Impact of Lactic acid bacteria (LAB*)* as probiotic against bacterial pathogen from *Cyprinus carpio* L.

J. K. H. Al-Faragi and S. A. A. Al-Saphar
College of Veterinary Medicine/ University of Baghdad

Abstract

The aim of the present study is to investigate the potential of lactic acid bacteria as a probiotic (biologicl dieases control). Six strains of lactic acid bacteria J_1, J_3, J_4, J_6, J_7, J_8 was Isolated from intestines of the common carp *Cyprinus carpio* were compared as to the antagonistic activity against fish pathogen bacteria *Aeromonas hydrophila, Pseudomonas luteola Pseudomonas aeroginosa Sarratia rabidaeae* and *staph sp.* Different method were used for measuring growth by optical density, agar well diffusion and cross- streak method also screened from bile salt and antibiotic tolerance. All strains of LAB showed different spectra of inhibition against pathogenic bacteria. The highest inhibition measured against *serratia rubidaeae and A. hydrophila*, moderate inhibition occure against. *P. luteola and P aeroginosa* and lower inhibition to *staph sp.* Also culture supernatant free fluid of all LAB showed no antagonistic activity against pathogenic bacteria moreover all strain show resistance to all antibiotic sensitivity OA2A–P disc except J_1, J_3, J_4 were sensitive to erythromycin in concentrate 60 mcg after incubation for 48 hr and only J_1, J_3, J_4, J_6 were exhibited tolerance reaction to bile salt at concentration not more than 3000 ppm the present study recommend to use lactic acid bacteria J6 as a good probiotic remedy to the fish culture in Iraq.

تأثير بكتريا حامض اللاكتيك LAB كمعززات حيوية ضد البكتريا المرضية في *Cyprinus carpio* L.

جمال خلف عطية الفراجي وسندس عون علي الصفار
كلية الطب البيطري/ جامعة بغداد

الخلاصة

هدفت الدراسة الحالية الى اختيار قدرة بكتريا حامض اللاكتيك كمعززات حيوية في السيطرة البايولوجية ضد البكتريا المرضية. قورنت ستة عزلات من بكتريا حامض اللاكتيك J1, J3, J4, J6, J7, J8 فيما بينها والمعزولة من أمعاء اسماك الكارب الشائع (*Cyprinus carpio*) في فعاليتها التضادية ضد البكتريا المرضية للأسماك *Aeromonas Serratia rabidaeae, Pseudomonas aeroginosa, Pseudomonas luteola,hydrophila* و *staph sp.* استعملت طرق مختلفة وهي قياس معدل النمو باستعمال تقنية درجة العتامة (O.D) والتثبيط باستخدام الانتشار بحفر الاكار agar well diffusion وطريقة النمو التضادي التعاكسي cross–streak method فضلا عن مقاومة البكتيريا اللبنية للمضادات الحيوية والاملاح الصفراء. أظهرت جميع عتر البكتريا اللبنية مستويات تثبيط تضادي مختلفة ضد البكتريا المرضية فكان اعلى تثبيط هو ضد بكتريا *Serratia rabidaeae* و*Aeromonas hydrophil*. أما التثبيط المتوسط فقد ظهر ضد بكتريا *Pseudomonas* aeroginosa و*Pseudomonas luteola* وادنى تثبيط ظهر ضد *staph sp* إلا ان السائل الطافي الخالي من

البكتريا (cff) لجميع عتر البكتريا اللبنية لم يظهر أي فعالية تضادية ضد البكتريا المرضية. أظهرت جميع العتر البكتريا اللبنية مقاومة ضد المضادات الحيوية المستعملة في قرص فحص الحساسية OA2A –P disc ماعدا J1, J3, J4 كانت حساسة للارثرومايسين بتركيز 60 mcg بعد حضانة 48 ساعة وعتر J1 ,J3, J4, J6 مقاومة لأملاح الصفراء بتركيز ليس اكثر من 3000 ppm. بينت الدراسة ان استعمال العزلة J6 من بكتريا حامض اللاكتيك كمعزز حيوي جيد للاستزراع السمكي في العراق.

Introduction

Various authors have shown that lactic acid bacteria are a part of the normal intestinal flora of fish (1). Most of the evidence comes from salmonid species like Arctic charr *(Salvelinus alpinu*s) and Atlantic salmon (*Salmo salar*), (2). (3) described the presence of lactic acid bacteria, including *Lactobacillus* in the intestines of various fish species at larval, fry and fingerling stages inhabiting ponds. However, it was discussed that some human activities like artificial feeding in ponds would have had an effect on the bacteria composition and load in some fish like carp (*cyprinus carpio*) which showed the highest content of lactic acid bacteria in the intestines Intensive aqua farming accompanies several disease problems often due to opportunistic pathogens as evident from general aquaculture. High stocking densities, high food inputs and other organic loads stimulate the selection and proliferation of opportunistic bacteria (4). Due to this negative balance of the microbial community in rearing water as well as in fish gut, the aqua culturists often face mass mortality of their stocks. The use of antibiotics and chemotherapy remains the method of choice as disease control strategy. The abuse of chemotherapeutics, especially antibiotics has resulted in development of multiple antibiotic resistant bacteria (5) Increased concern about antibiotic resistant microorganisms has led to several alternatives including the use of non-pathogenic microorganisms as probiotic (6). Probiotic concept has been widely applied for health promoting in farm animals, pets and aquatic animals (7, 8). Probiotics are usually defined as live microbial feed supplements which beneficially affects on the host animal by improving its intestinal microbial balance (9). Based on this definition, probiotics may include microbial adjuncts that prevent pathogens from proliferating in the intestinal tract (10). Lactic acid bacteria (LAB) are among the most important probiotic microorganism typically associated with gastrointestinal tract whereas they exercise beneficial effects. (11) suggested that probiotic bacteria would be found to be useful not only as food but also as biological controllers of fish disease and activators of nutrient regeneration.in the biological control in aquaculture emerge and since then the research effort has continually increased *Bacillus sp*.in often antagonistic against other fresh water fish pathogenic bacteria (12,13,14) was reported to the similar experiments have shown that the inoculation of some probiotic strains, mainly lactic acid bacteria, increase fish survival after being challenged with fish pathogens. (15) Showed inhibition of *Vibrio. vulnificus* by LAB and stimulation of the non-specific immune response resulting in resistance to disease in the prawn fed on LAB incorporated diets. Selection of probiotic strains is achieved by screening procedures for several characteristics *in vitro*, such as inhibitory activities against several fish pathogens and gastric and intestinal secretions (16). In the present study we compared the antimicrobial activity of probiotic bacteria (LAB) J1, J3, J4, J6, J7, J8 which isolated in previous study by (17) from gastrointestinal tract of common carp fish *Cyprinius carpio* against pathogenic bacteria using different methods such as growth monitored by measuring the optical density (O.D), cross streaking and well diffusion method.

Materials and Methods

- **Chemicals and media**: Analytical grade chemicals and dyes were obtained from Al-Kindi company for production of veterinary vaccines and drugs, bacteriological media were obtained from oxoide UK which include set of biochemical media, blood agar base, Nutrient agar, Nutrient broth tryptic soy agar, gas generating Kit and sense test disk from lamb GT. Manchester; England. MRS media were obtained from Himedia, and bile salt were supplied by sigma.
- **Bacterial strains**: bacterial strains includes:

1. *Pseudomonas aeroginosa* were obtained from central health laboratory/ Baghdad /ministry of health.
2. *Aeromonas hydrophila, Staph sp. Serratia rubidaeae, Pseudomonas luteola* were obtained from the laboratory of fish pathology veterinary college of Baghdad University. All bacterial strains were cultivated in 10 ml of Nutrient broth. Bacterial cultivation was performed at 30°C for 20 h. Approximately 1 ml of bacterial culture was transferred to 9 ml of liquid medium and incubated at 30°C for another 18 h, cell concentration was then adjusted to obtain final concentration of 106 CFU/ ml for determination of antibacterial activity.
3. lactic acid bacteria J1, J3, J4, J6, J7, J8 isolated from gastrointestinal tract of common carp fish in previous study by (17). Before use LAB strains were activated in MRS broth (18).

- **Bile Tolerance:** The modified method of (19) was used to determine bile tolerance of selected LAB. Before testing for bile tolerance, LAB strains were grown at 30°C for 24 hour in MRS broth without bile. One ml of the culture broth was poured on to MRS agar with bile salt concentrations of 2000, 3000 and 4000 ppm. Bacterial growth was determined after incubation at 30C0 for 48 hour.
- **Bacteriocin assay:** The isolates of lactic acid bacteria were propagated in MRS broth and incubated at 30°C for 48 hours. Cells were separated by centrifugation at 5000 rpm for 10 minutes The clear supernatants obtained were treated as follows:

1. Clear free fluid supernatants without any treatment (CFF).
2. Nutrelizing clear free fluid supernatants by adjusted to pH 6.5-7.0 with 2N NaOH (NCFF). Cell free supernatant was passed through 0.22µm membrane filter and evaluated for antimicrobial activity by agar well diffusion method (20).

The antagonistic effects of the culture supernatants of bacteriocin producing *Lactobacillus* were tested on various indicator organisms on Nutrient agar. All cultures were grown aerobically at 30°C for 48 hours. Inhibition zones around the wells were measured.

- **Inhibitory effect by agar well diffusion method:** The inhibitory effects of *Lactobacillus* strains on indicator organisms were carried by agar well diffusion assay. Petri dishes with nutrient agar that were previously inoculated with 0.1 ml of 24 hours old nutrient broth culture of individual test bacteria were poured. Once solidified, Petri dishes were stored for 2 hours at 4°C. Four wells of 5 mm diameter were made and filled with 50µl of culture supernatant. The inoculated plates were kept at 4°C for 2 hours and then incubated at 30°C for 24 hours. Inhibition zones around the wells were measured (20).
- **Inhibitory effect by O.D. measuring growth method:** According to (21) Five ml replicates of nutrient broth were individually supplemented with 2 ml of each of the

individual LAB strain supernatants. The each tubes were then inoculated with freshly grown culture of each indicator pathogenic bacteria respectively and incubated at 28 ± 2°C for 48 hr and growth was recorded by measuring the optical density at 540 nm. Control tubes comprised of nutrient broth inoculated with indicator pathogenic bacteria respectively

- **Inhibitory effect by cross – strack method:** All the three LAB strains were streaked on Tryptone Soya Agar (TSA) plates containing 1.0% sodium chloride and incubated at 28 ± 2°C for 48 hr. Freshly grown culture of five pathogen bacteria was streaked perpendicular to this growth and after incubation at 28 ± 2°C observed for antagonism according to (15).
- **Antibiogram of LAB isolates**: The isolates were inoculated into MRS broth individually and incubated for 24 hr about 25 ml of MRS agar was seeded with the cultures of LAB isolates10^6 CFU/ml mixed well. Poured in to sterile petriplates and stored at 4 c° for 1hr to solidify the media (OA21-P) antibiotics in a single ring were placed up side down pressed on the top of the agar plates and kept again at 4 c° for 1hr the plate were incubated at 30 C° for 24 hr and 48 hr resistance was defined as the absence of a growth inhibition zone around the discs.

Results and Discussion

- **Bile salt tolerance:** Six strain of LAB were isolated from gastrointestinal tract of common carp fish (*cyprinus carpio)* were tested for their ability to grow at bile sult of 2000, 3000, 4000 ppm in order to bile tolerant strains. Only J1, J3, J4, J6 strain were able to grow in MRS agar supplemented with 2000 and 3000 ppm bile salt while all strains were sensitive to grow in MRS ager supplemented with 4000 ppm bile salt (Table 1).

This is similar to the result obtained by (22) with the strains of *Pediococcus acidilactici* (P2), *Lactobacillus curvatus* (RM 10) and *Lactobacillus sake* (L2) were the most resistant to 3000 ppm bile salt at pH 6 (23) reported that the bile salt tolerance of the *Lactobacillus* strains were able to grow in MRS agar supplemented with 3000 ppm bile salt. It has been reported that certain strains of *Lactobacillus* are able to reduce this detergent effect by their ability to hydrolyze bile salt by bile salt hydrolase enzyme (BSH) (22), which are then readily excreted from the GI-tract (24). This particular enzyme decreases bile solubility and thus weakening its detergent effect.

Table (1) Bile salt tolerance of lactic acid bacteria isolated from common carp fish

Bile Salt Concentration (ppm)	Lactic acid bactria strains					
	J1	J3	J4	J6	J7	J8
2000	+	+	+	+	-	+
3000	+	+	+	+	-	-
4000	-	-	-	-	-	-

(+) tolerance (-) sensitive

- **Antibacterial activity against fish pathogens**: The antagonism of the six LAB strain J1, J3, J4, J6, J7, J8 was ascertained by as test tube well as by well diffusion and cross–streaking on TSA plates. Inhibitory effect by O. D. test tube nutrient broth containing cell free fluid supernatants (cff) of the six strains of LAB respectively failed to record turbidity after inoculating with the pathogen (*Aeromonas hydrophila, Pseudomonas luteola, Pseudomonas leutela, Serratia rubidaeae , Staph .sp.)* and incubating for 30 h

implying inhibition (Fig. 1, 2, 3, 4, 5) Turbidity measured as optical density was obtained in all the control tubes while tubes containing the LAB supernatants recorded low optical densities of the value of zero time. the culture media (supernatant) were used which showed highest inhibition effects of *Bacillus sp*. may be due to production of antibiotics bacteriocins, Lysozymes, proteases and hydrogen peroxide and the ulternative of pH values by the production of organic acid (25).

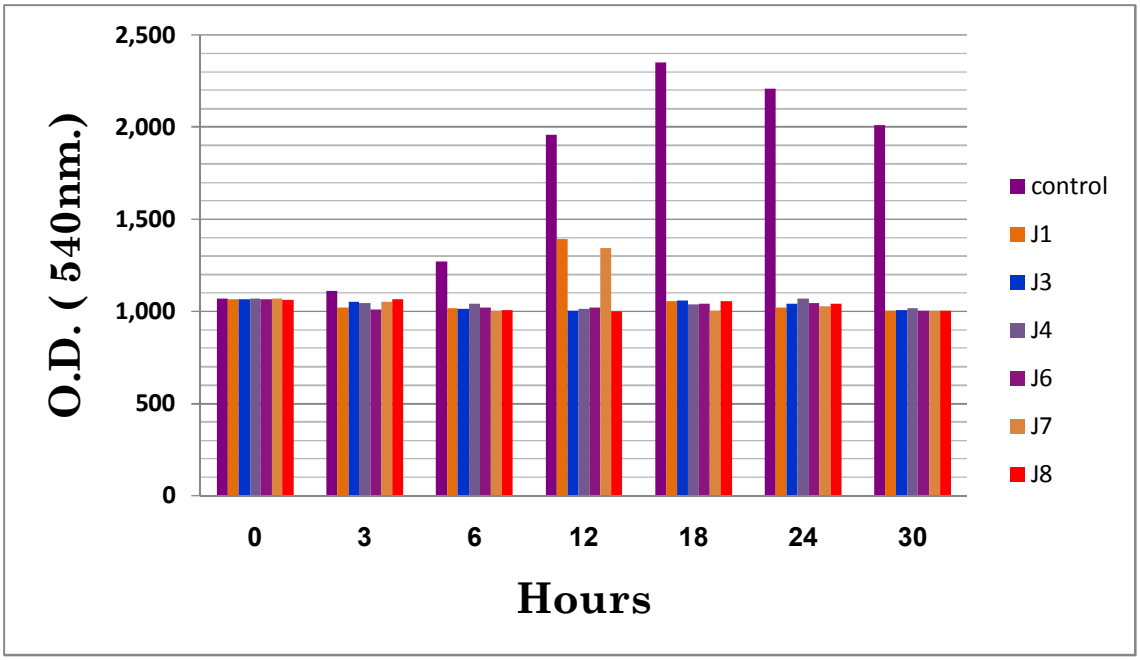

Fig. (1) Optical density of *Aeromonas hydrophila* (in test tube) *in vitro* containing six strains of LAB

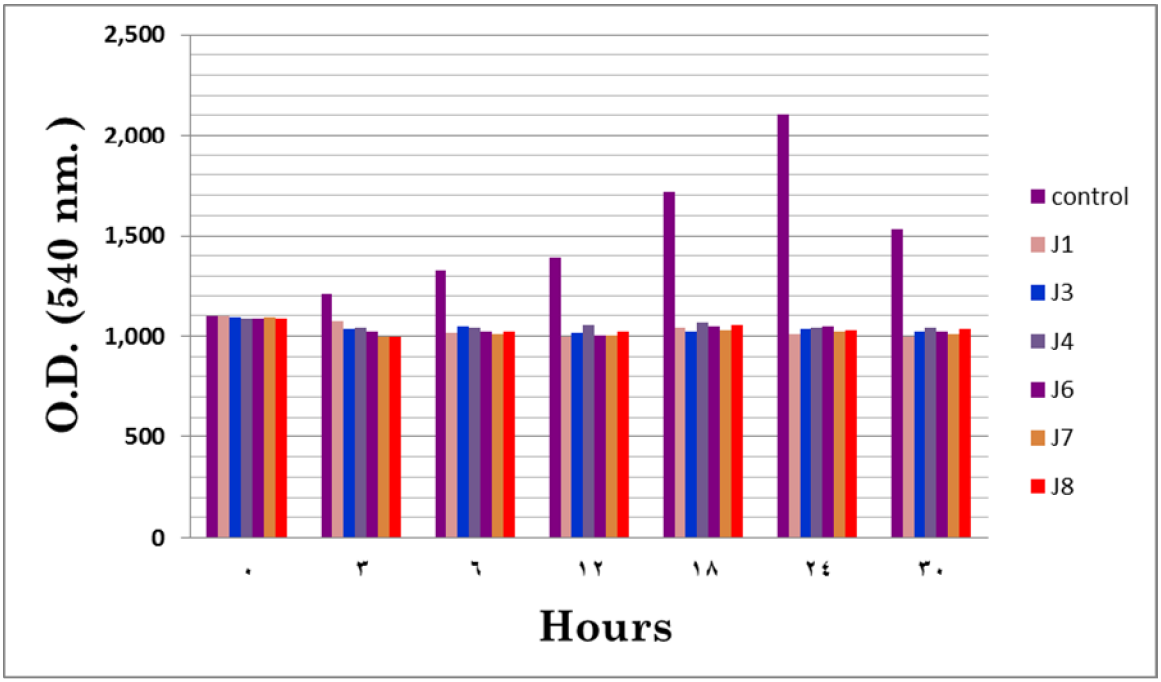

Fig. (2) Optical density of *Pseudomonas luteola* (in test tube) *in vitro* containing six strains of LAB

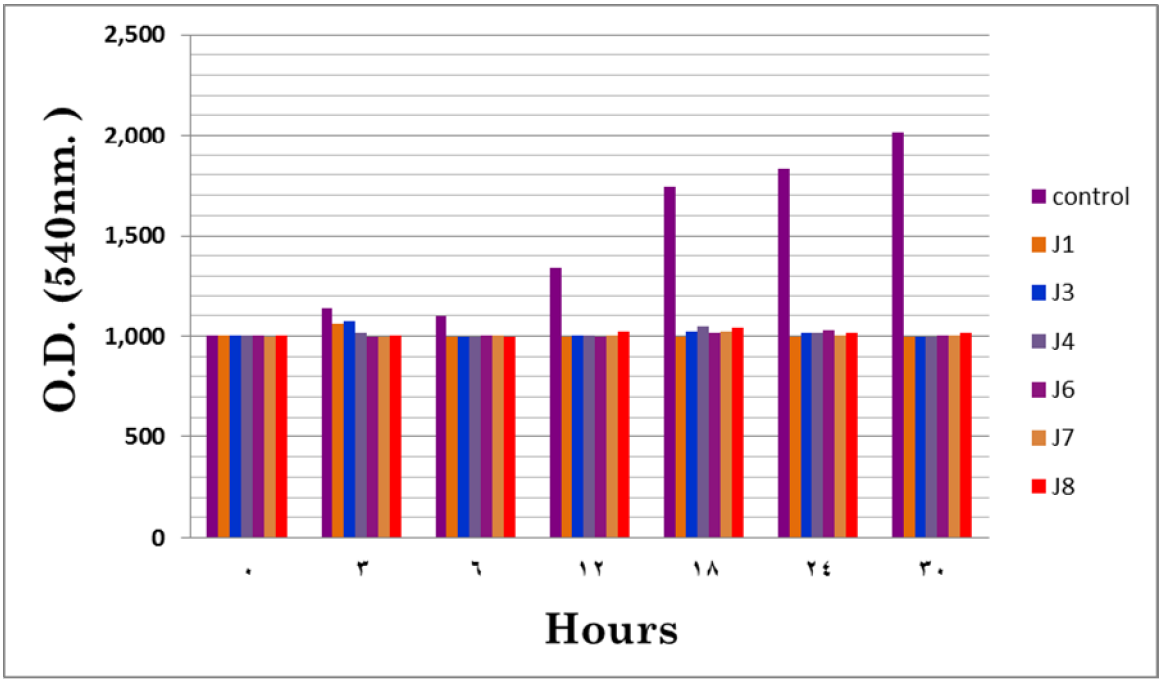

Fig. (3) Optical density of *Pseudomonas aeroginosa* (in test tube) *in vitro* containing six strains of LAB

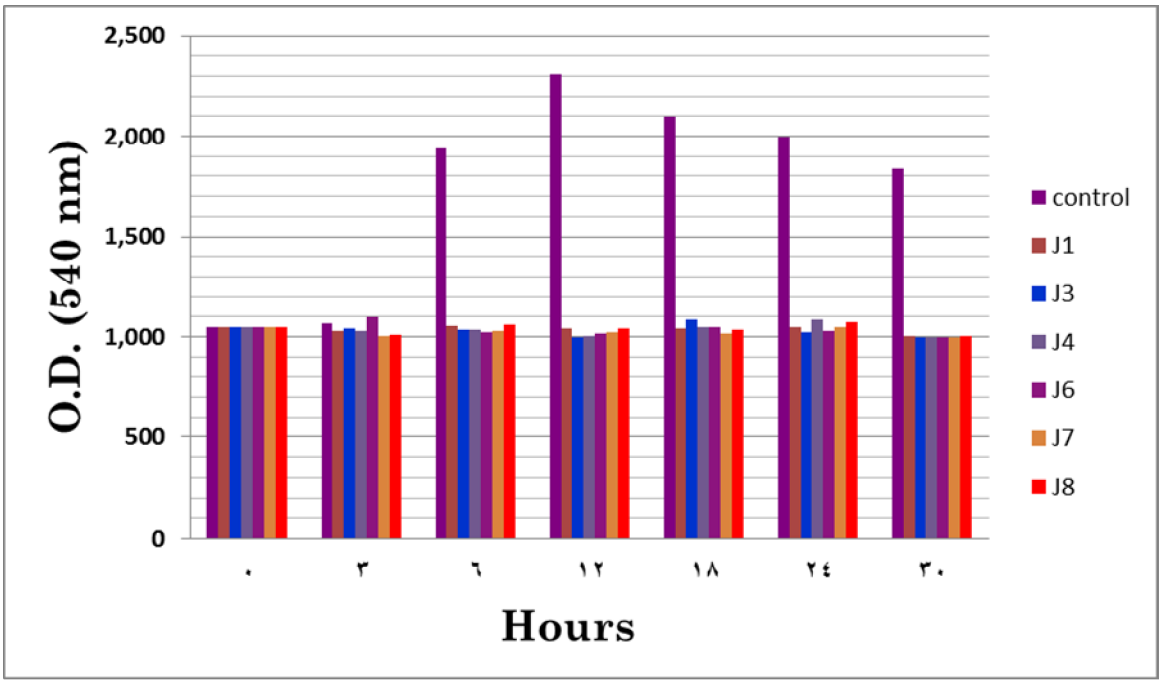

Fig. (4) Optical density of *Serratia rubidaeae* (in test tube) *in vitro* containing six strains of LAB

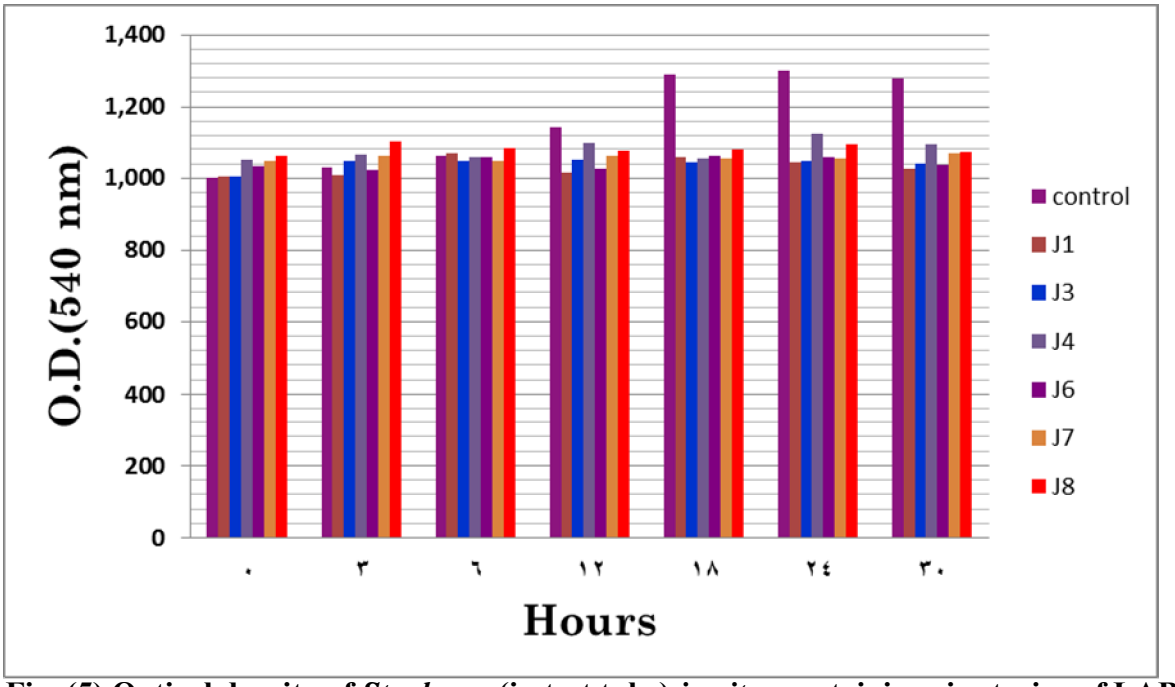

Fig. (5) Optical density of *Staph .sp.* (in test tube) *in vitro* containing six strains of LAB

- **Inhibitory effect by well diffusion and cross – streak method:** Six strains of LAB were assay for the ability to inhibit growth of (*Aeromonas hydrophila, Pseudomonas luteola, Pseudomonas aeroginosa, Serratia rubidaeae, Staph .sp.*) by agar well diffution method culture supernatants free fluid (cff) of LAB strains J1, J3, J4, J6, J7, J8 exhibited varying degree of inhibition activity against indicator (pathogen microorganism).

All LAB strain were exhibited highest antibacterial activity against *Serratia rubidaeae Aeromonas hydrophila* respectively Similar result were reported by (26) they showed the activity against *A. hydrophila* of 19 LAB strains including *Carnobacterium piscicola* and *Lactobacillus. plantarum*. The addition of freeze-dried *Carnobacterium. divergens* to compound feed did not improve the resistance of salmon fry challenged against pathogenic *A. hydrophila* (27). However, a similar dietary addition reduced the mortality rate of Atlantic cod fry when challenged against *Vibirio. anguillarum* (28). (29) found The inhibition zone 8 mm was observed on the plates inoculated with *Aeromonas hydrophila* by *Bacillus* sp. The moderate antimicrobial activity were against *Pseudomonas luteola.*

Table (2) Diameter of inhibition zone (mm) caused by antimicrobial activity of LAB strains against pathogen microorganisms

LAB strain	*Aeromonas hydrophila*		*Pseudomonas leutela*		*Pseudomonas aeroginosa*		*Serratia rubidaeae*		*Staph .sp*	
	CFF	CFBH	CFF	CFBH	CFF	CFBH	CFF	CFBH	CFF	CFBH
J1	20 mm	nil	12 mm	nil	10 mm	nil	28 mm	nil	8 mm	nil
J3	12 mm	nil	10 mm	nil	9 mm	nil	10 mm	nil	9 mm	nil
J4	20 mm	nil	13 mm	nil	11 mm	nil	18 mm	nil	8 mm	nil
J6	15 mm	nil	10 mm	nil	9 mm	nil	13 mm	nil	7 mm	nil
J7	8 mm	nil	7 mm	nil	6 mm	nil	9 mm	nil	5 mm	nil
J8	10 mm	nil	9 mm	nil	8 mm	nil	9 mm	nil	5 mm	nil
Chlormphinicol disc 30 mcg	25 mm		18 mm		16 mm		30 mm		nil	

Note: CFF = culture supernatant, NCCF = culture supernatant adjusted to pH 6.5-7.0 with 1M NaOH,

Which, *Pseudomonas aeroginosa* spoil food at low temperatures as a result of its lipolytic and proteolytic activity (30). Control of *P .aeroginosa* by bacteriocin activity of (31) has been reported that *P .aeroginosa* controlled by bacteriocin activity of *L.cacei* and *L.plantarum*. The low antimicrobial activity was against *Staph* .sp. There are many report about the antimicrobial activity of LAB most are against Gram positive bacteria (32) was reported to the isolated *Lactobacillus* sp., both homofermenters and heterofermenters, were able to inhibit the human and fish pathogens by acid production when using a high glucose concentration. A few strains also inhibited both gram-positive and gram-negative fish and human pathogens with low (0.2%) concentration of glucose in the medium. Inhibitory activities of these strains have been usually detected against related species such as *Staphylococcus aureus*, *Clostridium* and other fish pathogenic bacteria (33).

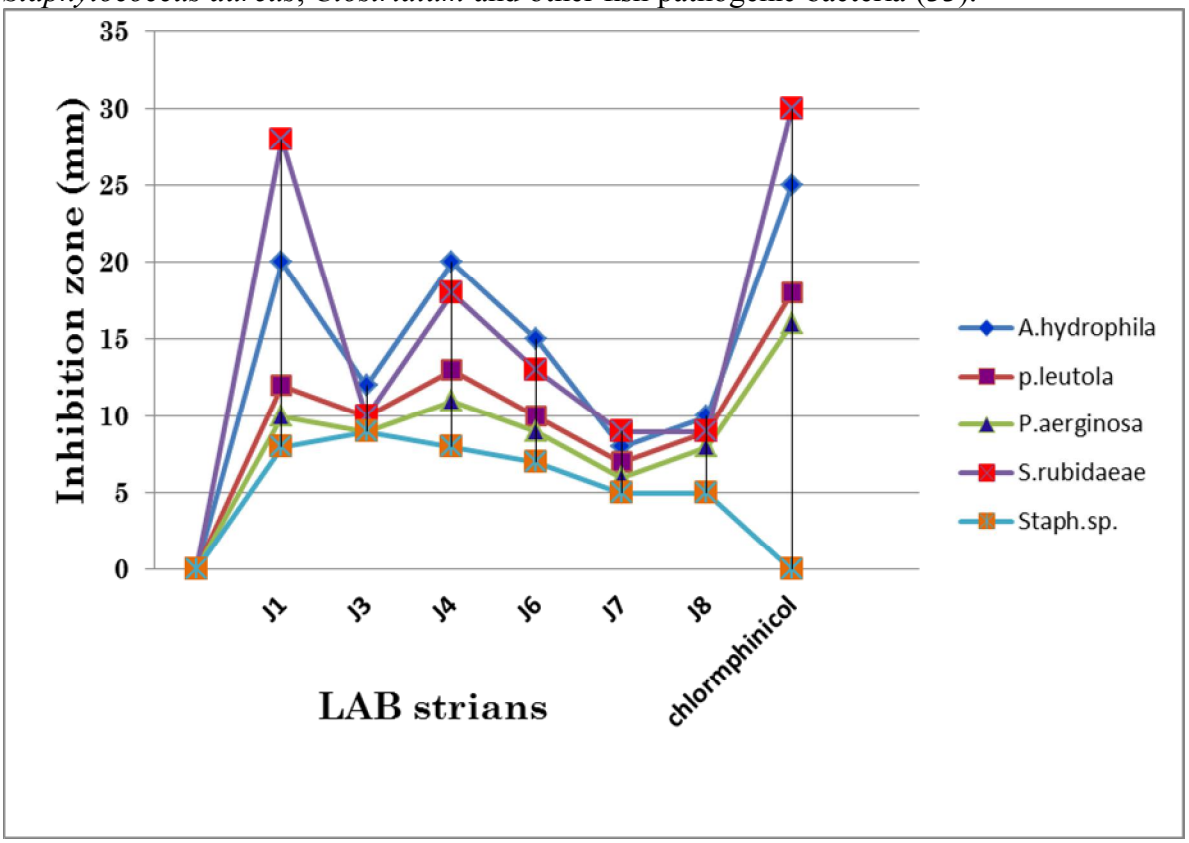

Fig. (6) Diameter of inhibition zone (mm) caused by antimicrobial activity of LAB strains against pathogen microorganisms

Neutralized culture supernatants (CFBH) of all strains exhibited no inhibition (Table 2, Fig. 6). The inhibitory effect of LAB may be due to acid or the bacitracin-like substances or combination of both (34). (35) have revealed that no correlation was found between bacitracin activity, lactic acid and hydrogen peroxide production. They reported that *Lactobacillus* strains 228, 345 and 431 produced H2O2 but did not demonstrate any inhibitory effect. Similar results were obtained (36) showed all strains of LAB may produce H2O2 but did not show any inhibitory effect.

- **Antibiogram of LAB activity:** Behavior of all LAB strains J1, J3, J4, J6, J7 and J8 were resistance to all antibiotic sensitivity OA2A-p disc except the J1, J3, J4 were sensitive to erythromycin in concentration 60 mcg after incubation for 48 hr (Table 3).

Table (3) Antibiogram of LAB isolates determined by antibiotic sensitivity
OA2A – PDisc

Antibiotics	After 24hr							After 48hr					
	Concen Tration (mg)	J1	J3	J4	J6	J7	J8	J1	J3	J4	J6	J7	J8
Erythromycin	60 mcg	R	++	++	R	R	+	+++	+ +	+ ++	R	R	+ +
Rifampicin	15 mcg	R	R	R	R	R	++	R	R	R	R	R	+ +
Colistinsulpht	150mcg	R	R	R	R	R	R	R	R	R	R	R	R
Penicillin	2unit	R	R	R	R	R	R	R	R	R	R	R	R
Kanamycim	1000mcg	R	+	+	R	+	+	+	+	+	R	+	R
Vancomycin	5 mcg	R	R	R	R	R	R	R	R	R	R	+	+

R resistance
R zone inhibition diameter less than 1 cm
+ zone inhibition diameter > 1 cm
+ + zone inhibition diameter > 2cm
+ + + zone inhibition diameter > 3cm

Besided the production of antimicrobial substances, bile salt tolerance and antibiotic resistant the great variety of mechanisms have been proposed for the action of probioticseg. Competition for adhesion receptors in the intestine, competition for nutrients and immune stimulation. Further investigations on these lines would throw more light into the actual mechanism of probiotic action in aquaculture. It was concluded that lactic acid bacteria do offer ample scope as probionts showing antagonism towards pathogenic bacteria. As these probionts were able to suppress pathogen growth in vitro and in vivo, it can be hypothesized that they have ability to colonize the gastrointestinal tract of prawn, which however, merits further confirmation. Consequently, they may prove to be suitable candidates for oral administration to farm fish, in commercial ventures to improve health and protect them against infection.

References

1. Ringø, E. & Gatesoupe, F. J. (1998). Lactic acid bacteria in fish: a review. Aquacult. 160: 177-203.
2. Gonzalez, C. L.; Enicinas, J. P.; Garcia Lopez, M. L. & Otero, A. (2000). Characterization and identification of lactic acid bacteria from freshwater fishes. Food Microbiol., 17: 383-391.
3. Kvasnikov, E. I.; Kovalenko, N. K. & Materinskaya, L.G. (1977). Lactic acid bacteria of freshwater fish. Microbiol., 46: 619-619.
4. Austin, B. L. F.; Stuckey, P. A. W.; Robertson, I. E. & Griffith, D. R. W. (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.
5. Sørum, H. (2006). Antimicrobial drug resistance in fish pathogens. In: Aarestrup, F.M.(Ed.), Antimicrobial Resistance in Bacteriav of Animal origin. ASM Press, Washington DC, PP. 213-238.
6. Salminen, S.; Ouwehand, A.; Benno, Y. & Lee, Y. K. (1999). Trends in Food Sci. Tech. 10: 107.
7. Nousiainen, J.; Javaninen, P.; Setala, J. & von Wright, A. (2004). Lactic acid bacteria as animal probiotics. P. 547-580. *In* Lactic Acid Bacteria Microbiological and Functional Aspects 3rd ed. Revised and Expanded.

8. Vaughan, E. E.; Heilig, H. G. H. J.; Ben-Amor, K. & de Vos, W. M. (2005). Diversity, vitality and activities of intestinal lactic acid bacteria and bifidobacteria assessed by molecular approaches. FEMS Microbiol.
9. Fuller, R. (1989). Probiotics in man and animal. J. Appl. Bacteriol., 66: 365-378.
10. Gatesoupe, F. J. (1994). Lactic acid bacteria increase the resistansc of Turbot larvae, Scopthalmus maximus, against pathogenic vibrio. Aquatic living resources. 7:277-282.
11. Yasuds, K. & Taga, N. A. (1980). Mass Culture Method For Artemia salina using bacteria as food.Mer.,18:53.
12. Gatesoupe, F.J.(1999).The use of probiotics in aquaculture. Aquaculture, 180: 147-165.
13. Rengipipat, S.; Rukpratanporn, S.; Piyatiratitivorakul, S. & Menasaveta, P. (2000). Immunity enhancement in black tiger shrimp (Penaeus monodon) by a probiotic bacterium (bacillus S11). Aquacult.,191:271.
14. Cai,Y.; Benno, Y.; Nakase, T. & Oh, T. K. (1998). Specific probiotic characterization of *Weissella hellenica* DS-12 isolated from flounder intestine. J. Gent. Applied Microbiol., 44:311-316.
15. De Man, J.; Rogosa, M. & Sharpe, M. E. (1972). A medium for the cultivation of lactobacilli. J. Appl. Bacteriol., 23: 130-135.
16. Byun, J. W.; Park, S. C.; Ben, Y. & Oh, T. K. (1997). Probiotic effect of *Lactobacillus* sp. DS-12 in flounder (*Paralichthys olivaceus*). J. Gent. Appl. Microbiol., 43:305- 308.
17. Alfaragi, J. K. & Alsaphar, S. A. A. (2011). Isolation of lactic acid bacteria (LAB) species as probiotic from intestinal contents of common carp *Cyprinus carpio* L. publication acceptance. J. Biotechnol Res. Center.
18. Arihara, K.; Ota, H.; Itoh, M.; Kondo, Y.; Sameshima, T.; Yamanaka, H.; Akimoto, M.; Kanai, S. & Miki, T. (1998). *Lactobacillus acidophilus* group lactic acid bacteria applied to meat fermentation. J. Food Sci., 63(3): 544-547.
19. Aslim. B.; Yuksekdag, Z. N.; Sarikaya, E. & Beyatli, Y. (2004). Determination of the bacteriocinlike substances produced by some lactic acid bacteria isolated from Turkish dairy products. LWT,1: 2004.
20. Tagg, J. R. (1991). Bacterial BLlS. ASM News Letters, P.57.
21. Erkkila, S. & Petaja, E. (2000). Screening of commercial meat starter cultures at low pH in the presence of bile salts for potential probiotic use. J.Meat Sci.,55: 297-300.
22. Pennacchia, C.; Ercolini, D.; Blaiotta, G.; Pepe, O.; Mauriello, F. & Villani, F. (2004). Selection of *Lactobacillus* strains from fermented sausages for their potential use as probiotics. J. Meat Sci., 67: 309-317.
23. Maragkoudakis, P. A.; Zoumpopoulou, G.; Miaris, C.; Kalantzopoulos, G.; Pot, B. & Tsakalidou, E. (2006). Probiotic potential of *Lactobacillus* strains isolated from dairy products. J. Int. Dairy, 16: 189-199.
24. Verschuere, L.; Rombaut, G.; Sorgeloos, P. & Verstraete, W. (2000). Probiotic bacteria as biological control agents in aquaculture. Microbiol. and Molecular Biol. Rev., 64: 655-671.
25. Lewus, C. B.; Kaiser, A. & Montville, T. J. (1991). Inhibition of food –borne bacterial pathogens by bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. Appl. Environmental Microbiol., 57:1683-1688.

26. Gildberg, A. A.; Johansen, J. & Bøgwald, J. (1995). Growth and survival of Atlantic salmon (Salmo salar) fry given diets supplemented with fish protein hydrolysate and lactic acid bacteria during a challenge trial with *Aeromonas salmonicida*. Aquaculture, 138:23-34.
27. Gildberg, A.; Mikkelsen, H.; Sandaker, E. & Ringø, E. (1997). Probiotic effect of lactic acid bac-teria in the feed on growth and survival of fry of Atlantic cod Gadu morhua. Hydrobiologia, 352,279-285.
28. Vijayabaskar, P. & Somasundaram S. T. (2008). Isolation of bacteriocin Producing lactic acid bacteria from fish gut and probiotic activity against common fresh water fish pathogen *Aeromonas hydrophila*. Biotechnol., 7: 124-128.
29. Unluturk, A. & Turantas, F. (1998). Food Microbiol. Mengi Tan Press, Cinarli, Izmir, P.605.
30. Kaya, M. (1992). Usage of different level of nitrite and different starter cultures in sucuk and their effects on the growth characteristic of *Listeri a monocytogenes* and the some other some quality criteria of sucuk. Ataturk university, Inst. Doc. TH., P.127.
31. Adolfo, B. G. (2004). *Lactobacillus plantarum* 44A as a live feed supplement for freshwater fish. Ph. D Thesis, The Netherlands with Summaries in English, Dutch and Spanish, Wageningen Universiteit, Wageningen.
32. Shillinger, U. & Lucke, F. K. (1989). Antibacterial activity of *Lactobacillus sake* isolated from.
33. Aslim, B.; Yuksekdag, Z. N.; Sarikaya, E. & Beyatli, Y. (2005). Determination of the bacteriocin-like substances produced by some lactic acid bacteria isolated from Turkish Dairy Products., LWT. 38: 691-694.
34. Aroutcheva, A.; Gariti, D.; Simon, M.; Shott, S.; Faro, J.; Simoes, J. A.; Gurguis, A. & Faro, S. (2001). Defense factors of vaginal lactobacilli. Am. J. Obstet. Gynecol. 185: 375-379. Cited by Yuksekdag, Z. N., Beyatli, Y. & Aslim, B. (2004). Determination of some characteristics coccoid forms of lactic acid bacteria isolated from Turkish kefirs with natural probiotics. LWT. 37: 663-667.
35. Nirunya, B.; Suphitchaya, C. & Tipparat, H. (2008). Screening of lactic acid bacteria from gastrointestinal tracts of marine fish for their potential use as probiotics Songklanakarin Sci. Technol., 30:141-148.