Assessment of arsenic sorption properties of lactic acid bacteria isolated from fecal samples for application as bioremediation tool

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Abstract
Arsenic intoxication through contaminated water and food is a challenging problem worldwide. The objective of the present study was to isolate the As-resistant lactic acid bacteria (LAB) and assess the As sorption stoichiometry of LAB to validate its practical application as a bioremediation tool. The present study isolated 50 As-resistant LAB colonies from human (HS1-25) and albino mice (MM1-25) fecal samples. Out of 50 As-resistant LAB, the HS12 isolate exhibited the highest As removal efficiency (0.021 mg/h/g). The As bioremediation kinetic study determined the contact time 10 min and the pH between 5 and 7 for optimum As biosorption from the water. The Langmuir isotherm model ($R^2 = 0.993$) was well fitted with the data than the Freundlich isotherm model ($R^2 = 0.876$). The As bioaccumulation and scanning electron microscopy studies proved that binding of As onto cell membrane (0.00037 mg/g) and within the cell (0.00036 mg/g) are the mechanism of As sequestration of LAB HS12. The biosorption of As (2.28–20.37%) from fruit juice, soft drink and coffee as well as multimetals (0.025–0.179 mg/l; 16.22–98.69%) along with As (0.00024 mg/l) from water validated the practical application potential of HS12. The phylogenetic analysis of 16S rDNA amplicon (500 bp) of isolated potential HS12 LAB strains showed 97% similarity to Lactobacillus reuteri. Due to having As biosorption efficiency from water and liquid foods, it can be concluded that the human origin identified L. reuteri HS12 strain could be employed as a novel candidate of As bioremediation to safe environmental and human health.

Keywords As contaminated water · As-resistant · Lactobacillus reuteri · Biosorption · As bioremediation · Food and drinks

Introduction
Arsenic (As) has recognized as a group I “known” carcino-gen (IARC 1987; WHO 2011), having highly toxic and poiso-nous effects on human and environmental health (Bhakta et al. 2016, 2009; Hemond 2004; Murphy et al. 1989) and is also responsible for various dreaded diseases Arsenicosis (Sengupta et al. 2008), hyperpigmentation, skin cancer, kidney damage, liver cancer, circulatory disorders, adverse pregnancy, neurotoxicity and other ailments (NAS 1977; NRC 1999; Quansah et al. 2015; Tolins et al. 2014). To overcome the severe impacts of As contamination and poisoning, the WHO and USEPA promulgated the new As rule that lowered the maximum contaminant level (MCL) in drinking water to 10 μg/l (10 ppb) for both community and nontransient, noncommunity water systems (http://www.epa.gov/safewater/arsenic.html/, https://www.epa.gov/sites/production/files/2015–09/documents/train1-background.pdf).

Arsenic-contaminated water and foods are the primary sources of As in the food chain and the human body. It is very much apparent that drinking water contamination of As is a widely known severe threat to different parts of the world during last few decades and has been envisaged as a global human health and environmental problem (https://www.who.int/water_sanitation_health/diseases-risks/diseases/arsenosis/en/, Bhakta et al. 2009; Ahmad et al.
The Bengal Delta Plain (BDP) is confronted with an elevated level of As in groundwater (Chatterjee et al. 1995; Rahman et al. 2003; Banerjee et al. 2010) and prolonged consumption of such contaminated groundwater has led to widespread As-related human health problems (Guha et al. 2010) in different districts (Nadia, 24 Parganas, Kolkata, Murshidabad, etc.) of West Bengal, India (Rajaković and Mitrović 1992; Bhakta et al. 2016). Additionally, the application of As-contaminated water for irrigation in agriculture leads to the accumulation of As in crops (FAO 2006; Akinbile and Haque 2012; Ruiz-Huerta et al. 2017; Shrivastava et al. 2017). Several types of research reported the contamination of various heavy metals (Cadmium, Lead, Zinc, Copper, etc.), including As in beverages (alcoholic—wine, spirits, and beers; non-alcoholic—soft drink, energy drinks, candies, chocolates and milk) at higher concentrations (Izah et al. 2017; Zucchi et al. 2005; Bingöl et al. 2010; Yentür et al. 2010). The primary sources of toxic metal(loid)s contamination in beverages and fruit juice are the used highly poisonous metal(loid)s containing water (Malhat and Nasr 2012; Abdel-Rahman et al. 2019) as well as vegetables and fruits produced by using contaminated soil and irrigated water (Antoine et al. 2017; Rahman et al. 2017; Al-Naggar et al. 2014; Kooner et al. 2014; Mutengwe et al. 2016) and excess pesticides and fertilizers (Kelepertzis 2014; Tóth et al. 2016; Srivastava et al. 2017; Fan et al. 2018; Wang et al. 2020; Alengebawy 2021).

From the above points of view, therefore, it is apparent that As contamination of water and food is a severe hazardous problem in human health as silent killers, since a large number of people across the world is exposed by As via water and food. To overcome this problem, several researchers developed various technologies, such as sorbent mediums, various filters, treatment devices, etc. (Rajaković and Mitrović 1992; Bhakta et al. 2013; Abbas et al. 2014; Bhakta et al. 2016; Ali and Bhakta 2019; Bhakta and Ali 2020) to remove the As from contaminated water. Unfortunately, these technologies are costly, not feasible, not working properly in the practical field, not user-friendly and not affordable for common people. For example, filters, absorbent mediums, treatment devices, etc. are suffered from poor As-removal efficiency and lack of technical backup after installation in the practical field, resulting in the As concentration in treated drinking water is significantly higher after a short period of installation of treatment device (Bhakta et al. 2016).

Recently, biosorption of various heavy metal(loid)s [such as—As, Cd (cadmium), Pb (lead), etc.] from aqueous solutions using various microorganisms has emerged as a promising ecotechnology in this respect. Bhakta et al. (2012a,b) identified some cadmium and lead-resistant lactic acid bacteria (LAB), *Enterococcus faecium* and *Lactobacillus reuteri* for applying as cadmium and lead biosorption from the aqueous phase. Few studies also explored As-resistant bacteria for As bioremediation in the environment (Abbas et al. 2014; Daya et al. 2016; Bhakta et al. 2018). Bhakta et al. (2010) reported the As removal capacity of some As-resistant LAB in a preliminary study. Halttunen et al. (2007a) worked on the As removal properties of *Lactobacillus* sp., whereas Elsanhoty et al. (2016) and Ameen et al. (2020) demonstrated the heavy metals (such as—cadmium, lead, etc.) removal capacity of some *Lactobacillus* sp. from water.

On account of the above discussion, it is apparent that the research on As biosorption potential of As-resistant LAB is too scanty. However, there is no such study concerning the isolation of potential As-resistant LAB from fecal samples and its application for As bioremediation from water, fruit juice, soft drink and coffee through the sequential characterizations and isotherm kinetic studies of As biosorption has not been performed so far. Therefore, the objective of the present study was to assess the As biosorption stoichiometry of LAB for application as a bioremediation tool through (1) screening of potential As-resistant and uptaking LAB, (2) assessment of As bioremediation kinetics, (3) determination of As bioaccumulation mechanism, (4) molecular identification and phylogenetic analysis of LAB and (5) validation of LAB for As bioremediation from fruit juice, soft drink and coffee for possible industrial application.

### Materials and methods

#### Fecal sample collection and processing

The fresh fecal samples (2 g) were aseptically collected in sterile falcon tubes (50 ml) from healthy humans of normal food habit (male, 44 years, 60 kg) and healthy six female albino mice (*Mus musculus*, 21 days, 9.6–11.35 g) reared aseptically at 24 °C in the cage (3 mice/cage) by feeding commercial food, drank water ad libitum and maintaining 12-h reversed light/dark cycle and 58% humidity in the animal house facility. The freshly collected fecal sample was processed properly by homogenizing and used as source samples to isolate the As-resistant LAB to employ in the subsequent study.

#### Arsenic (As) solution

Arsenic stock solution (2000 mg/l) was prepared by dissolving arsenic trioxide (As2O3, Cica-Reagent, Kanto Chemical Co., Inc., Tokyo, Japan) in MQ (Milli Q) water, autoclaved and stored in a capped glass bottle at 4 °C (Bhakta et al. 2010, 2014). Different concentrations of As solutions required for subsequent experiments were prepared by using this stock solution.
Enrichment culture of As-resistant LAB

The enrichment culture of LAB was performed following the method described by Bhakta et al. (2012a, 2018) to increase the population of As-resistant LAB in human (HS) and mice (MM) fecal samples.

Isolation and morphological study of As-resistant LAB

The MRS broth of enrichment culture (HS and MM) was serially diluted (10⁻¹–10⁻⁸) using PS, 100 µl of diluted broth was then inoculated over the As (100 mg/l)-supplemented and 0.017% bromocresol purple impregnated-MRS agar plates and anaerobically incubated in the anaerobic box at 37 °C for 24 h.

Morphologically different yellow colonies of HS (25) and MM (25) samples were randomly picked up by sterilized toothpick from the higher dilutions of As-supplemented (100 mg/l) agar plates to represent the As-resistant LAB and numbered (HS1-25 and MM1-25). Isolates were re-streaked two times on As-supplemented (100 mg/l) MRS agar plates. The morphological study of good growth pronouncing pure cultured isolates was performed under the phase-contrast microscope (Olympus, Tokyo, Japan). All high growth exhibiting isolates were preserved in MRS broth containing 20% glycerol at − 85 °C for subsequent studies.

Screening of potential As uptaking LAB

The potential As uptaking LAB isolate was screened from high growth exhibiting isolates by assessing their As-removal efficiency from the water following the method described by Pazirandeh et al. (1998) and Bhakta et al. (2012a) with some modifications. Eighteen hours fresh cultured of LAB isolates were harvested and washed thrice in 50-ml falcon tubes using MQ water and centrifuging (8000 g for 10 min). The cell pellet [100 mg/ml (wet weight)] of LAB was then resuspended in sterilized As solutions (0.5 mg/l) and incubated at 37 °C. After 14 h period of incubation, water samples were collected in 2 ml eppendorf tubes and the tubes were centrifuged at 8000 g for 10 min. One milliliter of supernatant water was carefully separated by pipette, and total As contents in water were analyzed by using the EXCEL program.

Arsenic bioremediation kinetics

The As bioremediation kinetic studies of selected LAB isolate were conducted following the batch biosorption experiments in order to characterize the optimum influencing parameters required for removing As from the ambience aqueous phase. The experiments on the effect of contact period, pH, concentrations of As and doses of LAB cell were performed in 50-ml capped falcon tubes with a known volume of As solution (20 ml) and weight of LAB cell. All experiments were conducted herein by using the wet weight of freshly cultured and washed (thrice) LAB cell and previously prepared As stock solution. The effect of contact period was determined by using the initial As concentration 0.3 mg/l, pH 7.4 and dose of LAB 5 g/l. The experiment for the effects of different initial pH (2.5–9) on the As removal of LAB cell was conducted by adjusting different pH (using 0.1 N HCl and 0.1 N NaOH) and maintaining the 0.3 mg/l initial As concentration and 5 g/l dose of LAB cell. The effects of different dosages of LAB cell were assessed by using 1, 5, 10 and 20 g/l weight of LAB cell and of 0.3 mg/l As solution with pH 7.6. To determine the effects of initial As concentrations, 0.15, 0.3, 0.4, 0.6 and 1.5 mg/l initial As concentrations with pH 7.6 and 5 g/l LAB cell weight were used. Each experiment used three replicates with identical conditions. The employed falcon tubes were shaken at the rate of 150 excursions/min at 37 °C within a shaker incubator.

The samples of each experiment were collected and centrifuged, and supernatant water was digested by nitric acid and analyzed (total As) using an ICP–AES (ICPS-1000IV; Shimadzu, Tokyo). The As biosorption capacity of the

$$\text{MRE} = \frac{C_i - C_f}{t_f - t_i} \times \frac{1}{M}$$  (1)
selected LAB strain was calculated by using the following equations (Bhakta et al. 2012b):

\[ q_e (mg/g) = \left( \frac{C_i - C_e}{M} \right) \times V \]  

(2)

where \( q_e \) is the sorption capacity of As (mg/g); \( C_i \) and \( C_e \) are the initial and final concentrations of As (mg/L), respectively; \( V \) is the volume of the As solution (L); and \( M \) is the weight of LAB strain (g).

### Arsenic bioaccumulation mechanism

The As bioaccumulation property was assessed by determining the As content in the outer surface of the cell membrane (OSM) and the intracellular space (ICS) of the LAB exposed to As solution following the method described by Bhakta et al. (2012a) with slight modifications. The LAB cell was first exposed to As fortified MRS medium, and samples were collected at 6, 14 and 24 h instead of 12, 24 and 48 h. The remaining methods were similar as followed by Bhakta et al. (2012a). The As-containing samples of supernatant water and cell pellet were digested by nitric acid and analyzed using an ICP–AES (ICPS-1000IV; Shimadzu, Tokyo) to determine the total As content in the OSM and ICS.

### Scanning electron microscopy (SEM) of LAB

Freshly cultured LAB were harvested in 2-ml Eppendorf tubes, centrifuged at high speed (13,000 g) to pellet the cells, washed thrice using MQ water and resuspended the cell pallet [10 mg/ml (wet weight)] in sterilized As (1 mg/l) solution. The LAB cell was harvested after 1 h period of As exposure by centrifugation and washed thrice using MQ water to remove free As and prepared for SEM study following the method described by Khan et al. (2016). The SEM and energy-dispersive spectroscopy (SEM–EDS) study was performed by using SEM equipment (JEOL-JSM-6500F, Tokyo, Japan) in the Center for Advanced Marine Core Research, Kochi University, Japan, using the process followed by Bhakta and Munekage (2013).

### Validation of LAB for industrial As bioremediation

It is apparent from the previous discussion that water and food may differently be contaminated by As at different concentration levels. As an example, therefore, the studies of As bioremediation from water and food-grade samples (such as fruit juice, soft drink and coffee) were performed in the following experiments to validate the use of LAB for industrial As bioremediation:

### Arsenic bioremediation from fruit juice, soft drink and coffee

In order to assess the practical application of selected LAB, the As removal study was performed following the method described by Pazirandeh et al. (1998) and Bhakta et al. (2012a) with slight modifications. To evaluate the As-bioremediation proficiency of selected LAB isolate from fruit juice, soft drink and coffee; the freshly cultured LAB cell was harvested, washed thrice and inoculated at the rate of 5 g/l in As fortified (at the rate of 0.2 mg/l) commercial orange fruit juice (40%), soft drink (Coca cola), and coffee (3%) collected from the local market in triplicates. The pH of As fortified fruit juice, soft drink and coffee solutions was adjusted to 7. A triplicate test tube set of As fortified (at the rate 0.2 mg/l) orange fruit juice (40%), soft drink (Coca cola) and coffee (3%) received no LAB cell considered as control. All tubes were shaken at the rate of 150 excursions/min at 37 °C within the shaker incubator for the period of 24 h. Samples were collected, centrifuged and supernatants were digested by a mixture (HNO3/HClO3 = 4:1). The total As content of the sample was analyzed using the ICP–AES as above mentioned.

### Multi-heavy metal(loid)s bioremediation from water

To evaluate the multi-heavy metal(loid)s bioremediation property, the selected LAB isolate was employed in the challenged solution of multi-heavy metal(loid)s including As. The composite solution of multimetal(loid)s was prepared by fortifying stock solutions of cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu) and zinc (Zn) at the rate of 1 mg/l, and As at the rate of 0.3 mg/l with the MQ water (pH 7). The six falcon tubes (50 ml) were filled with a prepared solution of composite multimetal(loid)s at the rate of 30 ml/tube and grouped into two (2 × 3)—control and treated groups. The freshly cultured cell of LAB isolate was harvested, washed thrice and mixed with composite multimetal(loid)s at the rate of 5 g/l/tube in the treated group. The control group received no LAB cells. All tubes were shaken at the rate of 150 excursions/min at 37 °C within a shaker incubator. The water sample was collected after 1 h period of experimentation and centrifuged at the speed of 8000 g for 10 min, and the supernatant was used for analyzing the multimetal(loid)s content using the AAS (AA-6800; Shimadzu, Kyoto, Japan) for Cd, Pb, Cr, Cu and Zn) and ICP–AES (for total As). The multimetal(loid)s removal rate was expressed in mg/g wet weight of the LAB cell.

### Molecular identification and phylogenetic analysis of LAB

Molecular identification of potential LAB was performed by polymerase chain reaction (PCR) and sequencing of DNA.
The genomic DNA of LAB was extracted by using chloroform/isoamyl alcohol (24:1) (Ruiz-Barba et al. 2005) and used as a template DNA in the PCR of 16S rDNA fragments using the universal primers (FProR, 5′-AGA GTT TGA TCC TGGCTCAG-3′ and R534, 3′-GGTCGTCGGCGCCATTAC-5′) following the detailed protocol as discussed by Bhakta et al. (2012a). The sequencing of the PCR product (DNA amplicons) was done by an automated DNA sequencer (Applied Biosystems, 3100-Avant Genetic Analyzer). The LAB was identified by searching the similarity in the Genbank DNA database using BLAST (Basic logical alignment search tool) at NCBI (http://www.ncbi.nlm.nih.gov/BLAST/).

After identification, the partial sequence (500 bp) of 16S rDNA of LAB strain was deposited in DDBJ GenBank for accession number and employed to construct the phylogenetic tree using the neighbor-joining method.

Statistical analysis

Obtained mean (three replicates) data of each experiment were considered for data analysis. Isotherm and regression analysis were performed by using the EXEL program. The test of the least significant difference was done using SPSS 10.

Results and discussion

Isolation and morphological study of As-resistant LAB

Out of 50 (HS1-25 and MM1-25), 26 As-resistant LAB isolates showed high growth in As supplemented (100 mg/l) MRS agar plate, whereas the remaining isolates showed poor growth patterns in re-streaked plate. The morphology of 26 LAB isolates was rod in shape. The well-grown 26 As-resistant LAB isolates were employed in the subsequent study. The results implied that fecal samples are the important source of LAB, which may be applied in the biotechnological industry for beneficial purposes.

Screening of potential As uptaking LAB

The As-removal of employed 26 As-resistant LAB isolates ranged from 0 to 29.82 µg/l (Fig. 1). Calculated MRE values were varied from 0 to 0.021 mg/h/g in 26 As-resistant LAB isolates (Fig. 1). Of twenty six, 9 As-resistant LAB isolates (HS8, HS11, HS12, HS16, MM8, MM10, MM12, MM14 and MM21) showed greater MRE values (0.012–0.021 mg/h/g) compared to that of the remaining 17 LAB isolates (0–0.005 mg/h/g). The HS12 isolate exhibited the highest MRE value (0.021 mg/h/g) among the 26 As-resistant LAB isolates.

The above results clearly evidenced that isolated 26 As-resistant LAB isolates have different As uptaking efficiencies. The variation of MRE values is probably due
to the variations of isolates, which apparently indicates that each isolate is supposed to be of different As-resistant LAB species and/or strains. Thus, the highest As uptaking HS12 isolate was screened as a potential LAB strain in this study. Bhakta et al. (2010) isolated a number of As-removing LAB from metagenomic mud and sludge samples. Various studies also isolated some heavy metal-resistant LAB from the environmental samples (Ameen et al. 2020, Huët et al. 2017; Bhakta et al. 2012a).

**Growth pattern of potential As-uptaking LAB**

The growth of control and treated LAB isolate-HS12 varied from 0.076 to 9.98 OD and from 0.076 to 7.25 OD, respectively (Fig. 2). The growth was increased with increasing time, and maximum growths of control (9.98 OD) and As treatment (7.25 OD) LAB isolate-HS12 were achieved at 18 h period of growth (Fig. 2). The As-treated LAB isolate-HS12 pronounced lower growth in different growth cycle phases (lag, log and stationary phases). The mean growth of As-treated LAB isolate-HS12 was remarkably lower (20%) than that of the control one. The highest growth inhibition of HS12 was 27% at the end of the log phase (maximum growth phase at 18 h).

The 20% reduced mean growth in the As-treated LAB isolate-HS12 apparently revealed the exertion of direct growth inhibition effects of As on LAB cells at the concentration of 5 mg/l. However, the growth inhibition of As is concentration dependent. Lead ions showed growth inhibition of

**Arsenic bioremediation kinetics**

**Effect of time (T)**

The effect of contact time on As removal of LAB HS12 strain is shown in Fig. 3a. The As removal ($q_e$) trend was rapidly increased and reached the maximum at 10 min period of experimentation, followed by a steady state thereafter. It can be considered that 10 min is necessary to sorb the maximum amount of As from ambience.

The initial rapid removal could be attributed to the high As affinity of cell membrane of the selected HS12 strain, since the number of vacant functional groups of the cell membrane for As was higher at the initial stage of biosorption. Due to the binding of As with the function group of the membrane, the number of vacant functional groups gradually decreased with time; therefore, As biosorption of HS12 strain was gradually decreased as time progressed. The rapid Cu(II) and Pb(II) biosorption rates of LAB biomass (L. rhamnosus, L. casei and L. plantarum) were occurred within 20 min (Wierzba 2015). Halttunen et al. (2007b) and Ameen et al. (2020) found 1-h contact period is best for optimum metals biosorption of Lactobacillus strains. The above findings indicate that the optimum contact time required for maximum metals biosorption of LAB is highly dependent on types of bacteria.

**Effect of pH**

Figure 3b represents the effects of the initial pH of the solution in removing As. The maximum As biosorption ($q_e$) was observed between pH 5 and 7, and the rate of As biosorption was lower at pH values < 5 and > 7.

Therefore, pH between 5 and 7 could be recognized as optimum for uptaking the highest rate of As from the surrounding environment. According to Mrvčić et al. (2009), the pH has a significant influence on heavy metals biosorption microbes. The pH is the important factor for interaction between microbe and metal(loid)s, especially in an aqueous medium in order to tackle the adverse impacts of metal(loid)s. The maximum metal biosorption capacity of LAB (L. rhamnosus, L. casei and L. plantarum) was achieved between pH values of 2 and 6 (Wierzba 2015), which strongly supports the present study. Hansen et al. (2006) reported the maximum As(V) biosorption of Lessonia nigrescens at acidic pH. Ameen et al. (2020) also observed pH 2 for highest Cd (1.46 ± 0.022 mg Cd mg−1) and Pb (0.91 ± 0.020 mg Pb mg−1) biosorption of L. plantarum MF042018. Moreover, several studies suggested lower or acidic (Yi et al., 2017) and 7 (Halttunen et al. 2007b) pH is optimum for maximum metal(loid)s (Cd, Pb and As, etc.) sorption of

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**Fig. 2** Growth pattern of selected As-resistant LAB isolate HS12 showing growth inhibition of HS12 in As-supplemented MRS medium compared to that of the control one
LAB. The above discussion clearly suggested that lower to neutral pH is favorable for maximum As and various heavy metals biosorption of LAB.

**Effect of dosage of LAB (M)**

The effect of dosage of LAB HS12 on the sorption of As from water is shown in Fig. 3c. The As biosorption capacity \( q_e \) of LAB HS12 showed a declining trend with increasing dosage up to 50 mg/l and followed by a steady state thereafter. In contrast, total As biosorption was increased with time. As expected, the \( q_e \) value decreased with increasing dosages for a given initial As concentration, since increasing dosages provide greater surface area or more adsorption site (Rengaraj and Moon 2002) and entire surface areas of higher doses are not exposed to As due to hindering process of a high density of HS12 cell. Khan et al. (2016) inferred that total Cd biosorption of *S. enterica* 43C is increased with increasing dosage, whereas the reverse response was found in biosorption capacity. Al-Garni (2007) also observed a similar response in the effect of biomass concentration on metal biosorption capacity.

**Effect of initial As concentration (Ce)**

Figure 3d depicts the effect of different As concentrations on the removal process of LAB HS12 in the aqueous phase. The LAB HS12 exhibited an increasing trend in biosorption of As from water with an increasing concentration of As.

The initial concentration-dependent increasement of As biosorption is probably due to the greater exposure of entire membrane surface of all cells to the As in the solution. Effect of initial Cd showed the increasing trend with increasing initial concentration of Cd in *S. enterica* 43C (Khan et al. 2016), which agrees with the obtained results of the present study.

**As biosorption isotherms**

Obtained results were analyzed by the Langmuir and Freundlich isotherm models to evaluate the As removal kinetics of LAB cells using the following equations (Eq. 3 and 4):

\[
q_e = \frac{q_m b C_e}{1 + b C_e}
\]  

(3)
where $q_e$ is the adsorbent amount (mg/g) of the As, $C_e$ is the equilibrium concentration of the As in solution (mg/L), $q_m$ is the monolayer adsorption capacity (mg/g), and $b$ is the constant related to the free energy of adsorption (L/mg).

\[
q_e = \frac{q_m}{1 + bC_e}
\]

Freundlich, \[q_e = K_f C_e^{1/n}\]  

where $q_e$ is the adsorbent amount (mg/g) of the As, $C_e$ is the equilibrium concentration of the As in solution (mg/L), $K_f$ is the adsorption capacity of Freundlich constant (mg/g), and $n$ is the adsorption intensity of Freundlich adsorption isotherm constant.

In As biosorption study, the parameters of the Langmuir and Freundlich isotherm models were calculated from the above equations and are presented in Table 1. The calculated parameters indicated that the obtained data of As biosorption process were more fitted with the Langmuir isotherm than with the Freundlich isotherm. The regression coefficient value ($R^2 = 0.993$) of the Langmuir isotherm is greater than the Freundlich isotherm ($R^2 = 0.876$), which confirms that a sorption equilibrium is favorable with the Langmuir model. The finding of the present study is strongly supported by Khan et al. (2016) and Ameen et al. (2020).

### Arsenic bioaccumulation mechanism

Figure 4 clearly exhibits the total As content in OSM and ICS of cells in different periods (6, 14 and 24 h) of experimentation. Total As content of OSM was decreased as time progressed, whereas an increasing trend of As content was observed in ICS with increasing time (Fig. 4).

A critical analysis of total As content in two major compartments of the bacterial cell (OSM and ICS) demonstrated the reverse relation with time progression. It can be explained the initial rapid As entrapment/binding onto the OSM by extracellular polymeric substances/polysaccharides (EPS) and gradual transportation (of As) towards ICS result in the gradual increment of As content. The EPS of bacteria acts as an excellent metal chelator, which helps to sequester the metal ions in microbial cell (Gadd 2010; Jarosławiecka and Piotrowska-Seget 2014; Bhakta 2016). Gadd (2010) and Bhakta et al. (2012a) reported that the cell walls of the gram-positive bacteria are efficient metal chelators and the carboxylic group of the glutamic acid of peptidoglycan was the major functional sites of metal deposition in *Bacillus subtilis* and *Lactobacillus* sp. According to Gupta and Dewan (2017), the EPS of bacteria can be of a potential agent of bio-detoxification of heavy metal-contaminated terrestrial and aquatic systems in a highly sustainable, economical and eco-friendly manner. The above findings allow for drawing a significant inference in favor of As binding and accumulation proficiency of selected HS12 LAB train.

### SEM of LAB

No distinct difference between SEM micrographs of control and As-treated HS12 strain was found, whereas EDS study counted different As content along with other constituents between control and As-treated HS12 strain (Table 2).

The presence of As in treated cell and absence of As in control cell further confirmed the As binding and accumulation facts of HS12 strain. It strengthens the results and statement as proposed in the study of As accumulation mechanism. Liu et al. (2019) used the SEM study in metal biosorption and showed substantial accumulation on the surface of *L. plantarum* YW11 treated with lead ions.
Validation of LAB for industrial As bioremediation

Arsenic bioremediation from fruit juice, soft drink and coffee

Arsenic biosorption capacity ($q$) of HS12 strain is presented in Fig. 5a. Arsenic removal percentages were 2.28, 20.37 and 7.39% in fruit juice, soft drink and coffee, respectively.

Results indicate the As uptake proficiency of HS12 in different liquid food mediums. Although the As removal rate is quite poor in the liquid food medium, it is apparent that the selected HS12 strain could be employed as an As sorbing agent in food in order to detoxify the As-contaminated food. The study of Halttunen et al. (2007a) removed 38.1% As in water at pH 7 using L. casei DSM20011, which supports the present study.

Multi-heavy metal(loid)s bioremediation from water

Figure 5b depicts the multimetals sorption performance of HS12. The removal of As, Cd, Pb, Cr, Cu and Zn was 0.00024, 0.178, 0.179, 0.043, 0.112 and 0.025 mg/l, respectively. The HS12 removed the significantly poor amount of As compared to that of the remaining metals employed in the study. A higher percentage of metal biosorption was found in Cd (98%), Pb (97%) and Cu (56%) compared to that of the remaining metal(loid)s. The order of metal(loid)s uptake of the HS12 was as follows: Cd > Cu > Zn > Pb > Cr > As. Ameen et al. (2020) observed 100% removal of Ni, Cr, Cd and Pb by L. plantarum MF042018 in a research, which agrees with the data of the present study.

Multimetal biosorption properties of HS12 indicate its differential affinity to the different metal(loid)s. It can be interpreted herein that the HS12 has a greater affinity to Cd, Cu and Zn than to the Pb, Cr and As. Importantly, it can be inferred that As removal process of HS12 is inhibited by the coexistence of other metals as revealed in the present study. However, the excellent multi-heavy metal(loid)s removal potential of HS12 indicates its significant efficiency and applicability in treating the water contaminated by As along with other heavy metals (Cd, Pb, Cr, Cu and Zn).

Molecular identification and phylogenetic analysis of LAB

The homology search of 16S rDNA amplicon (purified partial sequences 500 bp) of isolated potential HS12 LAB strains showed 97% similarity to Lactobacillus reuteri. The received accession number of the L. reuteri HS12 strain was LC521971. Phylogenetic analysis constructed a phylogenetic tree, which showed the position of selected HS12 strain in a phylogenetic tree along with closely related known strains of L. reuteri (Fig. 6) in Gene-Bank. The 16S rDNA sequence and its phylogenetic analysis revealed that isolated potential As-resistant strain is different from existing strains of L. reuteri in Gene-Bank. Therefore, potential As uptaking isolate explored from human fecal samples is identified as L. reuteri HS12 strain. A research isolated six LAB from the mud and sludge samples identified as the Pediococcus dextrinicus and P. acidilactici (Bhakta et al. 2010).

Conclusion

The present study explored Lactobacillus reuteri HS12 strain from the human feces identified as potential candidate of As biosorption among the 50 As-resistant LAB isolates for detoxification of As in water and food, since it showed the highest MRE value (0.021 mg/h/g). The 20% reduced growth pattern of selected L. reuteri HS12 was found due to inhibitory effect of As. Kinetic study for As sorption characterization revealed 10 min period and pH between 5 and...

Fig. 5  a Arsenic bioremediation capacity of HS12 strain from fruit juice, soft drink, and coffee; b Heavy metal(loid)s bioremediation capacity of HS12 strain from multimetals and As-contaminated water [inset shows the multimetal(loid)s removal percentage of HS12 strains]
7 are the optimum conditions in maximum removal of As from the aqueous phase. The As biosorption capacity \( (q_e) \) of LAB HS12 revealed a declining trend with increasing dosage, whereas it showed an increased response with increasing concentration of As. The As sorption kinetic of \( L. \) reuteri HS12 is well fitted with the Langmuir model than that of the Freundlich model, because the calculated regression coefficient value \( (R^2 = 0.993) \) of the Langmuir isotherm is greater than the Freundlich isotherm \( (R^2 = 0.876) \). The \( L. \) reuteri HS12 exhibited potential As biosorption \( (2.28–20.37\%) \) from fruit juice, soft drink and coffee as well as multimetals \( (0.025–0.179 \text{ mg/l}) \) along with As \( (0.00024 \text{ mg/l}) \) from aqueous solution, which validates the industrial application potential of HS12. Studied on As sequestration mechanism indicated the binding of As onto the surface and its accumulation within the cell. Taken together (1) the human origin of identified \( L. \) reuteri HS12 strain, (2) its As biosorption efficiency from water and liquid foods (fruit juice, soft drink and coffee) and (3) excellent multi-heavy metal(loid) s uptaking potential, the identified \( L. \) reuteri HS12 could be used as novel and potential biosorbent to decontaminate the As along with other heavy metals (Cd, Pb, Cr, Cu and Zn) in contaminated water and food in order to safe human and other animals severely exposed to As through food and water. Moreover, the As bioremediation properties of \( L. \) reuteri HS12 from foods (fruit juice, soft drink and coffee) revealed its possible industrial application proficiency and probiotic candidature of \( L. \) reuteri HS12 indicated the As bioremediation in the gastrointestinal tract of human and animals.

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**Availability of data and material** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. All data generated or analyzed during this study are included in this published article.

**Declarations**

**Conflict of interest** The authors declare that there is no competing interest in this regard.

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