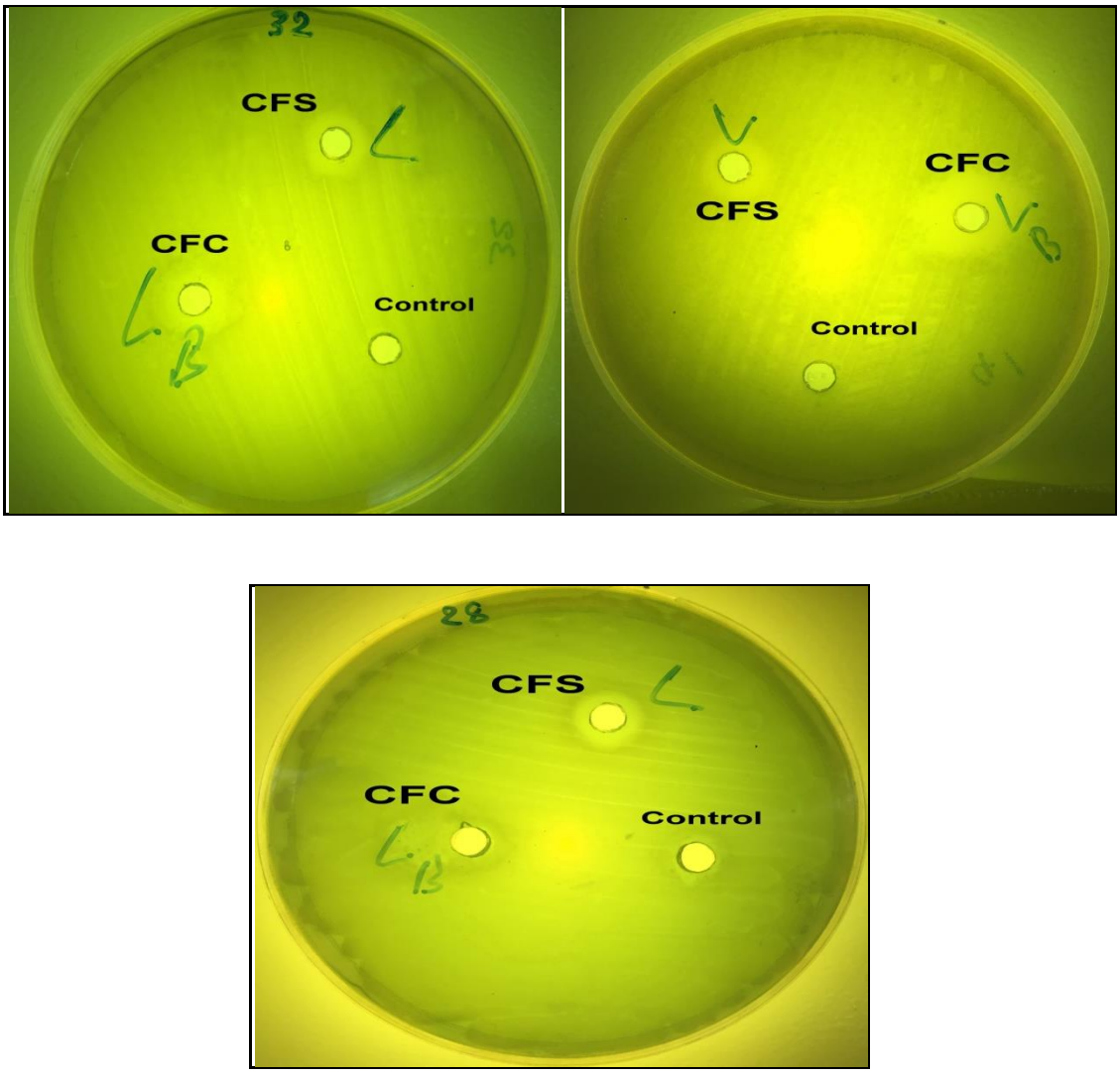
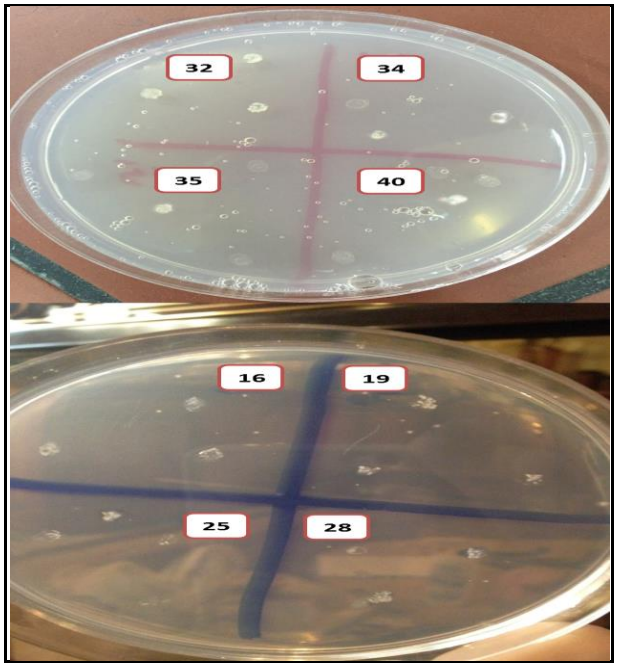


Figure (1): Anti- Pseudomonas growth activity of Lactobacillus CFS and CFC

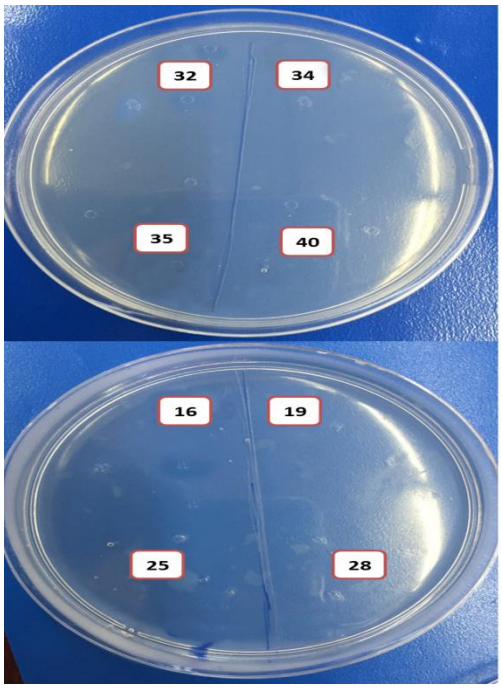
CFC of Lactobacillus, CFS of Lactobacillus, Control: Distill water.

CFS and CFC of Lactobacillus activity against siderophore P. Aeruginosa production:

P. aeruginosa isolates were inhibited by CFS and CFC when they mixed with medium (figure:2), in this figure there was no growth after treatment compared with before treatment. It means , these agents CFS and CFC could convert each of isolate from producing isolate to non siderophore producing isolates, Figure-2.



Growth of Pseudomonas in absence of CFC



No growth of Pseudomonas in presence of CFS

Figure (2): CFS and CFC of Lactobacillus against P. aeruginosa siderophore producing isolate.

reviously siderphore is considered as an important virulence factor of Pseudomonas it has been established that siderophore production is dependent on antigenic structure of P. aeruginosa and the type of isolate(kamel et al.,

2011) in our study 20 out of 40 P. aeruginosa isolates gave positive siderophore production , recorded 20 negative isolates were also isolated from patient ,our results revealed that there were no significant differences siderophore presence among the isolates that isolated from patients, So siderophore production must not always coincide as virulence marker presence in While ,our results agreed with other study(Al –Jassani.,2006)and (Muhisn et al., 2015). about the use of CFS and CFC of Lactic acid bacteria (among of them is Lactobacillus)to control pathogenic microorganism because that have an inhibitory effect by producing acids (organic acids) and antimicrobial substances that prevent microbial growth (bakari et al.,2011).The CFS and CFC that obtained from Lactobacillus could produce anti- P. aeruginosa substances which inhibited the growth of 16 important siderophore produced P. aeruginosa in varying degree. The main revelation in this study was that strain Lactobacillus CFS and CFC were active against P. aeruginosa.all P. aeruginosa isolates.

The inhibitory activity of Lactobacillus is mainly due to the accumulation of primary metabolites such as lactic acid , acetic acids, ethanol and carbon dioxide . Lactobacillus was also capable to produce antimicrobial compounds such as bacteriocins and other compounds with small molecular mass,(Sakaridis et al.,2012). thus it can be explained that some P. aeruginosa isolates could resist the CFS and CFC because of the production levels and proportions among these compounds depend on the biochemical properties of the strains used and physical and chemical conditions of growth (Pow thong and Suntornthiticharoen , 2013).

Antimicrobial activity is one of the most important selection criteria for probiotics (Chowdhury et al.,2012), Lactobacillus strain that used in this experiment has the first criteria of probiotics .In a study was achieved by Gharaei-Fathabad and Eslamifar (2011) a strain of Lactobacillus isolated from tea leaves showed strong inhibitory activity against some pathogenic. Bacteria strain of present study has almost , similar antibacterial capability , at the same time , it has better than these Lactobacillus isolate that isolated from dairy products and traditional drinking yoghurt in studies by Forouhandeh et al.(2010) and

Conclusions:

CFS and CFC of Lactobacillus could inhibit the growth and siderophore productivity of P. aeruginosa

Recommendations:

Further studies will be carried out to examine whether this strain will be able to adhere to epithelium cells , before predicting their use as probiotic in conferring health benefit to the host , also to alternate the antibiotic with natural probiotics that have safety side effect.Ali (2011) respectively .

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