Bioremediation of lead and cadmium and the strive role of *Pediococcus pentosaceus* probiotic

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Abstract

Consumption of food and water contaminated with heavy metals poses a huge threat to the life. Both of Lead (Pb) and Cadmium (Cd) are heavy metals and important environmental pollutants. Away from traditional treatments, the current study aims to adopt probiotic bacteria *Pediococcus pentosaceu* to treat heavy metal pollution. Present results indicated a good probiotic property of *P. pentosaceus,* where they were able to survive pH range from 3-9, during incubation periods 3 and 24 hours, and bile salt range 0.15-0.5% for the same period. The number of bacteria in gastric (pH 3) and intestinal juices (pH 8) after 24 hours of incubation was 390 and 205, respectively. Bacteria showed an inhibitory effect against pathogenic bacteria *Salmonella sp*. The antibiotic susceptibility test revealed them resistant to clindamycin, intermediate resistant against benzylpenicillin, ampicillin, and their sensitivity to the rest tested antibiotics. Isolated bacteria identified based on their morphology, biochemical characteristic in addition to the use of automated instrument for bacterial identification (Vitek II), and depending on the results bacteria were identified as *P. pentosaceus*. In bioremediation study, the lowest inhibitory concentration of lead and cadmium and (MIC) was done, followed by assay the removal capacity by *P. pentosaceus*, using atomic absorption spectrometry (AAS) analysis. Bacteria show high MIC (1800 and 150 ppm) for Pb and Cd respectively. With removal efficiency for Pb 62.10-68.39% in the concentrations 25 and 50 ppm, respectively, and for Cd 52.71-11.25% in the same concentrations. Depending on the present finding probiotic bacteria (*P. pentosaceus*) can apply in the bioremediation of heavy metals in the fish ponds when contamination occurs, in addition to their tradition used as safety additive to prevent fish disease and an enhancement agent .Finally the isolation of these bacteria from fish ponds can be considered as a good indicator for a healthy state of fish ponds in the studied area.

المعالجة الحيوية لكل من الرصاص والكادميوم والدور الفعال لبكتريا *Pediococcus pentosaceus*

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الخلاصة

يشكل استهلاك الماء والغذاء الملوثين بالمعادن الثقيلة تهديداً لكل من الحياة وتنوعها. يعد كل من الرصاص والكادميوم من أبرز المعادن الثقيلة الملوثة للبيئة وذلك لسميتها لكافة الأحياء. بعيداً عن طرق المعالجة التقليدية هدفت الدراسة الحالية الى استخدام بكتريا *Pediococcus pentosaceus* في معالجة التلوث بالمعادن الثقيلة. أثبتت نتائج الدراسة الحالية امتلاك هذه البكتريا صفات جيدة بالنسبة لمواصفات بكتريا البروبايتك، حيث كانت لها القابلية للعيش في أس هيدروجيني تراوح بين ٣-٩ خلال فترة حضن استمرت لمدة ٣ و٢٤ ساعة، وفي مدى من أملاح الصفراء تراوح بين ٠,١٥-٠,٥% ولنفس فترة الحضن. بينت النتائج أن نسب البقاء في كل من العصارة المعدية (أس ها ٣) والعصارة المعوية (أس ها ٨) وخلال ٢٤ ساعة كانت ٣٩٠ و ٢٠٥. أظهرت البكتريا فعالية تثبيطيه تجاه البكتريا

المرضية *Salmonella* sp. بينت نتائج فحص الحساسية الدوائية أن البكتريا كانت مقاومة لعقار كليندامايسين ومتوسطة المقاومة للامبسيلين والبنزايلبنسيلين، بينما كانت حساسة تجاه بقية العقاقير المدروسة. شخصت البكتريا تبعاً لكل من الصفات المظهرية والكيموحيوية ولتأكيد التشخيص تم استخدام جهاز تشخيص البكتريا VitekII ومن خلال النتائج شخصت البكتريا على أنها *P. pentosaceus*. في دراسة المعالجة الحيوية تم تحديد اقل تركيز قاتل بالنسبة لكل من الرصاص والكادميوم ومن ثم تم تحديد قابلية البكتريا على إزالة هذين المعدنين باستخدام جهاز الامتصاص الذري. أظهرت البكتريا قيمة عالية لأقل تركيز قاتل ١٨٠٠ و ١٥٠ جزء بالألف لكل من الرصاص والكادميوم وعلى التوالي مع قابلية إزالة لهذين المعدنين تراوحت بين ٦٢,١٠ ـ ٦٨,٣٩% و ٥٢,٧١ ـ ١١,٢٥% عند التركيزين ٢٥ و ٥٠ جزء بالألف وعلى التوالي. اعتمادا" على نتائج الدراسة الحالية يمكن استنتاج امكانية استخدام البروبايوتك بكتريا *P. pentosaceus* في المعالجة الحيوية للتلوث بالمعادن الثقيلة في احواض تربية الاسماك فضلا" عن استخداماتها التقليدية كمضافات امنه لحماية الاسماك من الممرضات وكمعززات للنمو . واخيراً تجدر الاشارة الى انه عزل البكتريا من احواض تربية الاسماك في منطقة الدراسة يعتبر مؤشر جيد لحالة الاحواض كونها بيئة قادرة على مواجهة المشاكل البيئية الطارئة.

Introduction

Both of Lead (Pb) and cadmium (Cd) are the two great toxic heavy metals in the environment, reported in the priority list of dangerous matter on the 2nd and 7th places, respectively (1). They are biologically nonessential and non-degradable and tend to accumulate in exposed organisms. The aquatic environment is very susceptible to heavy metal contamination, and the gradual increase of these metals in aquatic environments has become a major problem. Fish are the water inhabitants, that can be heavily affected by heavy metals. In spite of the constitutive efforts to protect the health from hazardous heavy metals, occupational and environmental exposures to Pb and Cd remain a serious problem in many countries (2). Several modified and sophisticated techniques have emerged, including many methods of biological treatment to improve the environment contaminated with heavy metals. More recently, the use of favorable microorganisms such as probiotics has been a field that is likely to be shown to scientists in the aquaculture field to care of aquatic animals and for the conservation of the aquatic environment. In most cases, probiotic bacteria, such as lactobacilli and bifidobacterial separated from environmental samples and the digestive tract have been used as an adjunct to improve nutrition, growth, disease controls (3,4), and the immune response (5). There are also many studies that have been shown that the Lactic acid bacteria (LAB) and particularly lactobacilli can bind heavy metals, so they can consider as a hopeful means for eliminating of heavy metals from food and water and may be the digestive tract as well, the extension of LAB applying in the food industry as a probiotic. Unlike classical remediation process, the binding of bacterial metal ions is nonspecific and environmentally benign, cheap, and active even at low metal ion concentrations (6). The removal of heavy metals with lactic acid bacteria (LAB) has been studied in numerous previous works (7-9). LAB is group of bacteria which give a positive reaction toward gram stains, under light microscope, they occur with rod or coccus shape. Their DNA has less than

55% mol from G+C, they don't form spore, cannot move, microaerophilic, they fermented carbohydrates to the lactic acid as their main end product. LABs are usually presented in an environment where the nutrient is being rich such as (milk, meat, vegetables), fermented decay material and the mucosal layer of the digestive tract of organisms (10). Probiotic bacteria are the majority of microbes that can be safely used in medical and veterinary applications (11), and there's many criteria the bacteria must be achieved to be perfect as a probiotic, such as the ability to survive at low pH in the stomach and high concentrations of bile salts available so as to reach the hindgut in good form and effort their advantageous properties (8). One of the important properties of probiotic is its ability to improve the immune performance of the host and therefore it must have antagonistic activity against pathogens, beside their ability to live with substances that act as antibacterial agents like organic acids, heavy metals, hydrogen peroxide and antifungal compounds (12).

From the above introduction, this study intends to isolate and individualize of probiotics from fish farming and demonstrate their *in vitro* bioremediation ability for Lead and Cadmium.

Material and methods

Collection of samples

Nine water samples were collected from three *Cyprinus carpio* (common carp) fish ponds located in Marine Science Center, Basra University, Al- Garma campus, using 500 ml glass bottles, during April 2018. Samples putted in ice box and transfer to the lab where saved under cooling 4 ºC till be used. The feed used in these ponds not contain any bacterial additive, and it was confirmed that no additional supplements were added to feed of fish in these ponds.

Isolation and enumeration of bacteria from water of the fish pond

A series of dilution was performed under severe sterilization for all water samples collected, then 0.1 ml of

each dilution planted on the surface of de Mann Rogosa Sharpe agar medium (MRS, Hi media) (13), and then incubated aerobically at 37 ºC for 48 hours. The clear bacterial colonies were taken from the plant medium to produce pure culture.

Characterize of bacteria

Morphological and biochemical tests were carried out for diagnosis the probable type of bacterial colony from MRS agar (14), and for certain the identification Vitek II (Biomerieux, USA), also has been used.

Probiotic properties of the isolate

Probiotic properties of identification bacteria, such as survive in acid, tolerance against bile salt, and tolerance to gastrointestinal juices have been studied as follows.

Affording to Acid

The acid tolerance test has been done using MRS broth with different pH 3, 5, 7, and 9, which prepared using HCl 1% (J. T. Baker) and NaOH 1N (Hi media) in addition to control flask (15), then autoclaved at 121 °C for 15 minutes (triplicate has been used for each test). 0.1 ml of overnight cultured bacteria in MRS broth has been used to inoculate each flask, which then incubated at 30 ºC. Optical density (OD) as the growth rate of bacteria was measured by a spectrophotometer (Shimadzu, UV-1800, Japan) at 600 nm after 3, and 24 hours incubation (9).

Bile tolerance (Oxgall treatment)

For this test MRS broth with different concentration of Oxgall bile salts (Difco) has been prepared 0.0, 0.15, 0.25, and 0.3% (w/v). Then each concentration inoculated with 0.1 ml from overnight cultured bacteria and incubated at 30 °C. The growth of bacteria in each concentration has been assessed after incubation at 37 °C for 4 and 24 hours by measuring the optical density at 600 nm using spectrophotometer (Shimadzu, UV-1800, Japan) (16).

Afford to simulate human gastrointestinal tract

To provide in vitro situation as those found in the digestive tract, both of gastric and pancreatic juices were prepared by dissolving 3 mg.ml^{-1} from pepsin (Sigma) and 1 mg.ml^{-1} from pancreatin USP (Sigma- Aldrich) in sterile sodium chloride solution (0.5%, w/v). Hydrochloric acid (3 mol.L^{-1}) and NaOH (1 mol.L^{-1}) have been used to revising the pH to 3 and 8 respectively. 0.2 ml of the overnight bacterial rinse in phosphate buffered saline (pH 7.0) were injected with 1.0 ml of simulated gastric or pancreatic juice and 0.3 ml NaCl (0.5%, w/v), then incubated at 37 °C. Colony forming unit (CFU /ml) was counted after incubation for 180 min for gastric tolerance, and 240 min for basic pH tolerance (17).

Antibacterial test

Freshwater fish pathogens, *Salmonella sp.* were used to study the antibacterial ability of probiotic bacteria using well diffusion techniques. Shortly, 1 ml of overnight culture of *Salmonella sp* in Tryptone soya broth (TSB, Hi media) was distributed through sterile loop on tryptone soy agar (TSA, Hi media). A probiotic suspension used as an antibacterial agent was obtained from a 24 hours bacterial culture after being centrifuged at 3000 rpm for 5 minutes (16).

Antibiotic susceptibility test

Automated way using Vitek II, were applied to study the sensitivity of probiotic against many antibiotics. The tests are run similarly on cards which have of attenuation of antimicrobials to appoint the break point, which mentioned to the minimum inhibitory concentration (MIC) of antibiotic.

Heavy Metal Tolerance Assays
Preparation of heavy metals concentrations

To prepare stock solutions from the heavy metal salts, the exact weight of Pb (NO$_3$)$_2$ and Cd (NO$_3$)$_2$ H$_2$O were dissolved in sterile deionized distilled water. Then suitable serial dilution of these stock solutions was used to make different concentrations of Cd (II) and Pb (II) (18).

The lowest inhibitory concentration

This test usually used to detect the ability of bacteria to tolerate heavy metals. Loopful of the overnight growing probiotic bacteria in MRS broth (MRS, Hi media), at 37 °C, was taken aseptically and streaked onto a plate of MRS agar supplemented with various concentrations of Cd, and Pb (25, 50, 100, 250, 500, 1000, 1500, 1800 and 2000 ppm). The plates were then incubated at 37 °C for 48 hours. A colony or limited growth was ignored. The lowest concentration of heavy metals leading to growth inhibition was determined to be the MIC. This procedure was carried out in triplicate, as one of them was control (19).

Biosorption study

2 ml of the overnight bacterial pending was used to vaccinate MRS broth appended with 25 and 50 ppm of Cd, and Pb, Then the broth nestles at 37 °C for 24 hrs. After that broth has been centrifuged at 3000 rpm for 20 min, the supernatant was used to assay Cd, and Pb removal by bacteria using flame atomic absorption spectrophotometer (AAS 6300, Shimadzu, Japan). This experiment has been done in triplicate as one of them was control. The bacterial efficiency in remove Cd, and Pb was calculated as percentage of removal using the equation below:
% Removal = (decline in HM concentration ÷ Initial HM concentration) × 100 Where, HM means heavy metal (19).

Results

Isolation and identification of *P. pentosaceus*

During the current search, only the isolate that gave positive reactions to gram stain and negative reaction to catalase were selected for additional diagnosis. The bacteria have been identified as *P. pentosaceus* through its phenotypic, culture characteristics, and based on biochemical tests. The colony properties of these bacteria were examined by aseptically handling, colony and transferred it to the selective medium (medium MRS) to monitor the growth pattern of the isolates. The colonies were creamy white, circular, low convex with a full rim were regarded as belonging to the genus *P. pentosaceus* (Table 1). and for more confirmation it's identified by Vitek II, with confidence degree 95%.

Table 1: Morphological, and biochemical properties of the bacteria

Colony Morphology	
Configuration	Round
Cell shape	Round
Motility	Non motile
Pigment	White Creamy
Gram reaction	+
Surface	Mucoid
Biochemical Tests	
H2S formation	-
Catalase	-
Nitrate reduction	-
Urease	-
Indole Production	+
Methyl red test	+
V. P. Reaction	+
Citrate utilization	+
Acid Production from	
Glucose	+
Lactose	+
Maltose	+

Acid tolerance test

The results in Table 2 showed that the growth of bacteria appeared during a range of pH 3-9, and during three and 24 hours, duration. These results indicated that *P. pentosaceus* having the most important feature of probiotic bacteria, which is their ability to alive in stomach where high acidity.

Tolerance against Bile salt

To be ensured from bile salts tolerance in the studied bacteria, they were analyzed to a greater extent for their ability to survive at different concentrations of bile salt (0,0.15, 0.25 and 0.5%), and during different incubation period (3 and 24 hours) results showed the capacity of the bacteria to survive under studied concentrations of bile salts, however, the growth of bacteria decreased with the increase concentration of bile salt also their survive were less in 24 hours of incubation than this in 3 hours (Table 2).

Probiotic survival in gastric and intestinal juices

The probiotic survival rate in both gastric and pancreatin juices was as shown in the table 2, results showed that bacteria could survive in both juices (pH 3 and pH 8) during 24 hours, with efficient survive in pH 3 than in pH 8.

Detection of antimicrobial activity

Result showed that the cell free solution of *P. pentosaceus* was able to inhibit the growth of pathogenic bacteria *Salmonella* sp.

Antibiotic susceptibility in *P. pentosaceus*

Table 2, shows the MIC values obtained for the different antibiotics tested in the studied bacteria. To determine whether an organism is sensitive, mild opponent or opponents to antimicrobials, the break value of MIC is adopted. From results we can conclude that the understudying bacteria was sensitive to the most studied antibiotics with differ in MIC value, whereas it was resistance to only inducible Clindamycin.

Metal resistant pattern

In order to evaluate the bioremediation ability of the isolates, bacteria were investigated for their tolerance to different concentrations of Pb, and Cd by assessing their minimal inhibitory concentrations (MICs). Results of MIC values were shown in the Table3. As it can be seen from the results, the value of MIC was greater for Pb 1800 ppm than this for Cd 150 ppm. In this context, it may be inferred although *P. pentosaceus* was resistant against Pb and Cd, but it was more resistant to Pb than Cd. In bioremediation study two concentration 25 and 50 ppm from Pb and Cd has been used and Results in (Table 3) indicated that the percentage of Pb removal were 62.10 and 68.39% for the concentration 25 and 50 ppm respectively, and for Cd they were 52.71 and 11.25%. Results also indicated that the studied bacteria were able to remove Pb more than their ability in removing Cd. Another conclusion can be added, the ability of bacteria to remove Pb increases with increased concentration, while Cd removal decreases with increased concentration, which means that Cd removal ability is concentration dependent, from the highest Cd concentration (50 ppm) a smaller fraction was removed than from the 25 ppm.

Table 2: Effect of both pH and concentration of bile salts in the survival of *P. pentosaceus*, the number of bacteria in gastric and intestinal juices, and Antibiotic sensitivity pattern of various antibiotics

Medium pH	Optical density (600 nm) (3 hours)	Optical density (600 nm) (24 hours)
3	0.564	0.327
5	0.886	0.639
7	1.629	1.687
9	1.511	1.645
Bile salt %	Optical density (600 nm) (3 hours)	Optical density (600 nm) (24 hours)
Zero	1.686	1.557
0.15	0.161	0.568
0.25	0.132	0.398
0.5	0.125	0.239
	Number of bacteria in gastric juice (pH 3) (24 hours)	Number of bacteria in intestinal juice (pH 8) (24 hours)
	390	205
Antimicrobial	MIC	Interpretation
Benzylpenicillir	1	I
Ampicillin	0.5	I
Cefotaxime	0.5	S
Ceftriaxone	1	S
Levofloxacin	2	S
Clindamycin		R
Erythromycin	<=0.12	S
Clindamycin	<=0.25	S
Linezolid	<=2	S
Vancomycin	0.25	S
Tetracycline	0.5	S

Table 3: The minimum inhibitory concentration (ppm), and heavy metals removal (%)

Heavy metals	MIC (ppm)	Heavy metals removed (%)/24h	
		25 ppm	50 ppm
Pb	1800	62.10	68.39
Cd	150	52.71	11.25

Discussion

Depending on the traditional way included the morphological and biochemical tests the isolated bacteria in present study has been identified as *P. pentosaceus*, and this finding also emphasis by using the Vitek II, which gave us result with confidence degree 95%. This results covenant with the results recorded by Ayo-Olalusi (6). Different feature related to good probiotic properties of bacteria has been studied. The high acidity in the stomach is considered one of the most important elements that can affect the survival of probiotics (20). Therefore, to be ensure from probiotic properties, testing their viability and functionality in acidic conditions must be defended, possessing this property is very important as probiotic bacteria have to pass through the stomach were strongly acidic condition found to arrive the intestines. Nevertheless, probiotic bacteria have different susceptibility to acidify depending on their types and strains and strain dependent (21).

Good probiotic sources must at least stay alive at pH 3 (22), because of the high acidity in the stomach. The present finding revealed that these bacteria were able to survive in extreme acidic condition, and these outcomes harmonize with the results reported by (23), and can be proved that the studied bacteria have one of the most selective standards for probiotic bacteria (24). Acid tolerance in probiotic bacteria also reported in other studies, Damayanti *et al.* (25) reported the viability of lactic acid bacteria *Lactobacillus plantarum* was 92.61% at pH 3 after 90 minutes. Raghad *et al.* (26) reported that the all isolates of *pediococcus* bacteria have the ability to survive in acidic conditions for 3 hour and 24 hours. The study of resistance to bile salts by probiotic bacteria consider extremely important, that the one of the most important features in the probiotic bacteria is their tolerance to the bile salts, because bile salts can act as antibacterial agents towards normal flora (21). Ability to survive in the bile salt also has been studied, and bacteria showed good ability to survive in a bile salt concentration 0, 0.15, 0.25 and 0.5%, but with an increase the bile salt concentration the bacterial survive decrease. Similar results were recorded by Allameh *et al* (9), where they find out, significant decrease in the growth of *L. mesenteroides* by increasing bile salt concentration. Also, Sukumar and Ghosh (27) reported that *Pediococcus* spp. which were isolated from an Indian fermented food showed meaningful bile tolerance. Shin *et al.* (28) finds out that the *P. pentosaceous* SH740 isolated from GIT of broiler chickens were survive in the 0.5% bile salts and stay viable after 2 hours at pH 3. The combined effect of the pepsin (pH 3) solution and the pancreatin solution (pH 8) was intended to simulate the gastric and intestinal juice respectively. Results indicated that the bacteria could survive in both solutions, however their survive was more in gastric solution than in pancreatin solution. Similarly, Grimoud *et al.* (29) reported that *Lactobacillus* strains have more survival rates under gastric conditions than the survival rate in the intestines. Tokatl *et al.* (30) also reported that they all studied strain of *L. plantarum* and *L. brevis* more resistance to intestinal conditions than gastric juice. Probiotic also showed good inhibition action towered pathogenic bacteria. The inhibition of pathogenic bacteria is due to the inhibitory action of the extracellular and to diffusible metabolites by *P. pentosaceus*. These findings are appropriate for those who have been indicated by (31,32). Antibiotic sensitivity pattern of *P. pentosaceous* against

various antibiotics has been studied and results indicated that the bacteria was sensitive to the most studied antibiotics with differ in MIC value, whereas it was resistance to only inducible Clindamycin, in this respect Klare *et al* (33) have been identified the MICs from 16 antimicrobials for 473 isolates of LAB, including *Lactobacillus*, *Pediococcus* and *Lactococcus*, and results proved that the vast majority of LAB were sensitive towards penicillin, ampicillin, ampicillin / sulbactam, quinupristin / dalfopristin, chloramphenicol and linezolid, where's, the resistant to streptomycin occur in three strains of the probiotic. Another study has found that *Lactobacillus* species are affected by many inhibitors of cell wall synthesis, such as penicillin and ampicillin. Munoz-Atienza *et al* (34) reported that *P. pentosaceus* LPP32, LPM83 and B5 were clindamycin resistant.

The MIC is defined as the minimum concentration of a heavy metal at which microbial growth is completely inhibited due to its effect (35). To assess the ability of bacteria to remediate heavy metals it's important to determine the minimum inhibitory concentration, in present study *P. pentosaceous* showed a high value of MIC for both Cd and Pb (150 and 1800 ppm) respectively, however the MIC was more for Pb than for Cd. This can be attributed to the high concentration of lead in the environment, which made the bacteria more resistant to Pb than Cd. Similarly, Bhakta *et al* (36) proved ability of six isolated from *Pediococcus dextrinicus* and *Pediococcus acidilactici* to tolerance, high concentration of Pb <1000, for all isolated, where's the MIC value for a Cd range from 50 to 250 for the six isolates. Li *et al* (37) reported that the MIC value for Pb by *L. bulgaricus* KLDS10207 was >1000. Since the bacteria under study showed high tolerance for both Pb and Cd, so they were used in the study of removing of these two metals using different concentration 25 and 50 ppm, and during 24 hours. Bioremediation ability of *P. pentosaceous* indicated ability of this bacteria to remove both Cd and Pb. Like other gram positive bacteria, *Lactobacillus* cell surface contains different charge group like carboxyl, hydroxyl, and phosphate which result from the complex component of their cell wall (thick peptidoglycan layer, (lipo) teichoic acids, polysaccharides, and proteins, including S-layer (glycol) proteins (19), and as a consequence they have large number of ligand that can binding cations such as Cd and Pb. Results also showed that *P. pentosaceous* was more efficient in remove Pb than Cd. This can be attributed to having a high MIC value for Pb than this for Cd, which may be responsible for developing the possibilities of bacteria in removing Pb. In the same context (36) showed that Pb and Cd resistant. *Lactobacillus* strains were more efficient in remove Pb than Cd. In the same context (38) reported that the Pb removal by *L. bulgaricus* KLDS10207 reach to 79.18% with the initial Pb concentration 50 ppm. Results indicated that the Cd removal by bacteria was

concentration dependent where it was decrease with increase the concentration, and this agree with (39). At a concentration of 10 μg/l up to 70% could be removed within 5 minute, and up to 90% after 1 hour, but at a concentration of 1000 μg/l between 5 and 30% was removed after 5 minute and between 20 and 55% after 1 hour (7), reported that the binding of both Cd and Pb by all studied stain of LAB, were ranged between 61.8-87.8% and 30.2-92.6%, respectively.

Conclusions

In this study, by morphological and biochemical investigation, isolated bacteria were identified as *P. pentosaceus* and the results were verified using VitekII. The bacteria undergo various tests to evaluate their probiotic characteristics. All were acids, bile and gastrointestinal tolerance. Analogous features of antibiotic susceptibility have been observed during this study and the bacteria have an antibacterial activity against the bacteria test indicator, and from the result can be concluded that they have perfect probiotic properties. And the results of bioremediation study indicated their ability to tolerate high concentration of Pb, and Cd, where the MIC was 1800 and 150 ppm respectively. Subsequently, an expanded flame AAS analysis was acted to report the removal of heavy metals by bacteria. The bacteria show a reasonable elimination of both Cd, and Pb. It can be concluded that *P. pentosaceus* has efficiency in removing both of Cd, and Pb from broth culture emended with different concentration 25 and 50 ppm of Cd, and Pb. Thus, the presence of these bacteria in fish ponds is of great importance in removing heavy metals as well as their role as the basis food supplements.

Acknowledgments

References

1. Agency for toxic substances and disease registry guidance for the preparation of a twenty first set toxicological profile. 2007. Available online: http://www.atsdr.cdc.gov/toxprofiles/guidance.pdf.
2. Jaafar R, Al-Sulami A, Al-Taee A, Aldoghachi F, Suhaimi N, Mohammed S. Biosorption of some heavy metals by *Deinococcus radiodurans* isolated from soil in Basra governorate, Iraq. J Bioremediat Biodegrad.2016;7(2). doi: 10.4172/2155-6199.1000332.
3. Fenchel T, Kofoed L. Evidence for exploitative interspecific competition in mud snails (Hydrobiidae). Oikos. 1976;27(3):367-376. doi:10.2307/3543455.
4. Sugita H, Okano R, Suzuki Y, Iwai D, Mizukami M, Akiyama N, Matsuura S. Antibacterial abilities of intestinal bacteria from larval and juvenile Japanese flounder against fish pathogens. Fish Sci. 2002;68(5):1004-1011.doi.org/10.1046/j.1444-2906.

5. Sugita H. Antibacterial abilities of intestinal bacteria from three coastal fishes. Aquacul Sci. 1998;46:563-568.

6. Ayo-Olalusi CI. Isolation and identification of probiotics *Pediococcus pentosaceus* from the gut of tilapia guineensis for use in aquaculture production. Intern J Res Stud Microbiol Biotechnol. 2017;3(1):15-20. doi.org/10.20431/2454-9428.0301004.

7. Halttunen T, Kankaanpa P, Tahvonen R, Salminen S, Ouwehand AC. Cadmium removal by lactic acid bacteria. Biosci Microflora. 2003;22(3):93-97. doi.org/10.12938/1996.2293.

8. Elsanhoty RM, Al-Turki I, Ramadan MF. Application of lactic acid bacteria in removing heavy metals and aflatoxin B1 from contaminated water. Water Sci Technol. 2016;74(3):625-638.doi.org/10.2166/wst.2016.255.

9. Allameh SK, Daud H, Yusoff FM, Saad CR, Ideris A. Isolation, identification and characterization of *Leuconostoc mesenteroides* as a new probiotic from intestine of snakehead fish (*Channa striatus*). Afr J Biotechnol. 2012;11(16):3810-3816.doi.org/10.5897/AJB11.1871.

10. Salminen, S. (Ed.), von Wright, A. (Ed.). Lactic Acid Bacteria. Boca Raton: CRC Press,2004. doi.org/10.1201/9780824752033.

11. .Rauta PR, Dhupal M, Nayak B. Screening and characterization of potential probiotic lactic acid bacteria isolated from vegetable waste and fish intestine. Int J Curr Microbiol App Sci. 2013;2:234-244.doi.org.10.1590/s1517-82832011000400039.

12. Ghosh S, Ringo E, Selvam ADG, Rahiman KM, Sathyan N, John N, Hatha AM. Gut associated lactic acid bacteria isolated from the estuarine fish *Mugil cephalus*: Molecular diversity and antibacterial activities against pathogens. Inter J Aquac. 2014;4(1):1-11. doi.org/10.5376/ija.2014.04.0001.

13. De Man J, Rogosa D, Sharpe ME. A medium for the cultivation of lactobacilli.JApplBacteriol.1960;23(1):130-135doi.org/10.1111/j.1365-2672.1960.tb00188.

14. Holt JG, Krieg N. Bergey's manual of systematic bacteriology. Baltimore: The Williams and Wilkins Co; 1984. 1-13 p.

15. Samelis J, Maurogenakis F, Metaxopoulos J. Characterization of lactic acid bacteria isolated from naturally fermented Greek dry salami. Int J Food Microbiol. 1994;23(2):179-196. doi.org/10.1016/0168-1605(94)90051-5

16. Balcazar JL, Vendrell D, de Blas I, Ruiz I, Muzquiz JL, Girones O. Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish. Aquac. 2008;278(1-4):188-191. doi.org/10.1016/j.03.014.

17. Charteris WP, Kelly PM, Morelli L, Collins JK. Selective detection, enumeration and identification of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in mixed bacterial populations. Int J Food Microbiol.1997;35(1):1-27.doi.org/10.1016/S0168- 1605(96)01222-6.

18. Etorki AM, El-Rais M, Mahabbis MT, Moussa NM. Removal of some heavy metals from wastewater by using of fava beans. Am J Analyt Chem. 2014;5(04):225. doi.org/10.4236/ajac/54028.

19. Huet MAL, Puchooa D. Bioremediation of heavy metals from aquatic environment through microbial processes: A potential role for probiotics? J Appl Biol Biotechnol. 2017;5(6):14-23.doi.org/10.7324/JABB/.506033.

20. Menconi A, Kallapura G, Latorre JD, Morgan MJ, Pumford NR, Hargis BM, Tellez G. Identification and characterization of lactic acid bacteria in a commercial probiotic culture. Biosci Microbiol Food Health. 2014;33(1):25-30.doi.org/10.12938/bmfh.33.25

21. Fontana L, Bermudez M, Plaza J, Munoz S, Gil A. Sources, isolation, characterization and evaluation of probiotics. Br J Nutr.2013;109(S2):S35-S50. doi.org/10.1017/S0007114512004011.

22. Fernandez M, Boris S, Barbes C. Probiotic properties of human *lactobacilli* strains to be used in the gastrointestinal tract. J Appl Microbiol.2003;94(3):449-55.doi.org/10.1046/j.1365-2672.2003.01850.x.

23. Davati N, Yazdi FT, Zibaee S, Shahidi F, Edalatian MR. Study of lactic acid bacteria community from raw milk of Iranian one humped camel and evaluation of their probiotic properties. Jundishapur J Microbiol. 2015;8(5):e16750.doi.org:105812/jjm 8(5)2015.16750.

24. Cakir I. Determination of some probiotic properties on *Lactobacilli* and *Bifidobacteria*. Ankara Univrsity thesis of Ph.D.2003.

25. Damayanti LI, Saragih JE, Purwoko T, Sardjono. Characterization of lactic acid bacteria as poultry probiotic candidates with aflatoxin B1 binding activities. Earth Environ Sci. 2017;12(3):1-9. doi.org/10.1088/1755-1315/101/1/012030.

26. Raghad S. Jaafar, Fadhil N. Al-knany, Bayan M. Mahdi and Asaad M.R. Al-Taee, Study the Probiotic Properties of *Pediococcus pentosaceus Isolated* from Fish Ponds in Basra City-South of Iraq, *J Pure Appl Microbiol*.,2019; **13**(**4**): doi: 10.22207/JPAM.13.4.

27. Sukumar G, Ghosh AR. *Pediococcus* spp. a potential probiotic isolated from Khadi (an Indian fermented food) and identified by 16S rDNA sequence analysis. Afr J Food Sci. 2010;4(9):597-602. doi.org/10.5897/AJFS2019.1826.

28. Shin M, Han S, Ji A, Kim K, Lee W. Isolation and characterization of bacteriocin-producing bacteria from the gastrointestinal tract of broiler chickens for probiotic use. J Appl Microbiol. 2008;105(6):2203-2212. doi.org/10.1111/j.1365-2672.2008.03935.x.

29. Grimoud J, Durand H, Courtin C, Monsan P, Ouarne F, Theodorou V, Roques C. In vitro screening of probiotic lactic acid bacteria and prebiotic glucooligosaccharides to select effective symbiotic. Anaerobe. 2010;16(5):493-500. doi.org/10.1016/j.anaerobe.2010.07.005.

30. Tokatl M, Gulgor G, Bagder S, Arslankoz N, Ozcelik F. In vitro properties of potential probiotic indigenous lactic acid bacteria originating from traditional pickles. Biol Med Res Inter. 2015;31(5):1-8.doi.org/10.1155/2015/315819.

31. Bartkiene VK, Antanaitis J, Kantautaite A, Kucinskas M, Ruzauskas L, Vaskeviciute R, Siugzdiniene J, Kucinskiene J, Damasius G, Juodeikiene S. Antimicrobial activity of lactic acid bacteria multiplied in an alternative substrate and their influence on physiological parameters of new-born calves. Vet Med. 2016;61:653-662. doi.org/10.17221/192/2015-VETMED

32. Dalia C, Grazina J, Algimantas P, Elena B. Antimicrobial activity of lactic acid bacteria against pathogenic and spoilage microorganism isolated from food and their control in wheat bread. Food Control2013;31:539-545. doi.org/10.1016/j.foodcont.2012.12.004.

33. Klare I, Konstabel C, Werner G, Huys G, Vankerckhoven V, Kahlmeter G, Hildebrandt B, Muller S, Witte W, Goossens H. Antimicrobial susceptibilities of *Lactobacillus*, *Pediococcus* and *Lactococcus* human isolates and cultures intended for probiotic or nutritional use. J Antimicrob Chemother. 2007;59(5):900-912..doi.org/10.1093/jac/dkm035.

34. Munoz E, Gomez B, Araujo C, Campanero C, del Campo R, Hernandez PE, Herranz C, Cintas LM. Antimicrobial activity, antibiotic susceptibility and virulence factors of lactic acid bacteria of aquatic origin intended for use as probiotics in aquaculture. BMC Microbiol. 2013;13(1):15.doi.org/10.1186/1471-2180-13-15.

35. Yilmaz. E. Metal tolerance and biosorption capacity of *Bacillus circulans* strain EB1. Res Microbiol. 2003:409-415. doi.org/10.1016/S0923-2508(03)00116-5

36. Bhakta JN, Ohnishi K, Munekage Y, Iwasaki K. Isolation and probiotic characterization of arsenic-resistant lactic acid bacteria for uptalking arsenic. Inter J Chem Biol Eng. 2010;3(4):167-74.

37. Li B, Jin D, Yu S, Etareri S, Muhammad Z, Huo G, Liu F. In vitro and in vivo evaluation of *Lactobacillus delbrueckii* subsp. bulgaricus KLDS10207 for the alleviative effect on lead toxicity.Nutr.2017;9(8):845.doi.org/10.3390/nu9080845.

38. Li J, Naidu R. Risk assessment of heavy metal contaminated soil in the vicinity of a lead/zinc mine. J Environ Sci. 2005;17(6):881-885.

39. Teemu H, Seppo S, Jussi M, Raija T, Kalle L. Reversible surface binding of cadmium and lead by lactic acid and bifidobacteria. Inter. J. Food Microbiol.2008;125(2):170-175.doi.org/10.1016/j.ijfoodmicro.2008.03.041.