INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

PROBIOTIC CHARACTERIZATION AND MOLECULAR IDENTIFICATION OF HEAVY METALS RESISTANT LACTIC ACID BACTERIA FROM SEWAGE AND EFFLUENTS

[a]Richa Sharma and [b*]Mahendra K. Gupta
[a]School of Studies in Microbiology, Jiwaji University, Gwalior-474011, Madhya Pradesh, India
[b*]School of Studies in Botany, Jiwaji University, Gwalior-474011, Madhya Pradesh, India
*Corresponding author email- mkgsac@yahoo.com

ABSTRACT
Heavy metals are poisonous substances generated during several industrial processes and accumulate in soil, air, water and have a tendency to accumulate in different food chains. Ingestion of heavy metal contaminated food and water leading to severe disease related to gastrointestinal disorders due to the destruction of gut microflora. Thus the present study was based on probiotic characterization and molecular identification of heavy metal resistant lactic acid bacteria (LAB) isolated from sewage and effluents that could be used as efficient heavy metal tolerating probiotics for *in-vivo* use by the human. Isolation was performed by spread plate technique after enrichment of samples using MRS Media. Lactic acid bacteria isolates were employed for sequential probiotic characterization after the screening of isolated LAB against six heavy metals (Cd, Pb, Zn, Cu, Cr and Hg) at varying concentration. Pathogenic assessment of LAB isolates has been done those were successful in probiotic activity. Efficient LAB isolates were identified by 16S rRNA sequencing. A total of 29 LAB isolates were obtained from 11 different sewage and effluent samples. After screening against six heavy metals, 23 LAB isolates were obtained and employed for probiotic characterization. Survival of 2 isolates namely Sw1b and Sw6c were found after passing through the sequential probiotic characterization. Finally, selected Sw6c isolate was found non-pathogenic in nature on blood agar and showed good probiotic properties thus identified on the basis of 16S rRNA sequencing. The sequence analysis of Sw6c strain showed 99% homology to *Pediococcus acidilactici*. Metal resistance pattern indicates that Sw6c isolate showed resistance for Zn, Cr and Pb up to 1 % concentration. Thus, Sw6c isolate could be used as efficient metal tolerating probiotic by human beings. Statistical analysis of the results was done by using SPSS 24.0 for the window (IBM).

KEYWORDS: Heavy metal, Probiotic, Lactic acid bacteria, Gut microflora, Gastrointestinal disorder.

INTRODUCTION

Heavy metal contamination is extensive and serious threat to the environment and living beings. Utilization of heavy metals contaminated food and water by the human beings are associated with the numerous diseases including gastrointestinal disorder. Gut microbiota provides protection against these harmful substances besides several other benefits but difficulty arises in their survival due to continuous exposure of heavy metals. Probiotic bacteria positively maintain intestinal balance thus taken by human beings. Recently, application of probiotics to reduce heavy metals toxicity in living beings has been paid much attention. Lactic acid bacteria particularly *Lactobacillus* has the ability to bind with heavy metals and other toxic compounds like aflatoxins from aqueous solution (Haskard *et al*., 2001 and Peltonen *et al*., 2001). It has been reported *Lactobacillus reuteri* as a potential cadmium and lead removal LAB probiotic for *in-vivo* challenge in the intestinal milieu of fish for the uptake and control of heavy metal bioaccumulation (Bhakta *et al*., 2012). Lactic acid bacteria based probiotics are an ideal organism to use as a helping tool to prevent or reduce the chances of heavy metals toxicity in human beings and prevent absorption of metals into the human body, so it is necessary to use the heavy metals resistant strain of probiotic to develop its long-term fruitful advantages. Thus, the present study was based on probiotic characterization and molecular identification of heavy metals resistant lactic acid bacteria isolated from sewage and effluents that could be used as efficient heavy metal tolerating probiotics for *in-vivo* use by the human.

MATERIALS & METHODS

Sample collection

A total of 08 sewage and 3 effluent samples were collected from different places of Gwalior (situated in 26.22° North Latitude and 78.18° East Longitude) India. Samples were collected in the aseptic condition in the clean pre-sterilized plastic bottles. All samples were kept at 4°C until further processed.

Isolation and morphological characterization

Preserved samples were thawed at room temperature and processed by the enrichment procedure. For this, 1 ml of each sample was inoculated in 9 ml of MRS broth (Hi-Media) and incubated for 48h at 37°C to enrich the population of lactic acid bacteria. After incubation, an aliquot of broth culture was serially diluted (10^{-1}-10^{-7}) in double glass distilled water, 100 µl suspension of diluted

broth was then spread over the MRS agar media plates and incubated at 37°C for 48h. Lactic acid bacterial isolates showing different colony morphology were streaked on the separate plate and obtained as pure culture and initially identified by gram staining. Later, all the pure culture of LAB isolates were used for catalase reaction and only catalase negative isolates were selected for further characterization. All pure isolates were maintained in 20% glycerol at (-) 70°C.

Screening of isolated LAB against heavy metals

Initial screening of isolated LAB for heavy metals (*e.g.* Zn, Cu, Cd, Hg, Cr and Pb) resistance had done by plate diffusion method (Hassen *et al*., 1998). MRS plates were swabbed with an overnight culture of each LAB isolates separately then wells are prepared with the help of sterile cork borer. 100 µl solution of appropriate heavy metal cadmium ($CdCl_2$), mercury ($HgCl_2$), zinc ($ZnSo_4.7H_20$), copper ($CuSo_4.5H_2O$), chromium ($K_2Cr_2O_7$), lead ($Pb(NO_3)_2$) at the concentration of 1%, 0.5%, 0.1% were poured in each well and plates were incubated at 37 C for 48h. After incubation, the diameter of a clear zone around the well (zone of inhibition) was measured in mm.

Probiotic characterization of heavy metals resistant LAB isolates

Acid pH tolerance

Heavy metals resistant LAB isolates were evaluated for their ability to grow in an acidic medium. 1 % (v/v) overnight grown culture of each heavy metals resistant LAB isolate inoculated in the test tube containing MRS broth adjusted at pH 2, 3, 4 and 6.5 (Control) with 5N HCl and incubated at 37 C for 4 h. After incubation MRS broth adjusted at pH 3, 4 and 6.5 were diluted up to 10^{-2}, 10^{-5}, 10^{-7} respectively except pH 2 which showed less growth of LAB at low pH. Then 100 µl suspensions from each tube were spread over the MRS agar plate and incubated at 37 C for 48h. The acid tolerant LAB was assessed in terms of viable colony count in plate observed after 48h of incubation.

Bile salt tolerance

Bile tolerance test was performed only for the LAB, those were successful in acid tolerance (Gilliland *et al*., 1984). MRS broth containing 0.2% concentration of bile salt was inoculated with 1% v/v overnight grown culture of acid tolerant LAB isolates and incubated for 24h at 37 C. Control was subjected as without bile salt. After incubation growth was measured in terms of optical density at 600 nm.

Survival under the condition of synthetic human stomach juice

Transit tolerance of acid and bile resistant LAB isolates in upper gastrointestinal tract was performed by using *in-vitro* model simulating juices (Pedersen *et al*., 2004). For this purpose, synthetic gastric juice was prepared according to the composition (In g/l- protease peptone 8.3, glucose 3.5, NaCl 2.5, potassium-di-hydrogen phosphate 0.6, $CaCl_2$ 0.11, KCl 0.37, Bile 0.05, lysozyme 0.1, pepsin 13.3 mg, pH 2.5 set with 1M HCl) and heated to 37 C for 30 min. and filtered. 1 % inoculum of an overnight culture of selected LAB isolates was added to 15 ml of the prepared juice separately. The growth was measured in terms of OD at 600 nm after 1, 2, 3 and 4h of incubation.

Biofilm Formation

Qualitative determination of biofilm formation was studied by congo-red agar method (Freeman *et al*., 1989). Selected LAB isolates were inoculated on the surface of Congo-Red Agar plates (0.8g congo red with 36g saccharose in 1 liter Brain Heart Infusion Agar) and incubated for 24h at 30 C under aerobic condition. After overnight incubation, the slime producing bacteria showed black color colonies considered for further study.

Antibiotic sensitivity assay

The antibiotic sensitivity of the selected LAB isolates was determined by using the NCCLS modified Kirby-Bauer disc diffusion method (Bauer *et al*., 1966). Following clinically important antibiotics were used in this study divided into three category on the basis of their mode of action: Group-A antibiotics (inhibit cell wall synthesis) namely imipenem (IPM) (10 mcg/disc), vancomycin (VAN) (30 mcg/disc), methicillin (MET) (5 mcg/disc) and ampicillin (AMP) (10 mcg/disc), Group-B antibiotics (inhibit protein synthesis) namely erythromycin (ERY) (15 mcg/disc), gentamycin (GEN) (10 mcg/disc), tetracycline (TET) (30 mcg/disc) and streptomycin (STR) (10 mcg/disc), Group-C antibiotics (inhibit DNA synthesis) namely ciprofloxacin (CIP) (5 mcg/disc), levofloxacin (LVX) (5 mcg/disc), norfloxacin (10 mcg/disc) and trimethoprim (TMP) (5 mcg/disc). An overnight culture of each LAB was swabbed over Mueller Hinton Agar plates then prepared discs (Hi-media) of each antibiotic put over the plate and incubated at 37ºC for 24-48h. A clear zone around the disc measured in mm which indicates the sensitivity of respective culture to that antibiotic. Zone of inhibition was measured in mm.

Antimicrobial activity assay

The antimicrobial activity of selected LAB isolates against gastrointestinal pathogen was assayed by well diffusion method. Indicator strain like *Salmonella enterica typhimurium* (MTCC 98), *Bacillus cereus* (MTCC 1272), *Staphylococcus aureus* (MTCC 3160) and *Escherichia coli* (MTCC 443) was procured from IMTECH, Chandigarh (India). According to this method, overnight cultures of LAB isolates in MRS broth were centrifuged at 12000 rpm for 5 min. Supernatant was filtered with 0.2 µm nitrocellulose membrane in order to obtained bacterial free supernatant. Nutrient agar plates were swabbed with indicator bacteria (~10^7 CFU/ml) and wells are prepared with the help of sterile cork borer. Then 100 µl bacterial free supernatant of the selected LAB was poured in each well separately and incubated at 37°C for 24-48h. The diameter of a clear zone around well was measured in term of mm.

Assessment of pathogenic potential

It was determined by hemolytic activity on blood agar plates. For this, the selected LAB culture was streaked on blood agar plate (Nutrient agar containing 5% blood of sheep) and incubated at 37°C for 24-48 h and observed for hemolysis. The type of hemolysis was identified as α, β and γ on the basis of the appearance of the brownish zone around colonies, complete hemolysis with a clear zone

around colonies and no change in color around the colonies respectively.

Molecular identification of efficient LAB by 16S rRNA sequencing

Genomic DNA was isolated for amplification and sequencing of 16S rRNA gene of efficient heavy metal resistant LAB isolates by the method of CTAB-phenol-chloroform-isoamyl alcohol extraction method (Ausubel *et al*., 1997). 16S rRNA fragment of efficient LAB isolate was amplified by using universal primer 1510R (5' ACG GYT ACC TTG TTA CGA CTT 3') and 7F (5' AGA GTT TGA TYM TGG CTC AG 3') for lactic acid bacteria with thermocycler (Palm cycle, Genetix, Biotech Asia Pvt. Ltd.). The PCR reaction mixture (25 µl) consisting 2.5 µl of 10X PCR buffer, 0.6 µl of dNTPs, 0.6 µl of dream Taq polymerase (Fermentas), 2 µl of each primer (Reverse and forward primer), 15.8 nucleases free water and 1.5µl of template DNA. PCR reaction was performed with the following conditions: 4 min at 95 °C, followed by 35 cycles of 1 min at 95 °C, 30 sec at 52 °C and 90 sec min at 72 °C, 6 min at 72 °C and hold at 4ºC. PCR products were resolved in 1.2% agarose to confirm the amplification of the desired gene. 16S rRNA gene amplicon was purified by using DNA gel extraction kit (GeneJET gel extraction kit, Fermentas). Purified PCR product was used for sequencing as standard method of Sanger di-deoxy method with an automated DNA sequencer (Applied Biosystems (ABI), 3500 XL Genetic analyzer RUO). The sequence obtained were analyzed and compared with the database of the already sequenced organism by using BLAST (Basic Local Alignment Search Tool) (http://www.ncbi.nlm. nih. gov/blast/Blast). The sequence was submitted to the Genbank of National Center for Biological Information (NCBI).The multiple sequence alignment and construction of phylogenetic trees was done by using MEGA7 software (Kumar *et al*., 2016).

Statistical analysis

All experiments were performed three times independently. Results were expressed in term of mean ± standard deviation by using SPSS 24.0 for the window (IBM).

RESULTS & DISCUSSION

Isolation and morphological characterization

A total of 29 LAB isolates from 11 different sewage and effluent samples were obtained. Microscopic examination found that all isolates were gram-positive cocci or bacilli. All the isolates were found catalase negative thus, used for heavy metals screening.

Screening of isolated LAB against heavy metals

After isolation, all the 29 LAB isolates were tested for their resistance to heavy metals at 3 concentration viz. 1%, 0.5%, 0.1% in term of zone formation. Out of 29 isolates, only 01 LAB isolate showed resistance against cadmium chloride and mercuric chloride at 1%, 10 LAB isolates showed resistance against copper sulfate, 21 showed resistance against zinc sulfate, 10 showed resistance against potassium dichromate, and 22 showed résistance against lead nitrate up to 1 % concentration (Table 1). In total, 23 isolates were showed resistance up to 1 % concentration of at least one of the heavy metal was selected for probiotic characterization.

Probiotic characterization of heavy metal resistant LAB isolates

Acid and bile salt tolerance

Heavy metal resistant LAB isolates were employed for their acid and bile tolerance activity. Out of 23, only 05 LAB isolates were able to survive at pH 2, 3 and 4 thus, selected for bile salt tolerance activity. Total viable count (log CFU/ml) of these 05 LAB isolates mentioned in Figure 1. Results of bile salt tolerance indicated in Figure 2 concluded that all 05 isolates tolerated 0.2% concentration of bile salts. Thus, these 05 acid and bile salt tolerant LAB isolates used for further characterization.

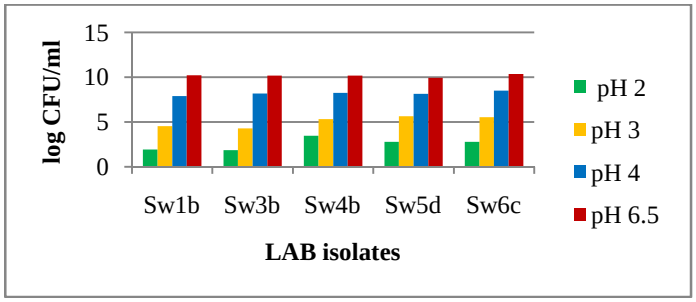

FIGURE 1. Viable count of selected heavy metals resistant LAB isolates in log CFU/ml

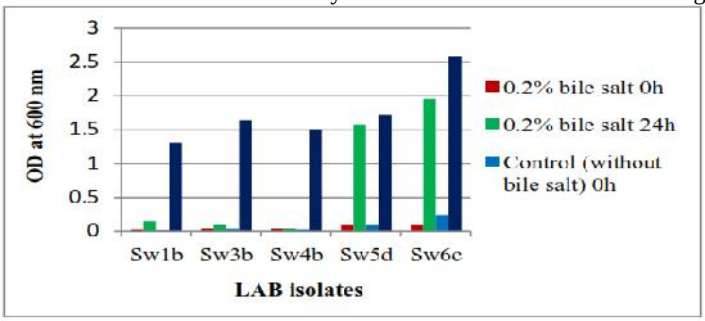

FIGURE 2. Optical density of selected heavy metal resistant LAB isolates with and without bile salt (Control)

Survival under the condition of human synthetic stomach juice

Growth of selected 05 acid and bile tolerant LAB isolates in synthetic human stomach juice was determined by measuring OD after 1, 2, 3 and 4h. Figure 3 indicated that Sw1b, Sw4b, and Sw6c showed the increase in OD up to 4 h while OD of Sw3b increases up to 3h Sw5d isolate showed the decrease in OD with time.

Biofilm formation capacity

LAB isolates that produce slime layer prevent the attachment of the pathogenic microorganism to the gut epithelium. 04 LAB isolates that showed survival under the condition of synthetic human stomach juice namely Sw1b, Sw3b, Sw4b and Sw6c were employed for this test. 03 LAB isolates Sw1b, Sw3b and Sw6c showed positive while Sw4b isolate showed negative results in this test.

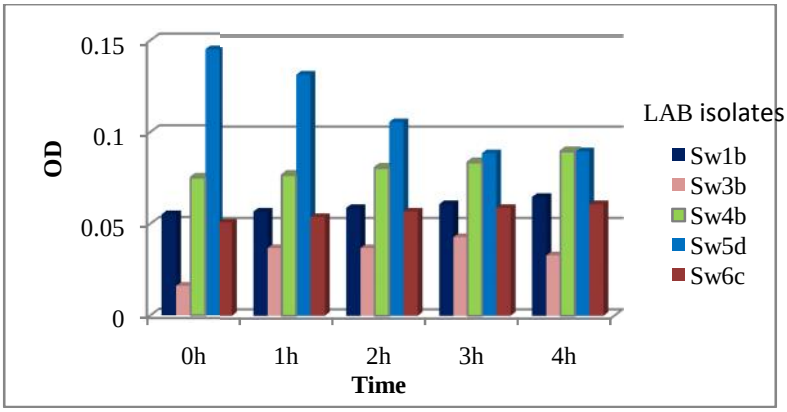

FIGURE 3. Optical density of acid and bile tolerant heavy metal resistant LAB isolates for survival under condition of synthetic human stomach juice

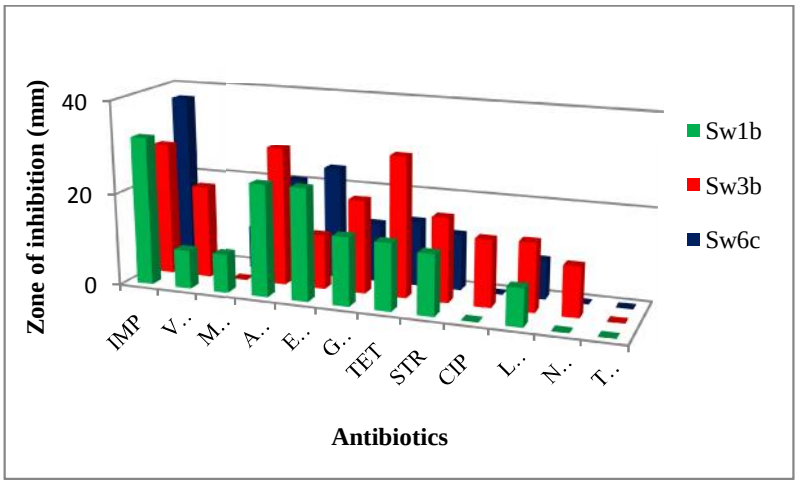

FIGURE 4. Zone of inhibition of selected heavy metal resistant LAB isolates against different group of antibiotics

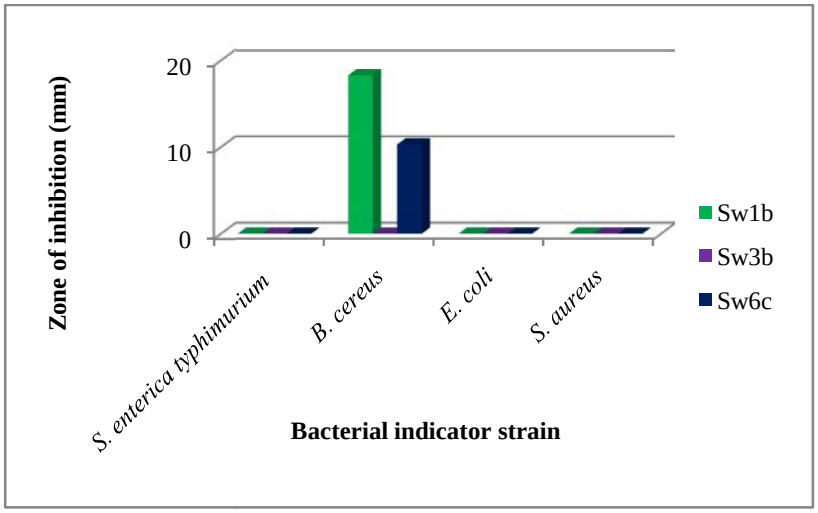

FIGURE 5. Antimicrobial activity of selected heavy metal resistant LAB isolates in terms of zone of inhibition

Antibiotic sensitivity assay and antimicrobial activity assay

Results of antibiotic sensitivity assay of LAB isolates mentioned in figure-4, concluded that all 03 LAB isolates show resistance against trimethoprim antibiotic. In addition, Sw1b isolate showed resistance against ciprofloxacin and norfloxacin, Sw3b isolate showed resistance against methicillin while Sw6c isolate showed resistance against the maximum number of antibiotic (04) vancomycin, ciprofloxacin, and norfloxacin.

Production of antimicrobial substance by the LAB and their use in food industries is very significant. For this, selected 03 LAB isolates were employed for their antimicrobial activity and results suggested (Fig. 5) that Sw1b and Sw6c isolates showed growth inhibition activity against *Bacillus cereus* (MTCC 1272). No zone of inhibition was found against *Salmonella enterica typhimurium* (MTCC 98), *E. coli* (MTCC 443) and *Staphylococcus aureus* (MTCC 3160). Sw3b isolates did not show antimicrobial activity against any pathogen.

Assessment of pathogenic potential

Selection procedure for potential probiotic also includes safety aspects like origin, identity and lack of harmful activities. Safety characteristics include the absence of hemolytic activity and transferable antibiotic resistance of the strain (FAO/WHO, 2006). The result of pathogenic analysis revealed that Sw6c isolate showed gamma hemolysis on blood agar thus non-pathogenic in nature.

Molecular identification by 16S rRNA sequencing

In order to identify species, nucleotide sequence obtained after 16S rRNA sequencing was analyzed by BLAST on NCBI. Results revealed that Sw6c isolate showed 99% homology to that of the reference strain. Then the sequence was submitted to Genbank and assigned the Accession number SUB3942065 Sw6c MH248378.

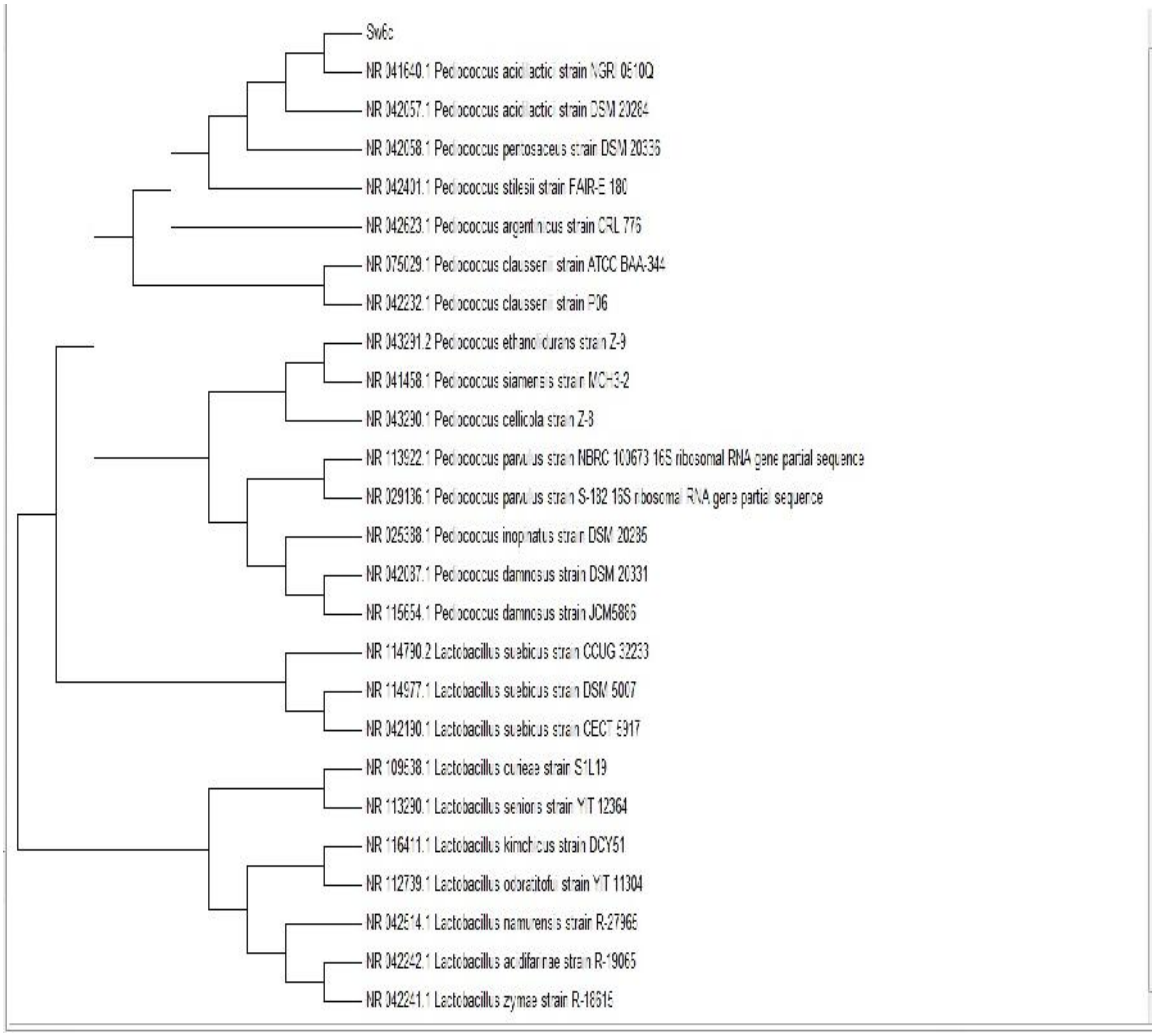

FIGURE 6 Phylogenetic tree based on 16S rRNA sequence analysis constructed by neighbor joining method by using MEGA 7

.

TABLE 1: LAB isolates showing zone of inhibition (in mm) against different heavy metals at different concentration

S. No.	Name of isolates	Cadmium ($CdCl_2$)			Mercury ($HgCl_2$)			Copper ($CuSo_4.5H_2o$)			Zinc ($ZnSo_4.7H_2o$)			Chromium ($K_2Cr_2O_7$)			Lead ($Pb(No_3)_2$)		
		In percent solution																	
		0.1	0.5	1	0.1	0.5	1	0.1	0.5	1	0.1	0.5	1	0.1	0.5	1	0.1	0.5	1
1.	Sw1a	12.66 ±1.15	15.66 ±1.15	16.66 ±1.15	11 ±1	14.66 ±1.15	17.33 ±0.57	R	R	R	R	R	R	R	R	13.33 ±1.52	R	R	R
2.	Sw1b	5 ±4.35	17.66 ±0.57	21.33 ±0.57	R	7.66 ±1.15	9.66 ±1.15	R	R	R	R	R	R	R	R	R	R	R	R
3.	Sw2a	22.33 ±0.57	26 ±0	27.66 ±0.57	6.66 ±1.52	8 ±1.73	9.33 ±1.15	R	R	R	R	R	R	R	11.33 ±1.52	13.66 ±1.15	R	R	R
4.	Sw2b	17.33 ±0.57	20.33 ±0.57	22.33 ±1.15	R	R	11.66 ±0.57	R	R	R	R	R	11±1	R	13.66± 3.78	17.66 ±3.78	R	R	R
5.	Sw2c	14.33 ±3.05	18.33 ±3.05	20.33 ±2.88	R	R	R	R	R	R	R	R	R	17.33 ±0.57	23.66 ±0.57	31 ±1	R	R	R
6.	Sw3a	30.66 ±2.08	32.66 ±2.08	35.33 ±1.52	16 ±1	21 ±1	24.66 ±1.52	R	R	10.66 ±2.08	R	R	R	R	R	R	R	R	R
7.	Sw3b	R	R	R	12.66 ±0.57	12.66 ±0.57	17.66 ±0.57	R	R	R	R	R	R	10.33 ±1.52	19.33 ±2.08	26.66 ±1.52	R	R	R
8.	Sw3c	27.33 ±1.15	28.66 ±0.57	30 ±0	24.33 ±0.57	27.33 ±0.57	29 ±1	13 ±2	19 ±2.64	24 ±2.64	5.66 ±4.93	15.33 ±1.52	17.33 ±1.52	15.33 ±0.57	17.33 ±0.57	18.33 ±0.57	R	16 ±1	17.33 ±1.15
9.	Sw4a	33.33 ±0.57	34.66 ±0.57	36.33 ±0.57	11.66 ±0.57	15.33 ±1.15	24.33 ±4.04	R	R	9.66 ±1.15	R	R	R	R	12 ±2	21.66 ±2.08	R	R	R
10.	Sw4b	22.33 ±0.57	31.33 ±0.57	34.33 ±0.57	R	8.66 ±0.57	16.33 ±0.57	R	11 ±1	15.33 ±0.57	R	R	R	R	R	11.66 ±1.52	R	R	R
11.	Sw5a	22.33 ±0.57	26.66 ±1.15	32 ±1	6.33 ±5.50	23 ±1	24.33 ±0.57	R	R	R	R	R	R	R	R	R	R	R	R
12.	Sw5b	19 ±1	25.33 ±0.57	32.66 ±1.52	15.33 ±0.57	23.66 ±1.15	28.33 ±0.57	R	R	R	R	R	R	R	R	R	R	R	R
13.	Sw5c	22.33 ±1.52	24.66 ±2.08	26.66 ±2.08	14.33 ±0.57	16 ±1	18.33 ±1.52	11.33 ±1.52	13 ±2	16 ±2	15.66 ±2.51	21.33 ±2.08	25.66 ±1.52	22.33 ±2.08	27.33 ±2.08	29.33 ±2.08	13.66 ±1.52	15 ±2	18.66 ±1.52
14.	Sw5d	R	14.66 ±1.15	16.66 ±1.15	R	16.33 ±1.15	17.33 ±1.15	R	R	R	R	R	R	13 ±1	15 ±1	17 ±1	R	10 ±1	17.33 ±1.52
15.	Sw6a	R	R	11.33 ±1.52	12.33 ±0.57	15 ±0	16.66 ±0.57	R	R	11.66 ±2.51	R	16.66 ±3.05	19 ±2.64	5.33 ±4.61	17.66 ±1.15	18.33 ±0.57	R	15 ±2	17 ±2

Continue in next page------

16.	Sw6 b	22.33 ±0+.57	25 ±0	27 ±0	R	R	13.33 ±2.08	R	R	R	R	R	R	R	R	R	R	R
17.	Sw6c	24.66 ±3.05	29.66 ±3.05	32.66 ±2.51	13.33 ±0.57	22.33 ±1.15	25 ±1	4.66 ±4.04	20 ±1.73	21.66 ±1.15	R	R	R	R	R	R	R	R	R
18.	Sw7a	23.33 ±1.15	30.66 ±0.57	35.66 ±0.57	R	11 ±1	16 ±1	R	13 ±2	18.66 ±1.52	R	R	R	R	R	R	R	R	R
19.	Sw7b	23.66 ±0.57	28 ±1	29.66 ±1.15	13.66 ±0.57	15.33 ±0.57	17.33 ±1.52	9.66 ±0.57	16.66 ±0.57	18.66 ±1.15	R	09 ±1	11.33 ±1.52	R	R	R	R	R	R
20.	Sw7c	14.33 ±2.51	15.66 ±3.05	17.33 ±2.51	9.66 ±0.57	12.33 ±1.15	16 ±1.73	R	11.66 ±1.15	13 ±1	R	R	R	R	R	R	R	R	R
21.	Sw7d	32±2	33.33 ±1.52	34.33 ±0.57	14 ±0	16.33 ±0.57	20.66 ±0.57	R	14.66 ±1.52	17 ±2	R	R	R	R	R	R	R	R	R
22.	Sw8a	14.33 ±0.57	23.66 ±1.15	27.66 ±1.15	24.66 ±0.57	28.66 ±0.57	31.33 ±0.57	21 ±2	25 ±1.73	29.33 ±2.08	13.33 ±2.08	19.33 ±2.88	22.66 ±2.08	11.66 ±1.52	17 ±1.73	21.33 ±2.08	26 ±1	31.33 ±0.57	34.66 ±0.57
23.	Ef1a	5.66 ±4.93	23.33 ±0.57	26 ±1	13 ±1	15.33 ±0.57	18 ±1	R	R	10.66 ±1.15	R	R	R	4 ±3.46	23.33 ±1.52	28 ±1.73	R	R	R
24.	Ef1b	5.66 ±4.93	23.33 ±0.57	26 ±1	R	16.66 ±1.15	21.66 ±1.15	R	R	13 ±2	R	R	12.33 ±1.52	R	11.66 ±1.52	15 ±1.73	20 ±1	22 ±1	24.33 ±1.15
25.	Ef1c	R	R	13.66 ±0.57	R	13.66 ±0.57	17 ±1	R	R	14 ±2	18.66 ±2.08	23 ±2	26.66 ±1.52	R	11.33 ±1.52	16 ±1	12 ±2	14 ±2	18.66 ±1.52
26.	Ef2a	19.33 ±0.57	22 ±1	26 ±1	10.33 ±0.57	13.33 ±0.57	15 ±1	R	11.66 ±2.08	15 ±1.73	R	R	R	12.33 ±2.08	17.33 ±2.08	21 ±1.73	R	R	R
27.	Ef2b	13.33 ±1.52	15 ±1	17.33 ±0.57	12.33 ±0.57	14 ±1	15.33 ±1.15	10.66 ±1.15	18 ±1	22 ±1	R	R	R	R	12.33 ±1.15	14.33 ±1.15	R	R	R
28.	Ef2c	17.33 ±0.57	28.33 ±0.57	31.66 ±1.15	10.33 ±0.57	13.33 ±0.57	19.66 ±1.15	R	4.33 ±3.78	17.66 ±2.51	R	R	R	14.66 ±0.57	24.66 ±0.57	28.33 ±0.57	R	R	R
29.	Ef3a	R	10.66 ±0.57	12 ±0	10.66 ±1.15	12.66 ±1.15	15.66 ±1.15	R	10.66 ±1.52	13.33 ±2.08	R	R	R	26 ±1	32.66 ±1.52	34.66 ±1.52	R	R	R

R- Showing resistance against heavy metals

Data are mean ±standard deviation of three independent experiments.

CONCLUSION

The interaction of bacterial species with metals and their use to remove metals from contaminated sites represent an exclusive process. Bacteria have the capability to carry metals across the cell, reduces the chances of damage to the cells by minimizing the cellular concentration of toxic metals. Biosorption and bioaccumulation of heavy metals by the LAB is a booming approach to detoxify the human body from heavy metals. LAB can be used as a dietary supplement for people at risk of heavy metal exposure. Thus, the present study provides the facts that newly identified *Pedicoccus acidilactici* Sw6c strain possess all desirable probiotic properties in addition to heavy metal resistance against three heavy metals namely zinc, chromium and lead. Thus, this strain could be used as metal tolerating probiotic for *in-vivo* use by human where it solves the problem of heavy metals contamination and bioaccumulation.

ACKNOWLEDGEMENT

This work was financially supported by Madhya Pradesh Council of Science and Technology, (MPCST), Bhopal, (India). The Authors wishes to thank the Head of the Department of Molecular Human Genetics, Jiwaji University, Gwalior (India) for providing sequencing facilities.

REFERENCES

Ausubel, F.M., Brent, R., Kingstone, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, K. (1997) Current protocols in molecular biology. Green Publishing Associates, New York and Wiley Interscience, New York.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. (1966) Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathology 36, 493-496.

Bhakta, J.N., Ohnishi, K., Munekage, Y., Iwasaki, K., Wei, M.Q. (2012) Characterization of lactic acid bacteria-based probiotics as potential heavy metal sorbents. J. Appl. Microbiol. 112(6), 1193-1206.

FAO/WHO (2006) Probiotics in Food. Health and Nutritional Properties and Guidelines for Evaluation, FAO Food and Nutrition, Paper no. 85 Roma, Italy.

Freeman, J., Falkiner, F.R., Keane, C.T. (1989) New method for detecting slime production by coagulase negative staphylococci. J. Clin. Pathol. 42, 872-874.

Gilliland, S.E., Staley, T.E., Bush, L.J. (1984) Importance in bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct. J. Dairy Sci. 67, 3045-3051.

Haskard, C., El-Nezami, H., Kankaanpaa, P., Salminen, S., Ahokas, J. (2001) Surface binding of aflatoxin B1 by lactic acid bacteria. J. Appl. Environ. Microbiol. 67, 3086-3091.

Hassen, A., Saidi, N., Cherif, M., Boudabous, A. (1998) Resistance of environmental bacteria to heavy metal. Bioresour, Tech. 64, 7-15.

Kumar, S., Stecher, G. and Tamura, K. (2016) MEGA 7: Molecular evolutionary Genetics Analysis, ver. 7.0 for bigger datasets, Molecular biology and Evolution, 33, 1870-1874

Peltonen, K., El-Nezami, H., Haskard, C., Ahokas, J., Salminen, S. (2001) Aflatoxin B1 binding by Dairy strain of *Lactic acid bacteria* and *Bifidobacteria*. J. of dairy Sciences 84, 2152-2156.

Pedersen, C., Jonsson, H., Lindberg, J.E. and Roos, S. (2004) Microbiological characterization of wet wheat distillers grain, with focus on isolation of *Lactobacilli* with potential as Probiotics. Appl. Environ. Microbiol. 70, 1522-1527.