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ORIGINAL PAPER

Isolation and identification of cadmium- and lead-resistant lactic acid bacteria for application as metal removing probiotic

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Abstract The purpose of the present study was to isolate and identify the metal-resistant lactic acid bacteria from sediments of coastal aquaculture habitats for removal of cadmium and lead from ambience. Collected sediment samples were used to isolate the cadmium- and lead-resistant bacterial colonies by spread plate technique using agar media (De Man, Rogosa and Sharpe) supplemented with cadmium or lead at 50 mg/l. Isolates were identified by bacterial colony polymerase chain reaction and sequencing of 16S ribosomal deoxyribonucleic acid. Metal removing probiotic was determined by characterizing the lactic acid yield in culture media, viability in fish intestine, metal-resistant and metal-removal efficiencies. 16S ribosomal deoxyribonucleic acid sequencing data of five (Cd10, Cd11, Pb9, Pb12 and Pb18) and other all isolates clearly showed 99 % similarities to *Enterococcus faecium* and *Bacillus cereus,* respectively. The Pb12 exhibited higher lactic acid yield (180 mmol) than that of the remaining *E. faecium* strains and excellent viability without pathogenicity; therefore, further study was carried out using Pb12 strain. The selected Pb12 strain showed elevated metal resistant (minimum inhibitory concentrations 120 and 800 mg/l for cadmium and lead, respectively) and removal efficiencies [Cadmium 0.0377 mg/h/g and lead 0.0460 mg/h/g of cells (wet weight)]. From the viability and metal removal points of view, it can be concluded that isolated metal-resistant *E. faecium* Pb12 strains might be used as potential probiotic strains for removing heavy metals from fish intestinal milieu to control the progressive bioaccumulation of heavy metals in the fish.

Keywords Bioaccumulation · *Enterococcus faecium* · Heavy metal · Removal efficiency · Sediment

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Introduction

The increasingly high rate of heavy metal contamination in aquatic environment due to various anthropogenic (agricultural, industrial and military operation) and geogenic (weathering process) activities and its hazardous impacts on the aquatic as well as terrestrial lives are of growing concern worldwide. In general, heavy metal is biologically nonessential and poisonous to plants, animals and humans (Gupta and Gupta 1998). Non-biodegradable and persistent types of heavy metals can easily accumulate in soil (Nwachukwu et al. 2010), sediment, plant (Ashraf et al. 2011; Ling et al. 2011) and aquatic flora and fauna leading to biomagnifications in the food chain. The heavy metal-containing wastewaters have detrimental effects on all forms of life upon direct discharge to the environment (Aksu 2005; Kratochvil and Volesky 1998). Among hazardous heavy metals, cadmium (Cd) and lead (Pb) are most priority pollutants in the environment and causes various dysfunctions in living organisms. According to Campbell (2006), Cd is a 'priority pollutant' not only from the human health perspective, but also from a broader ecosystem

viewpoint. Cd enters into the body of animals and humans through food chain and causes various diseases (Belimov et al. 2005), such as, osteomalacia, lung and renal cancer, cardiovascular system, liver, and reproductive system disorders (Hrudey et al. 1996; USEPA 1992).

Intake of Pb also causes central and peripheral nervous systems damage, memory deterioration, diminished intellectual capacity in children and skeletal disorder (Jarup 2003).

Moreover, application of poorly/partly treated contaminated sewage and wastewater in wastewater-fed aquaculture as a cheap source of nutrients is a very common practice in developing countries (Bunting et al. 2010; Edwards 2005a, b; Jana 1998; Phuong and Tuan 2005) to gain a high production benefit. The heavy metal emanating from effluents of various industries (such as tanneries, dye producers, textile dyeing plants, metal engineering, electroplating) may lead to the problem of heavy metal bioaccumulation in aquatic plants (Kara 2005) and fish (Hollis et al. 2001; Long and Wang 2005; Marcussen et al. 2007). It has been reported that heavy metals can accumulate in different tissues of freshwater fishes (Amundsen et al. 1997; Ruangsomboon and Wongrat 2006) and marine fishes (Kalay et al. 1999; Wong et al. 2001).

Recently, application of favorable bacterial species as probiotic is a potentially emerging field to the scientists of aquaculture industry for the welfare of aquatic animals as well as conservation of aquatic environment. Generally, bacteria of lactobacilli and bifidobacteria are commonly isolated from the environmental samples and gut content of fish and used as probiotic in nutritional, growth, disease control (Macey and Coyne 2005; Mohideen et al. 2010) and immunological (Sugita et al. 1998, 2002) aspects. Besides these, *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces boulardii,* and *Enterococcus faecium* are also employed, among others. Besides the above stated works, further investigations on the removal of heavy metals from aquatic environment using bacterial agent (*E. coli, Staphylococcus aureus, B. subtilis* etc.) have been carried out (Brown and Lester 1982; Min-sheng et al. 2001). In this context, it is also apparent that heavy metal contamination induces the development of resistant ability in microbial communities of the environment. Heavy metal-polluted soil affects the structure (qualitative and quantitative) of microbial communities, resulting in decreased metabolic activity and diversity (Giller et al. 1998). It is also reported that many soil bacteria are tolerant to heavy metals and play important roles in mobilization of heavy metals (Gadd 1990; Idris et al. 2004). From the above understanding of probiotic applications as well as metal-resistant properties of bacteria, it is obvious that study relating to isolation of metal-removing lactic acid bacteria (LAB) from metal-resistant bacterial community of the environment has not been investigated so far. Therefore, the present study has been undertaken for isolation and identification of Cd- and Pb-resistant LAB from the sediment of aqua-farming zones using 16S ribosomal deoxyribonucleic acid (16S rDNA) sequencing for their application as metal-removing probiotic. The study was carried out in 2009 at Department of Environmental Engineering and in 2010 at Research Institute of Molecular Genetics, Kochi University, Kochi, Japan.

Materials and methods

Study sites and sampling

Coastal regions consistently receive a wide variety of land-based wastewater containing a vast array of potentially environmental health risk posing metal pollutants resulting in the enrichment of pollutants load in this habitat. This leads to many significant changes in the structure of microbial community. Therefore, the present study considered four coastal aqua-farming zones of Vietnam, Vunh Tau (10°21′0″N and 107°4′0″E), Nha Trang (12°13′40″N and 109°11′38″E), Da Nang (16°2′38″N and 108°11′58″E) and Hue (16°28′0″N and 107°36′0″E), for collection of sediment samples (Fig. 1). The most industrially developed province Vunh Tau comprises petroleum (as major),

Fig. 1 Map of Vietnam showing the sediment sampling stations in the present investigation

electricity, power plant, petrochemicals (urea plant, polyethylene, steel and cement industries), fishery and shrimp as well as tourism as important industries. Aquaculture, tourism and a number of light industries are predominant in the remaining three coastal habitats. Location of mountain is also a specific geographical criterion of these four sampling zones of Vietnam.

Forty sediment (0–5 mm) samples (10 samples/station) were collected by specific sampler from randomly selected four sampling stations and preserved at −20 °C.

Processing of sample

Equal proportions (at 10 g) of all sediment samples were thoroughly mixed and pooled into one for bacterial culture. Ten grams of pooled sediment sample was aseptically weighted (wet weight), suspended in 90 ml of sterile 0.85 % physiological saline (PS) and homogenated using rotary shaker (10 min) for detaching bacteria from sediment to solution.

Metals (Cd and Pb)

Cd and Pb solutions were prepared from standard Cd (Cd 1,000) and Pb (Pb 1,000) (Cica-Reagent, Kanto Chemical Co., Inc., Tokyo, Japan) solutions, respectively, and sterilized by autoclaving for using in different experiments.

Isolation of resistant bacteria

Isolation of metal-resistant colonies was performed by spread plate method using De Man, Rogosa, and Sharpe (MRS, Difco) agar media (specific for LAB) supplemented with metals (Cd and Pb separately) at 50 mg/l. Appropriate tenfold serial dilutions (10^{-1} to 10^{-3}) of homogenated sediment solution were prepared in sterile PS. After inoculation of 1 ml aliquot of sample, 20 ml MRS agar was molten in petri plate at ~40 °C and plates were incubated at 37 °C for 24 h anaerobically using the Anaero-pack Rectangular jar with an Anaeropack-Anaero sachet (Mitsubishi Gas Chemical Company, Tokyo, Japan).

Identification of metal-resistant bacteria by 16S rDNA sequence

Morphologically different colonies (20) were randomly picked by sterile toothpick from each Cd- and Pb-supplemented MRS agar plates of higher dilutions to represent the metal-resistant LAB community of the collected samples. DNA of isolated LAB was extracted following the methods described by Ruiz-Barba et al. (2005) using chloroform/isoamyl alcohol (24:1) and applied for polymerase chain reaction (PCR).

Fragments of bacterial 16S rDNA were amplified by PCR using the universal primers FProR (5′-AGAGTTTGATCCTGGCTCAG-3′) and R534 (3′-GGTCGTCGGCGCCATTA-5′) (invitrogen) with the thermocycler PC818 (ASTEC programme temperature control system). The PCR reaction (20 µl) included 10 µl of AmpliTaq Gold® 360 Master Mix with 0.5 µl of 360 GC Enhancer (Applied Biosystems), 1 µl of each primer, 2.5 µl of nuclease free water and 5 µl of template DNA. The thermocycle program was as follows: 95 °C for 10 min; 30 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min; and a final extension step at 72 °C for 7 min. After reaction, the PCR products were detected by electrophoresis on a 1.2 % agarose gel, staining with ethidium bromide and visualizing under UV light.

The 16S rDNA gene amplicons containing band part of agarose gel was separated gently and purified by DNA gel extraction kit (Wizard® SV Gel and PCR Clean-Up System, Promega) according to the manufacturer's instruction. Purified DNA suspension was employed for sequencing PCR using BigDye with the R534 primer. The thermocycle program was as follows: 98 °C for 1 min; 40 cycles of 98 °C for 10 s, 50 °C for 5 s, and 60 °C for 2.5 min. Nucleotide sequencing was performed with an automated DNA sequencer (Applied Biosystems, 3100-Avant Genetic Analyzer). Bacterial identification was carried out analyzing the nucleotide sequence in the Genbank DNA database using basic logical alignment search tool (BLAST) at NCBI.

Measurement of lactic acid

One milliliter of each fresh cultured bacterium was inoculated in 9 ml MRS broth and incubated at 37 °C for 24 h, anaerobically. Broth was centrifuged, filtered by 0.25 µm filter (Advantec, Tokyo, Japan) and used for measuring the concentration of lactic acid. Lactic acid was measured using high-performance liquid chromatography (HPLC, Jasco liquid chromatography solvent delivery system and UV/Vis detector, Tokyo, Japan) with cosmosil packed column (5C18-PAQ) at UV 210 nm using 20 mmol H_3PO_4 as mobile phase. Next study was conducted considering the isolates showed lactic acid production in MRS broth.

Viability of LAB within fish intestinal milieu

The present study used the identified LAB for examining the viability within the fish intestinal milieu as a model experiment of probiotic application. This study used five aquariums (15 l) filled with tap water (pH 7.4). Pre-acclimatized (10 days) gold carp (goldfish, *Carassius auratus*) fingerlings (4 ± 1 cm) introduced at 9 fingerlings/aquarium and employed for each identified LAB. Continuous

aeration facility was provided during the total 16 days period of experimentation. LAB-supplemented feed was prepared according to Panigrahi et al. (2005) with some modifications. LAB was cultured in MRS broth, harvested, blended with sterile commercial feed at $\sim 10^6$ cfu/g, and preserved at −30 °C throughout the period of experimentation. Before application, LAB-supplemented feed was brought to normal temperature and applied to aquarium at 3 % of fish body weight/day. A control aquarium received feed without LAB administration.

Total gut samples of three fishes/tank were collected at 0 (before application of LAB mixed feed), 7 and 14 days, homogenized in PS, serially diluted (10^{-1} to 10^{-3}) and inoculated aseptically in triplicates as described previously. Viable LAB colonies were enumerated after 24 h incubation at 37 °C and identified by PCR. Fish mortality, growth, water pH and temperature were monitored throughout the period of experimentation. Further study was carried out using the LAB survived in the probiotic application test.

Metal-resistant pattern

Minimum inhibitory concentrations (MICs) of Cd and Pb against isolated LAB were determined by agar disc dilution method. Aseptically, a single colony was transferred to a 100 ml sterilized MRS broth by a sterilized loop, cotton plugged and placed in incubator overnight at 37 °C anaerobically. After 12 to 18 h of incubation, the cultures were diluted with sterile PS to bring the final inoculum size $\sim 10^7$ cfu/ml. Diluted 100 µl bacterial culture was inoculated on the MRS agar plate to form the loan of bacterium for MIC test. Different concentrations of Cd (50–300 mg/l) and Pb (50–1,000 mg/l) were prepared and dispensed at 50 µl in each disc (8 mm diameter) of the bacterium-seeded agar plate and were incubated at 37 °C for 24 h anaerobically. The diameters of the growth inhibition zones were recorded in different concentrations. MIC value was assessed by determining the lowest metal concentration of bacterial growth inhibition.

Metal removal by resistant isolate

The metal removal ability of the identified LAB was determined by measuring the metals (Cd or Pb) uptake of LAB cells following the method described by Pazirandeh et al. (1998) with some modifications. Freshly cultured LAB was harvested in 1.5 ml microcentrifuge tubes, centrifuged at high speed to pellet the cells and washed pellet thrice by sterilized MQ water. Cells [40 mg (wet weight)] of LAB resuspended in 5 ml sterilized Cd or Pb solution (calculated initial concentration 1,000 µg/l) prepared from previously mentioned stock solutions and incubated at 37 °C with three replicates. One control receiving no LAB was considered to determine the reduction rate of metal in solution. Samples were collected at 0, 1 and 2 h periods, centrifuged to pellet the cell and analyzed for heavy metal content using the AAnalyst 200 atomic absorption spectrophotometer (Perkin Elmer).

Antibiotic-resistant profile

Likewise MIC assay, bacterium-seeded MRS plates were prepared for antibiotic susceptibility test using the disc-diffusion method. The discs of 8 mm (Advantec, Toyo Roshi Kaisha Ltd.) were then placed onto the bacterium-seeded agar plates. Solutions of different concentrations (0.1, 1, 5, 10, 50, 100, 200, 300, 400, 500 and 1,000 mg/l) of each antibiotic (trimethoprim, sulphamethoxazole, chloramphenicol and streptomycin) were prepared and 50 µl of solution was pipetted onto each disc. Each concentration was employed in triplicate and incubated at 37 °C for 24 h. Antibiotic resistant profile was assessed by measuring the diameter (mm) of the clear zone around the disc.

Statistical analysis

All data were expressed as mean (±SE) and statistically analyzed by one-way ANOVA. Statistical test was considered significant at 5 % probability level using statistical package EASE and M-STAT.

Results and discussion

Identification of metal-resistant bacteria by 16S rDNA sequence

The PCR-amplified 16S rDNA of metal-resistant bacterial isolates resulted in the synthesis of characteristic single band of about 500 bp using the primers FProR and R534. The sequencing data (460 bp) of purified 16S rDNA amplicons of all isolates were employed for bacterial identification. Five (Cd10, Cd11, Pb9, Pb12 and Pb18) and remaining all isolates clearly showed 99 % similarities to *Enterococcus faecium* and *Bacillus Cereus,* respectively. Cd10 and Cd11 grew in Cd-supplemented plate, whereas Pb9, Pb12 and Pb18 were examined in Pb-supplemented plate. All other isolates also grew in both Cd- and Pb-containing MRS agar plates.

It indicates that lowland coastal bottleneck region receives an excessive amount of heavy metal-loaded wastewater from the upper part of the land leading to enrichment of the various metal pollutants, which is responsible for the development of metal-resistant

properties of bacteria inhabiting in the sediments investigated. Since, aqua-farming zones receive water from the rivers and canals carrying huge amount of pollutants containing wastewater from different anthropogenic as well as geogenic sources, therefore, the bacterial communities of the coastal aqua-farming are likely to be exposed to high metal-loaded effluents. Giller et al. (1998) reported that heavy metal contaminating soils affect the structure (qualitative and quantitative) of microbial communities, resulting in decreased metabolic activity and diversity. As a result, many soil bacteria are tolerant to heavy metals (Gadd 1990; Idris et al. 2004). According to Qing et al. (2007), *B. cereus* and *Enterobacter cloacae* were identified as Cd-resistant strains based on morphological, physiological, and biochemical characteristics and 16S rDNA sequencing.

Measurement of lactic acid

Among the identified bacterial isolates, only Cd10, Cd11, Pb9, Pb12 and Pb18 strains of *E. faecium* showed higher lactic acid concentration (70–180 mmol) in the MRS broth culture media (Fig. 2) ($P < 0.05$, ANOVA), whereas no or very negligible amount of lactic acid yield was detected in the remaining isolates. Rengpipat et al. (2008) also observed that lactic acid bacterium, *Weissella confusa,* produced 730 mmol lactic acid in 24 h culture period which is comparatively higher than that of the strains isolated in the present study. This proposition indicates the lower lactic acid yielding property of the isolated strains of *E. faecium* compared to other LAB.

Results of lactic acid measurement study clearly revealed that five strains of *E. faecium* were LAB because they synthesized lactic acid in MRS media under anaerobic condition. It is also fact that all remaining isolates have no capability to synthesize the lactic acid. Hence, these five LAB strains may be used as probiotic candidates. In this context, it should be mentioned, though some strains of *Bacillus cereus* are used as probiotic but most of them have severe pathogenic effects. Therefore, five LAB, Cd10, Cd11, Pb9, Pb12 and Pb18 strains of *E. faecium* were considered for further viability study. *Enterococcus* such as *E. faecium* and *Pediococcus* such as *P. acidilactici* are mainly Gram-positive bacterial strains which were used in animal feed in the European Union (EU) (Anadon et al. 2006).

Viability of bacteria within fish intestinal milieu

Except fish reared with *E. faecium* Pb12 strain-supplemented feed, no bacterial colonies were observed in the gut content of the fish cultured with remaining LAB supplemented feed and control groups. The mean count was varied from 0 to $52 \pm 8 \times 10^3$ cfu/g (Fig. 3) in all LAB isolates. There was no fish mortality in all treatments. Daily growth rate of fish (0.5–0.54 g/day) did not differ between control and treated groups ($P > 0.05$, ANOVA) (Fig. 3). Lack of growth difference was probably due to the short duration of experiment, age of employed fish and fish species specificity of LAB. These factors should be considered in further study to assess the specific effect of isolated LAB on growth criteria of fish. Though it is not clear from the present study, it indicates the functional specificity of probiotic strains. Temperature and pH of water ranged from 23.5 to 25 °C and 6.9 to 7.7 in five aquariums, respectively.

In this respect, it should be mentioned that pathogenic effects of *E. faecium* are the most significant aspects for the

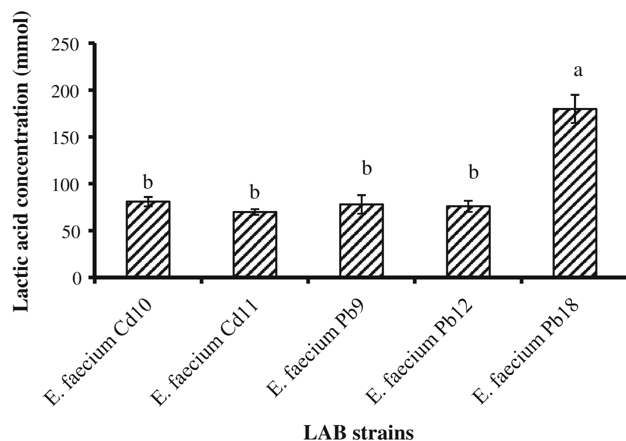

Fig. 2 Lactic acid concentration in the supernatant of five isolated *E. faecium* strains (Cd10, Cd11, Pb9, Pb12 and Pb18) cultured MRS broth media (*same scripts over the bars* indicate no significant difference)

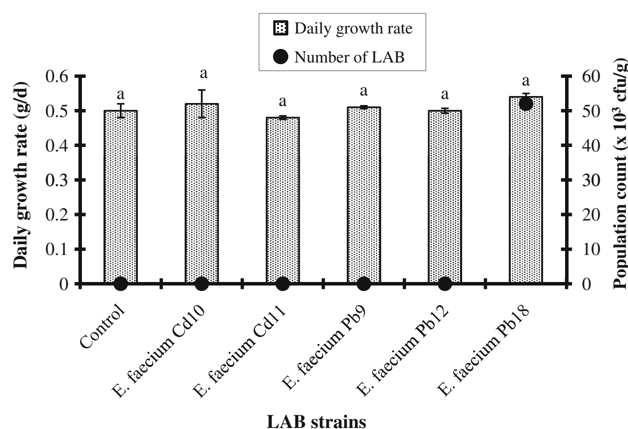

Fig. 3 Mean population count of five *E. faecium* strains (Cd10, Cd11, Pb9, Pb12 and Pb18) in fish intestinal milieu and daily growth rate of fish (*same scripts over the bars* indicate no significant difference)

selection as probiotic, which was not observed when it was applied in the fish through feed. The above viability and non-pathogenic properties clearly demonstrated that *E. faecium* Pb12 strain could be incorporated in the fish feed as a probiotic agent. Musikasang et al. (2009) isolated LAB, *E. faecium* from chicken gastrointestinal digestive tract as potential probiotic. Herranz et al. (2001) observed among LAB including some *E. faecium* strains as non-pathogenic with an ability to produce lactic acid and bacteriocin. In animal management, *E. faecium* have received more attention as probiotic preparations, such as, in pigs (Macha et al. 2004; Scharek et al. 2005; Taras et al. 2006) and poultry due to having immune system stimulating properties in host and protect animals from gastrointestinal diseases (Taras et al. 2006; Vahjen et al. 2002).

Since *E. faecium* Pb12 strains survived in the fish intestine without exhibiting the pathogenicity, further experiments were carried out using only this LAB strain among five.

Metal-resistant pattern

The MIC values of isolated *E. faecium* Pb12 were 120 and 800 mg/l for Cd and Pb, respectively. Though hollow zones around the disc exhibited increasing trend with increasing the concentration of Cd (11–19 mm) but no noticeable increasing trend was recorded in Pb (10–12 mm) (Fig. 4).

Results signified that isolated *E. faecium* Pb12 acquired strong resistant ability against two tested metals and Pb-resistant capacity was higher compared to that of the Cd (Fig. 4). It may be of interest to note that identified LAB are supposed to be resistant to other heavy metals also. Though Cd-resistant *B. cereus* and *E. cloacae* exhibited 1,200 and 2,000 mg/l MIC values respectively, high resistant ability was also found against several other heavy metals compared to control bacterial species, *B. cereus* and *E. coli* (Qing et al. 2007). It is obvious that the isolated *E. faecium* Pb12 strains might have survived in significantly higher Cd- and Pb-polluted environment.

Metal removal by metal-resistant LAB

A significant metal removal response of *E. faecium* Pb12 was registered from both Cd- and Pb-contaminated water. The mean concentration of metals sharply decreased from 973 ± 1 to 369.5 ± 4.5 μg/l (measured value) in Cd and from 906 ± 1.5 to 168.5 ± 8.5 μg/l (measured value) in Pb during 2 h periods of experimentation (Fig. 5).

Total metal removal of isolated LAB *E. faecium* Pb12 were 603.5 ± 4.5 and 737.5 ± 8.5 μg/l in Cd and Pb, respectively. Metal removal efficiencies of *E. faecium* Pb12 were also 0.0377 mgCd/h/g and 0.0460 mgPb/h/g of cells (wet weight). It becomes apparent from above results the higher resistant ability is probably responsible for the removal of greater amount of Pb from the aquatic environment than Cd. The higher Pb affinity of the *E. faecium* Pb12 over the Cd can also be interpreted in this respect. However, it is apparent that probiotic LAB *E. faecium* Pb12 could be applied for uptaking the Cd and Pb.

Antimicrobial resistance patterns

In vitro disc-diffusion assay for antibiotic resistant profile of isolated *E. faecium* Pb12 pronounced a significant antibiotic-dependant response ($P < 0.05$, ANOVA). The values of growth inhibition zones varied between 12 and 25.5 mm in all antibiotics tested in the experiment. Growth inhibition zones were not recorded up to antibiotic concentration of 100 mg/l in Trimethoprim and sulphamethoxazole as well as up to 10 and 500 mg/l concentrations in chloramphenicol and streptomycin, respectively (Table 1). Hollow zones for both trimethoprim and sulphamethoxazole started developing at the concentrations of 200 mg/l, whereas it appeared at the concentrations 50 and 1,000 mg/l in chloramphenicol and streptomycin, respectively.

This resistant profile study clearly showed that *E. faecium* Pb12 has acquired not only metal-resistant ability but also a broad spectrum of antibiotic resistance proficiency in

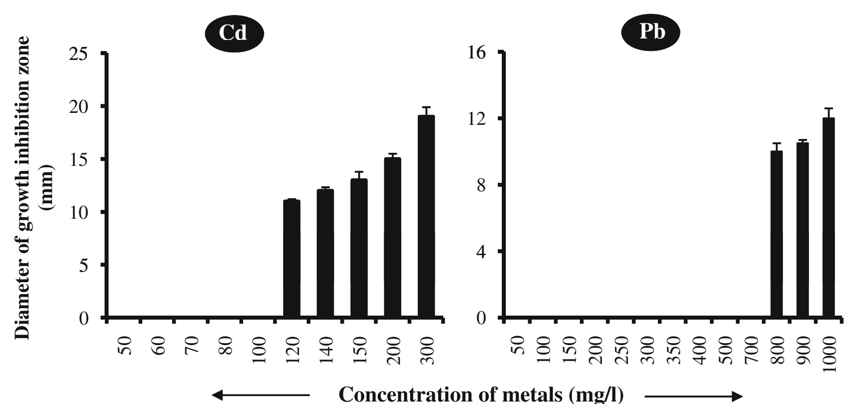

Fig. 4 Growth inhibition zones at different metal concentrations illustrating the resistant pattern of *E. faecium* Pb12 against Cd and Pb

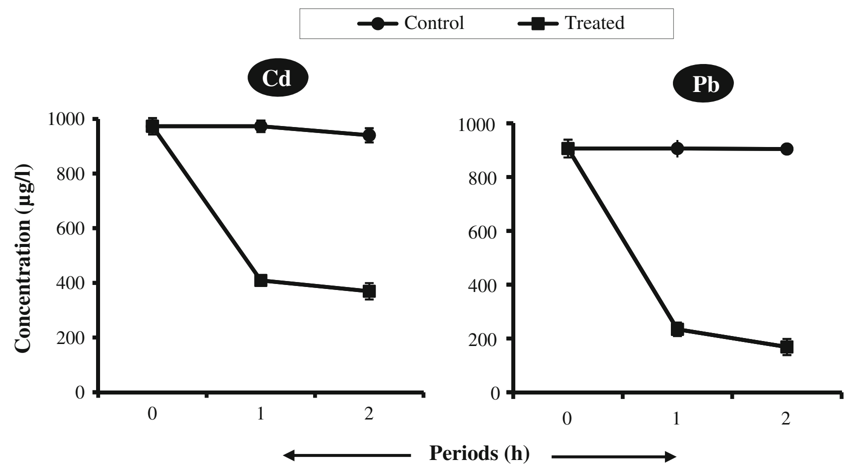

Fig. 5 Cd and Pb removal responses of identified LAB *E. faecium* Pb12 from water phase

Table 1 Mean values (±SE) of the diameter (mm) of growth inhibition zones around the disc representing the antibiotic resistant profile of LAB, *E. faecium* Pb12 isolated from sediment of coastal aquaculture habitat

Antibiotic	Concentration of antibiotic (mg/l)										
	0.1	1	5	10	50	100	200	300	400	500	1,000
Trimethoprim (mm)	0	0	0	0	0	0	17 ± 0.7	19 ± 0.3	21 ± 1	22 ± 0.32	25.5 ± 0.1
Sulphamethoxazole (mm)	0	0	0	0	0	0	16 ± 0.4	19 ± 0.2	22 ± 1	24.5 ± 0.5	24 ± 0.22
Chloramphenicol (mm)	0	0	0	0	12 ± 0.3	15 ± 0.5	16.5 ± 0.7	19 ± 0.6	22 ± 0.1	25 ± 0.5	25.5 ± 0.3
Streptomycin (mm)	0	0	0	0	0	0	0	0	0	0	12 ± 0.5

aqua-farming area, which would be a potential criteria for surviving as probiotic by withstanding the antibiotic-contaminated environment.

Conclusion

On the basis of above discussion, it enables us to draw the following conclusions: (1) 16S rDNA sequencing data clearly identified that five metal-resistant LAB isolates are *E. faecium* and remaining all are *B. cereus.* (2) The properties of lactic acid production and viability without pathogenicity clearly suggested the *E. faecium* Pb12 strain could be applied as potential probiotic agent. (3) Identified LAB strain, *E. faecium* Pb12 acquired high magnitude of metals (Cd and Pb) and antibiotics resistant capacities in coastal aquaculture sediments with elevated metal removing efficiency.

Moreover, probiotic, metal-resistant, metal-removal and antibiotic-resistant criteria implied that *E. faecium* Pb12 of aqua-farming sediments could probably be used as potential probiotic strain with feed for the removal of heavy metals from intestinal milieu of aquatic organisms especially fish to control progressive bioaccumulation of heavy metals. Further experiment should be studied in natural condition to ascertain the exact metal-removal capacity of identified LAB.

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