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RESEARCH ARTICLE

Mitigation of arsenic accumulation in crop plants using biofertilizer

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Abstract

Elevated levels of arsenic in crop plants have been found in various regions worldwide, especially where agricultural soils have been affected by arsenic-enriched aquifers and human activities including mining, smelting, and pesticide application. Given the highly toxic nature of arsenic, remediation should be carried out immediately to reduce this potentially toxic element transport from soil to crop plants. This study focused on the utilization of biofertilizer which is a combination of arsenic-accumulating microorganisms and adsorbent (carrier) in order to achieve high efficiency of arsenic immobilization and ability to apply in the field. Thirty-two bacterial strains were isolated from 9 soil samples collected from the Dongjin and Duckum mining areas in Korea using a nutrient medium amended with 2 mM sodium arsenite. Among isolates, strain DE12 identified as *Bacillus megaterium* exhibited the greatest arsenic accumulation capacity (0.236 mg/g dry biomass) and ability to resist up to 18 mM arsenite. Among the three agricultural waste adsorbents studied, rice straw was proved to have a higher adsorption capacity (0.104 mg/g) than rice husk and corn husk. Therefore, rice straw was chosen to be the carrier to form biofertilizer together with strain DE12. Inoculation of biofertilizer in soil showed a reduction of arsenic content in the edible part of lettuce, water spinach, and sweet basil by 17.5%, 34.1%, and 34,1%, respectively compared to the control group. The use of biofertilizer may open up the potential application in the field for other food plants.

Keywords Arsenic · Accumulation · Bacteria · Biofertilizer · *Bacillus megaterium* · Soil remediation

Introduction

Grain and vegetables have long been recognized as important sources of food for the world's population. Therefore, cultivating such plants on contaminated land can potentially lead to the accumulation of toxicants including arsenic in edible parts of plants, subsequently in the human body. Unfortunately, numerous studies have reported elevated concentrations of total arsenic in crop plants worldwide due to high levels of arsenic in soil and irrigation water (Das et al. 2004; Bhattacharya et al. 2010; Li et al. 2017; Nguyen et al. 2019; Hoang et al. 2021). While the Chinese National limit for As is 0.5 mg/kg in vegetables (Clever and Jie 2014) with a maximum level of total arsenic at 0.3 mg/kg in rice (Codex Alimentarius Commission 2014), arsenic contents from rice and vegetable samples from contaminated sites have exceeded these thresholds. Additionally, some of the effects of arsenic poisoning on human health including melanosis, keratosis, gangrene, and skin cancer have been noticed among populations in South and South-eastern Asia, especially in arsenic-affected countries (Ahmed et al. 2016). Therefore, it is necessary to perform proper treatments immediately to reduce arsenic transport from soil and irrigation water to food chains.

For soil remediation, biological methods are considered greener compared with chemical or physical technologies. However, they are less stable as they depend on microbial adaptability to the local environment. Recently, biofertilizer has received considerable attention because of its potential to provide a better survival rate for inoculant microorganisms. Biofertilizer is defined as a fertilizer consisting of microorganisms with specific functions and organic materials (or carriers). Therefore, it has effects on both microorganisms and organic carriers (Sun et al. 2020). Biofertilizer has been long investigated for many purposes, mostly for enhancing nutrient supplements and plant biomass (Mukhtar et al.

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2017; Sohaib et al. 2020; Naher et al. 2021). Several studies have also reported the use of biofertilizer for the immobilization of heavy metals in soil to increase biomass and decrease toxicants such as Cd, Hg, Pb, Cr, Co, and Ni concentrations in edible parts of lettuce and wheat compared with the control (Hassan et al. 2017; Wang et al. 2017a). However, few such studies have been conducted about arsenic.

There are several mechanisms behind the way microorganisms interact with arsenic compounds. However, these bioactivities will eventually lead to either decrement or enhancement of As bioavailability in the environment. Various studies have been conducted to examine the role of specific microorganisms in protecting crops by making As less bioavailable in cultivating soil. For instance, inoculation of *Trichoderman asperellum*, an As-resistant fungal strain, can increase As availability in Chenzhou soils and As content in water spinach (Su et al. 2017). Meanwhile, *Brevundimonas diminuta*, an As-accumulating bacterial strain, can help reduce arsenic uptake in edible parts of rice plants and effectively sequester As in root systems (Singh et al. 2016). As-tolerant bacteria including *Ralstonia eutropha*, *Rhizobium tropici*, and *Exiguobacterium aurantiacum* can alleviate As and Cd contents in edible parts of Chinese cabbage and radish (Wang et al. 2017b). However, microbial inoculants generally have a short shelf life as the population of microorganisms will soon decline after inoculation due to unpredictable abiotic stresses (such as pH, moisture, temperature, salinity, and elevated levels of toxicants) and biotic stresses as they have to compete with indigenous micro-flora and protozoans for energy, carbon, and nutrients (Siddiq et al. 2018). Therefore, the presence of carrier material plays a very important role in microbial survival. In this study, agricultural wastes including rice husk, rice straw, and coconut husk were examined as carriers for biofertilizer. The main reason for that is because lignocellulosic fibers are porous and can swell in contact with water (Budd and Herrington 1989), meaning that they can provide habitats for inoculant bacteria and possess good moisture adsorption capacity. Besides, they are low-cost, renewable materials and available in adequate amounts. Furthermore, lignocellulosic materials have shown to be promising biosorbents of arsenic via main interactions including complexation, electrostatic attraction, ion exchange, and precipitation (Maia et al. 2021), which could help fortify the purpose of biofertilizer in this study. In addition, open burning is one of the most common waste straw management practices performed by farmers all over the world, leading to the emission of lethal greenhouse gases (Singh et al. 2021). Therefore, the utilization of agricultural waste can help reduce the environmental burden.

The objectives of this study were to screen for arsenite-resistant bacteria and suitable carriers for biofertilizer and to examine the potential applicability of biofertilizer in contaminated cultivating soil. The application of biofertilizer could be a useful strategy for immobilizing arsenic in soils and preventing the accumulation of this element in crop plants.

Materials and methods

Site description and sample collection

Sampling sites for this study were arsenic-contaminated mining areas in South Korea (Fig. 1). The Dongjin Au–Ag–Cu mine is situated in Jinan-gun, Jeollabuk-do

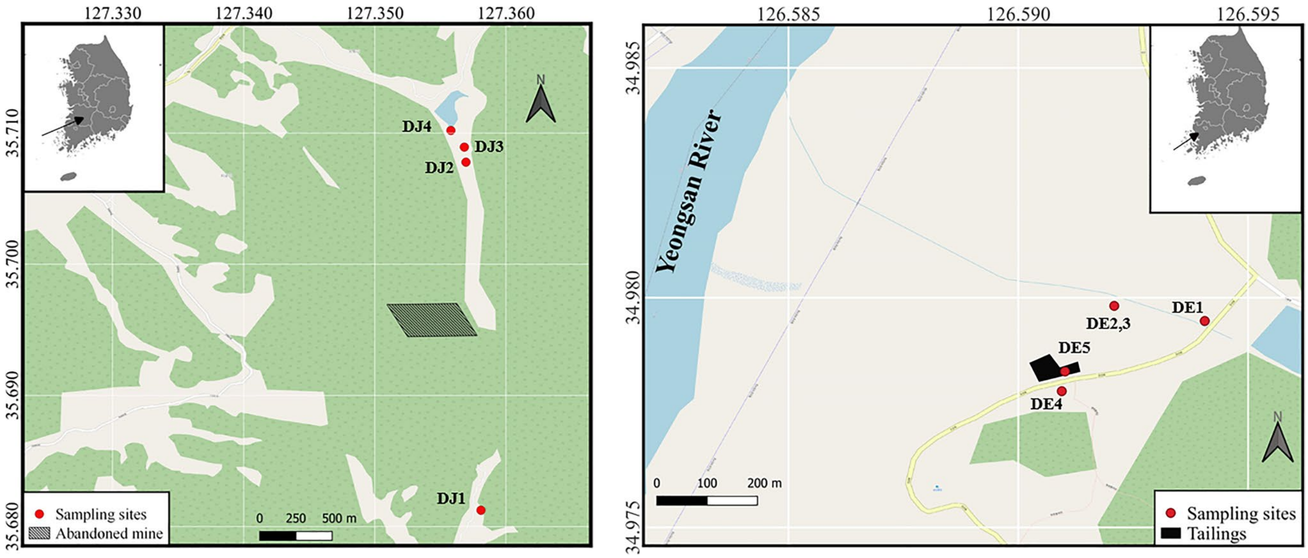

Fig. 1 Locations of soil sampling sites from the Dongjin (left) and Duckum (right) mining areas

with such ore minerals as chalcopyrite, galena, sphalerite, arsenopyrite, and pyrite. Small-scale mining activities had been carried out in this area until 1965 (Lee et al. 1996). However; several studies have thus far proclaimed significant levels of heavy metals and metalloids in the vicinity due to mine waste (Na et al. 1997; Yoo et al. 2014). The Duckum Au–Ag mine is located in Naju-si, Jeollanam-do. After it was abandoned, this mining area was left behind with 645,160 m^3 of tailings and 225,000 m^3 of waste rock piles, threatening the surrounding environment (Kim et al. 2002).

Soil samples were collected and sealed in sterilized plastic bags and kept at 4 °C until further analysis. For arsenic content analysis, soil samples were dried, sieved through 200-µm mesh, and digested in aqua regia. The mixture was then centrifuged at 3000 rpm for 10 min and filtered through a 0.45-µm syringe filter before it was analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Ko et al. 2020). Arsenic levels in collected soil samples are shown in Table 1.

Isolation of arsenite-resistant bacteria

After 0.1 g of fresh soil sample was added to 100 mL saline solution, serial dilution was performed. Then, 100 µL aliquot of each diluted sample was spread onto nutrient agar (peptone 5 g/L, NaCl 5 g/L, yeast extract 2 g/L, agar 15 g/L) amended with 2 mM of sodium arsenite. After incubating at 30 °C for 3 days, distinct colonies were selected based on morphology and subcultured on fresh media (Dey et al. 2016). The cellular morphology of each isolate was observed using a bright-field microscope. In addition, screening of all isolates on blood agar plates (KisanBio) was performed to examine hemolytic activity.

Table 1 Total arsenic concentrations in soil samples

Sampling sites		Depth (cm)	Coordinates	Arsenic concentration (mg/kg)
Dongjin	DJ1	0–20	35.6812; 127.3581	18.8
	DJ2	0–20	35.7078; 127.3569	38.6
	DJ3	0–20	35.7090; 127.3568	52.8
	DJ4	0–20	35.7102; 127.3558	269.0
Duckum	DE1	0–20	34.9797; 126.5920	5.5
	DE2	0–20	34.9794; 126.5942	8.6
	DE3	20–30	34.9794; 126.5942	
	DE4	0–20	34.9782; 126.5909	39.4
	DE5	0–20	34.9785; 126.5910	45.08

Evaluation of arsenite resistance and accumulation

In order to evaluate arsenite resistivity, minimum inhibitory concentrations (MIC) of all isolates were determined. MIC was determined as the lowest arsenite concentration that inhibited visible microbial growth. One percent of the 24-h grown culture of each isolated strain was inoculated into the nutrient broth (peptone 5 g/L, NaCl 5 g/L, yeast extract 2 g/L) amended with different concentrations of sodium arsenite (2–20 mM) in a 96-well plate. Bacterial growth was then tested through the presence of a colony on a nutrient agar plate (Banerjee et al. 2011).

To determine arsenite accumulation by bacterial cells, 1% of the 24-h grown culture of each isolate was inoculated into 5 mL nutrient broth in the presence of 2 mM sodium arsenite. Bacterial biomass was harvested by centrifugation (3000 rpm, 10 min) at room temperature and washed twice with saline solution. After the supernatant was carefully removed, cell pellets were then air-dried for 2 days and dissolved in 200 µL of concentrated nitric acid for 2 days at room temperature (Kostal et al. 2004). Arsenic content in biomass was then determined by inductively coupled plasma mass spectrometry (ICP-MS).

Identification of selected bacterial strain

Those strains capable of tolerating and accumulating arsenite were selected for identification by analyzing their 16 rRNA genes. PCR amplification of 16 s rRNA gene fragments was done using 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACCGTTACCTTGTTACGACTT-3′) primers. DNA fragments were sequenced with primers 785F (5′-GGATTAGATACCCTGGTA-3′) and 907R (5′-CCGTCAATTAMTTTRAGTTT-3′). Nucleotide sequences were compared to sequence databases in the NCBI GenBank using nucleotide BLAST. Bacterial 16S rRNA gene sequences obtained from this study were aligned with other sequences from the database, and phylogenetical analysis was made by the neighbor-joining method with 1000 bootstrap replicates using MEGA 11 software.

The physiological characteristics of bacterial colonies were examined using biochemical test kits (KisanBio). Different biochemical properties of bacterial isolates such as catalase activity (Cat), Voges-Proskauer's test (VP), and utilization of different carbon sources including arabinose (Ara), lactose (Lac), mannitol (Man), glucose (Glu), and decarboxylation of amino acid including ornithine (Orn), lysine (Lys), and arginine (Arg) were tested.

Adsorption capacity of carrier

Adsorbents (rice straw, rice husk, coconut husk) were washed thrice with DI water, dried, and passed through a 200-μ m mesh sieve. Then, 10% adsorbents (w/v) were added to the solution of 10 mg/L sodium arsenite (pH 6). These solutions were mixed at room temperature on a rotary shaker for 24 h. At the end of the experiment, the residual concentration in the supernatant liquid was analyzed by ICP-OES (Podder and Majumder 2015). The adsorption capacity of each carrier was calculated with the following equation:

$$q_e = \frac{(C_0 - C_t)V}{m}$$

where,

q_e adsorption capacity of adsorbent (mg metal/g dry weight).

C_0 the initial concentration of arsenic in the solution (mg /L).

C_t the final concentration of arsenic in the solution (mg /L).

V the volume of the metal solution (L).

m the dry weight of the adsorbent (g).

Biofertilizer formation

Selected adsorbent and bacteria strains were used to formulate biofertilizer. To become a carrier, the adsorbent was washed thrice with deionized water, dried, ground, and sieved through 200-μ m mesh before autoclaving twice. Then, 40 mL of overnight grown culture of selected strain was added to 40 g of sterilized carrier material stored in an aseptic container and incubated at 30 °C for 2 days. After incubation, biofertilizer portions in all containers were mixed together and kept in a sterilized plastic bag. Measurement of colony forming units (CFU) in formulated biofertilizer was made after 7 days of packaging. Observation of viability was carried out using the spread plate method, and the number of colonies was calculated using the total plate count (CFU/mL) method (Hassan and Bano 2015).

Plant cultivation and analysis

Preparation of cultivating soil

Contaminated soil was collected in the Duckum mining area. Cultivating soil was attained by mixing contaminated soil and purchased fertile soil at a ratio of 1:1. Arsenic concentration of contaminated soil and mixed soil analyzed by ICP-OES was 128.3 mg/kg and 64.09 mg/kg, respectively. Mixed soil was then homogenized with coconut peat at a ratio of 3:1 to improve water retention capacity and increase available nutrient content. The mixture was then air-dried at room temperature for 7 days for cultivation. Biofertilizer was then inoculated in cultivating the soil with particular ratios to obtain the same bacterial concentration in the soil. The mixtures were then left in shade to settle for 2 days. Treatment comprised fertile soil (FS) (non-contaminated soil), contaminated soil (CS), and biofertilizer + contaminated soil (BF-CS). For the BF-CS group, 10% (w/w) biofertilizer was added to contaminated soil before cultivation.

Seed preparation and germination

Seeds of lettuce (*Lactuca sativa* Linn), water spinach (*Ipomoea aquatica* Forssk), and sweet basil (*Ocimum basilicum* Labiatae) were surface-sterilized with 95% ethanol for 2 min followed by shaking with 10% chlorox for 2–3 min (Hassan and Bano 2015). A pot experiment was carried out in a greenhouse of the School of Earth Sciences and Environmental Engineering, Gwangju Institute of Science and Technology, South Korea. Disinfected seeds germinated in cultivating soil for 1 week. After germination, uniform seedlings were thinned to four plants per pot with triplicates for each treatment. All plants were cultivated under ambient temperature and irrigated without leachate with a light and darkness regime. Pots were placed randomly in the greenhouse and moved every day to ensure that they received the same light. As water spinach is a semi-aquatic vegetable, the submerged condition was applied only to this plant type. Lettuce and water spinach plants were harvested after 40 days, while sweet basil plants were collected after 60 days of cultivation.

Arsenic analysis

Collected crop samples were rinsed thrice with deionized water to remove soil particles or dust on the surface and separated into roots and shoots (edible parts). All samples were then air-dried for 4 days and homogenized using a mortar and pestle. Then, 0.05 g of each sample was digested with 3 mL of concentrated nitric acid (65%) at 220 °C for 20 min in a microwave digestion system. After digestion, the extract was filled up with deionized water to 10 mL, and the solution was filtered through a 0.45-µm syringe filter. Total arsenic and heavy metal concentrations were then detected by ICP-MS (Park and Choi 2013).

Data quality control and statistical analysis

All samples were digested in triplicate. The accuracy of the digestion method was evaluated by the analysis of standard reference materials (SRM 2711a, SRM 1573a) in the same way as the treated sample**.** Recovery rates for SRM 2711a and SRM 1573a were 104% and 91%, respectively. Calculation and statistical data analyses were performed using Microsoft Excel 2016. The sampling map was built with QGIS (version 3.24) software. Figures were created with OriginPro 2021.

Results and discussion

Isolation of arsenite-resistant bacteria

Thirty-two bacterial strains were isolated from soil samples collected from the Dongjin and Duckum mining areas. Most colonies had a white to milky color. The rest had a yellow to orange color. Glistening, water-like colonies were observed for half of the isolates. This is a character of a bacterial colony that produces slime or a capsule. These microbial surfaces can function as a protection layer for cells against dewatering or toxic substances (Sheng et al. 2010). Gram-staining results varied among isolates. Spore formation was found for DJ24, DJ25, DJ33, DE12, DE13, and DE14. Cellular shapes of all strains were mostly bacilli except for DJ31, DE16, DE42, and DE52 which were cocci (as shown in Fig. 2). Of all bacterial isolates, DJ36, DJ44, DE17, DE21, DE41, and DE51 were found to have hemolysis activities. Thus, they were not used for further experiments.

Evaluation of arsenite resistance and accumulation by bacterial cells

To examine the potential arsenite resistivity of isolates, minimum inhibition concentrations (MICs) are determined and recorded in Table 2. Overall, bacterial strains isolated from the Dongjin mining site exhibited greater abilities to tolerate arsenite over those from Duckum contaminated areas, which might be due to higher concentrations of total arsenic in general in the Dongjin soil samples. Among these strains, DJ22 and DJ41 thrived the best in the presence of arsenite with the ability to sustain their growth in the presence of over 20 mM of sodium arsenite. Interestingly, these two strains had several similar characteristics: smooth, yellow colonies; gram-positive; and cellular size of 0.5 × 0.6 µm. Strains DJ25 and DE12 also exhibited excellent resistance ability, with MIC values of 20 mM and 18 mM, respectively. Gram-staining of these two strains results showed the presence of spore formation, an attribute of several bacteria to resist extreme external conditions. Microscopic observation of several strains revealed capsular material surrounding these bacteria, which could be one of the reasons for their high resistance to arsenic. All strains that were able to tolerate above 4 mM of arsenite were used for further experiments.

Arsenite accumulating abilities of isolated strains are illustrated in Fig. 3. Isolate DJ24 showed the greatest arsenic accumulation ability (0.247 mg/g dry biomass). Results of MICs for arsenite and arsenic accumulation suggested that bacterial response toward arsenite varied among isolates. Particularly, strains DJ22 and DJ41 with the highest resistant abilities were among the bacteria that accumulated the lowest amount of arsenic, which indicated that these strains had mechanisms other than accumulation to survive in the presence of arsenite. Isolate DE12, which possessed high arsenic resistance and accumulating abilities (0.236 mg/g dry biomass) was then selected for species identification.

Identification of selected bacterial strains

Based on the results of 16S rRNA gene sequence and phylogenetic analysis, DE12 was designated as a species belonging to *Bacillus* genera and shared high similarity with *Bacillus megaterium* as shown in Fig. 4. Detailed characterization of DE12 was also performed to help assure its identification and to explore its biotechnologically important traits. Table 3 displays results for particular biochemical tests of strain DE12 and other bacteria identified as *B. megaterium* from previous studies. It also describes the biochemical characteristics of *B. megaterium* in Bergey's manual (Whitman 2009). Physiological characteristics of strain DE12 were found to be similar to those of other bacteria listed. In addition, DE12 was found to be a gram-positive bacterium with an average size of 1.5 × 2.3 µm. It was able to form spores, a dormant form of bacteria to resist physical and chemical influences. This characteristic could help assist the survival of DE12 in an environment with elevated levels of As and other toxicants. There has been no evidence of pathogenic traits of this species. Moreover, *B. megaterium* has been claimed to be of economic importance due to its ability to produce several crucial enzymes (Sura and Hiremath 2019). Therefore, strain DE12 was chosen to be the bacterium for biofertilizer formation.

Adsorption capacity of carrier

Although agricultural wastes, which are carbonaceous materials, are usually used for biochar formation to enhance the adsorption capacity of pollutants, the production cost of biochar could make it harder for biofertilizer to be applied on a large scale in paddy fields. In this study, the experiment was conducted only on raw materials. Rice straw was chosen for its high adsorption capacity to be the carrier to form biofertilizer together with strain DE12. Coconut husk exhibited the lowest adsorption capacity at 0.016 mg/kg among the studied materials. Adsorption capacity of coconut husk in this study was lower than that of coconut fiber in the study

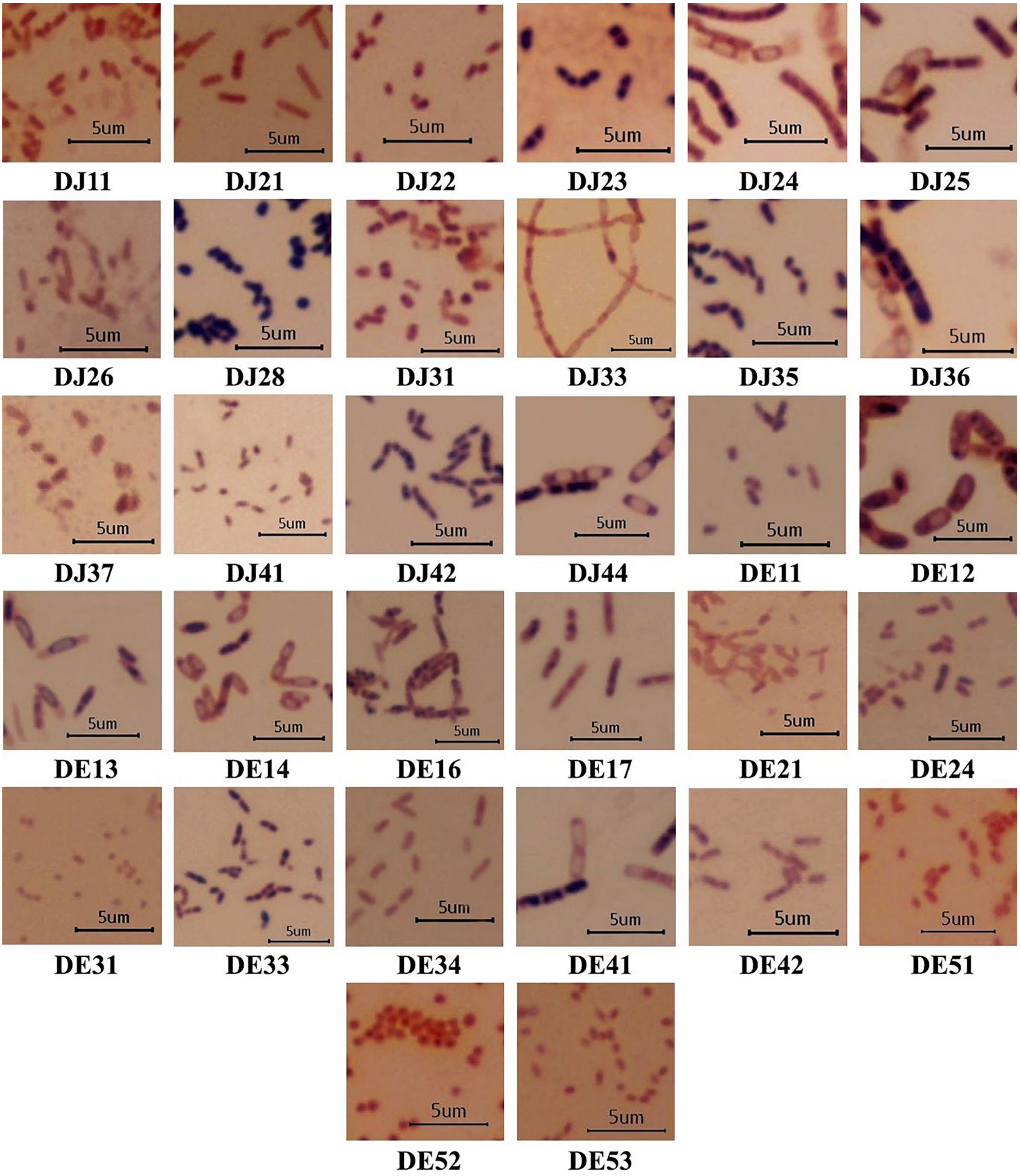

Fig. 2 Cellular morphology of isolated strains under bright-field microscope (× 1000 magnification)

by Nashine and Tembhurkar (2016) at over 0.03 mg As (III)/ g adsorbent with the same adsorbent dose (10 g/L) but higher initial arsenite concentration (0.380 mg/L) (Nashine and Tembhurkar 2016). Rice straw was found to have higher adsorption capacity (0.104 ± 0.008 mg/g) than rice husk (0.066 ± 0.009 mg/g). This could be explained by differences in chemical compositions between rice straw and rice husk. Lignin and silica are two components presented in

Table 2 Minimum inhibition concentrations (MICs) of arsenite for bacterial isolates

Isolates	DJ11	DJ21	DJ22	DJ23	DJ24	DJ25	DJ26	DJ28	DJ31
MIC (mM)	10	4	> 20	4	6	20	16	16	4
Isolates	DJ33	DJ35	DJ37	DJ41	DJ42	DE11	DE12	DE13	DE14
MIC (mM)	4	12	10	> 20	8	12	18	4	4
Isolates	DE16	DE24	DE31	DE33	DE34	DE42	DE52	DE53	
MIC (mM)	14	6	6	6	6	4	16	6	

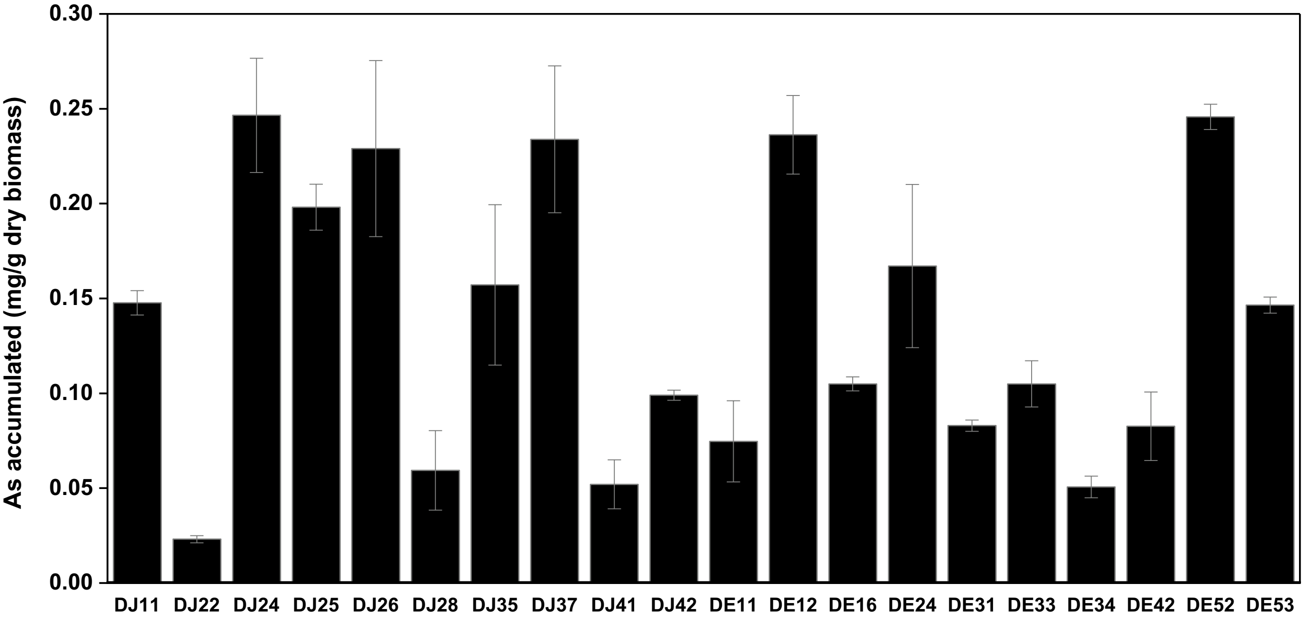

Fig. 3 Arsenic-accumulating abilities of isolated strains

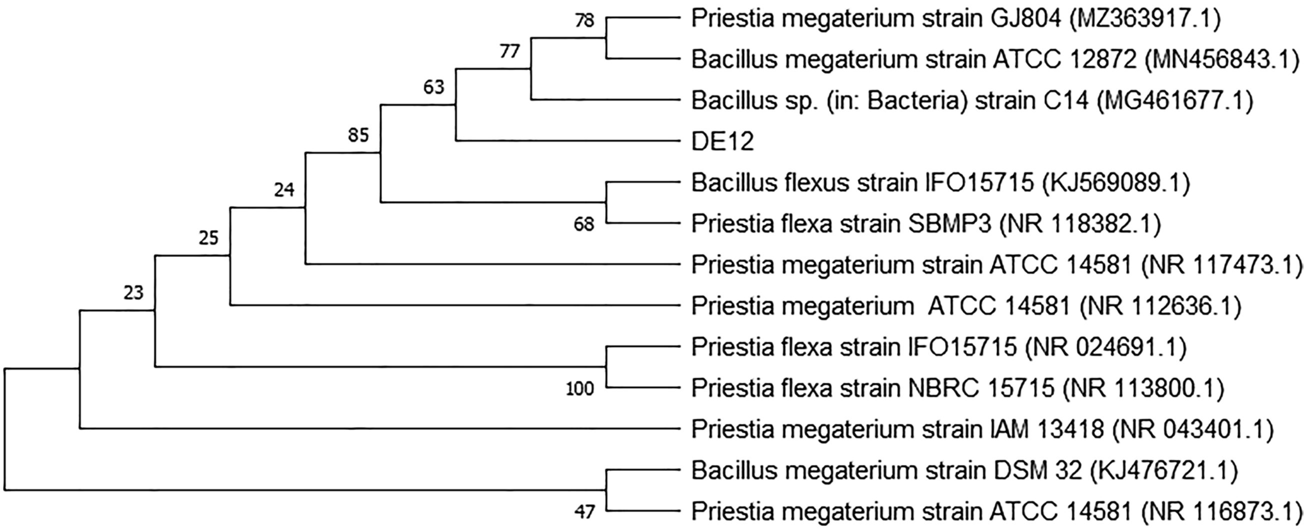

Fig. 4 Phylogenetic tree of strain DE12 constructed based on 16S rDNA sequences

Table 3 Physiological characteristics of strain DE12 and *Bacillus megaterium* in other studies

Reference	Size (µm)	Spore	Gram	Cat	Ara	Lac	Man	Glu	VP	Orn	Lys	Arg
This study (DE12)	1.5 × 2.3	+	+	+	+	+	+	+	−	−	−	−
Sura and Hiremath 2019	≥ 0.9 × (2–4)	+	+	+		+	+	+	−			
Andriani et al. 2017		+	+			+	+	+	−			
Bergey's manual	(1.2–1.5) × (2–5)	+	+	+	+	+	+	+	−	−	−	−

+, positive reaction; −, negative reaction

significant amounts in these parts of the rice plant. Both of these components can reduce the binding between accessible functional groups on rice husks and rice straw surfaces and adsorbate ions/molecules (Chakraborty et al. 2011). According to a previous study, the lignin content in rice husk amounted to 22.5%, which was significantly higher than the lignin content in rice straw (13.5%) (Rosado et al. 2021). Silica was also found to be more abundant in rice husk (93%) than in rice straw (82%) (Damanhuri et al. 2020). Therefore, the presence of high content of lignin and silica can hinder rice husk from becoming a potential adsorbent material.

Biofertilizer formation and plant analysis

Strain DE12 and rice straw were selected as components of the biofertilizer. Biofertilizer is generally recommended to be free from contaminants and should contain a microbial load of approximately 10^7 cells/g carrier to achieve the best support for plant growth (Sethi and Adhikary 2012). For measurements made at 7 days after incubation, the formulation contained 1.5×10^9 CFU/g, which could be applied to the field. This result is also similar to previous studies on biofertilizer preparation containing 13×10^8 CFU/g and 19×10^8 CFU/g of *Pseudomonas moraviensis* and *Bacillus cereus*, respectively (Hassan and Bano 2015). For the BF-CS group, 10% (w/w) biofertilizer was introduced to mixed soil and left in a shade for 2 days before being used for cultivation.

Plant samples were collected after a particular cultivation time. The difference observed between the lengths of plants in the cultivated group was not significant. The study by Egodawatta et al. (2018) has shown a poor relationship between both length and biomass (in root and shoot) of water spinach and exposure to As concentration under 100 mg/kg in naturally contaminated soils. Codling (2014) has also reported an insignificant reduction in lettuce yield when lettuce is cultivated in naturally As-contaminated soil (133–153 mg As/kg).

Overall, As accumulated the most in roots of all three cultivated plant types (Fig. 5). High arsenic and metal concentrations are usually expected in roots because of their direct contact with these elements. Concentrations of toxic elements are generally decreased sharply from roots to shoots and stems. Kumwimba et al. (2013) studied As adsorption in five lettuce cultivars and found that the average As concentration in roots was 12–31 times higher than that in shoots (Kumwimba et al. 2013), which is quite similar to the present study (9–25 times for lettuce). These results indicate the role of roots in restricting the transport of these compounds from soil to aerial parts of these plants.

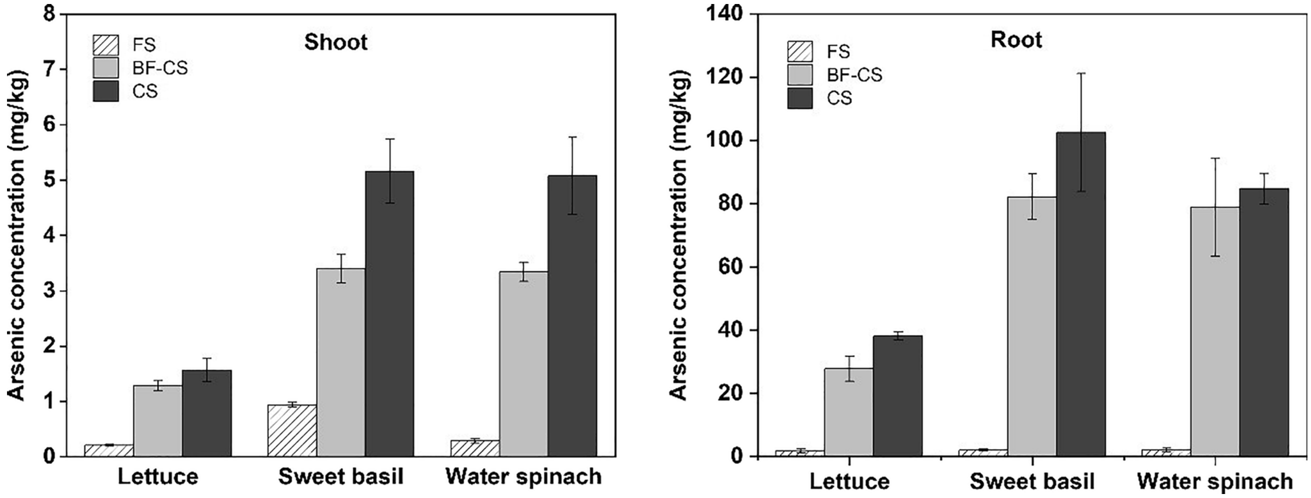

Fig. 5 Total arsenic concentrations in shoots and roots of studied plants between different cultivating treatments (FS: fertile (non-contaminated soil), BF-CS: biofertilizer + contaminated soil, CS: contaminated soil)

In addition, cultivating leafy vegetables in soil with elevated concentrations of potentially toxic elements can result in alarming levels of these compounds in shoot and root samples. Contents of arsenic in the CS group were 6–18 times higher than those in the FS group which was cultivated in non-contaminated soil. In addition, China has released the National Food Safety Standard of Maximum Levels of Contaminants in Foods and set a limit for As at 0.5 mg/kg in vegetables (Clever and Jie 2014). Results showed that concentrations of these elements in all samples cultivated in the CS group (cultivated in contaminated soil) exceeded this standard. Therefore, these vegetables are not recommended for fresh consumption.

Interestingly, As contents in edible parts of sweet basil harvested after 60 days were comparable to those in water spinach, which was cultivated in contaminated soil for 40 days. Amounts of arsenic compounds accumulated in plant biomasses of these two plants were also noticeably higher than those in lettuce. This could be due to the promoted arsenic accumulation under flooding conditions of water spinach. In this study, arsenic contents in shoots and roots of the CS group were 5.08 mg/kg and 84.73 mg/kg, respectively. These levels were quite similar to arsenic content in shoots of water spinach at 5.18 mg/kg dry weight when cultivated in a flooding area with soil containing 72.1 mgAs/kg in a previous study by Liao et al. (2021). The study also reported that arsenic content in water spinach cultured in aerobic conditions with the same soil was 1.57 mg/kg. This result demonstrated that higher As concentration in pore water of flooding soil could increase the accumulation of As in roots and shoots compared to that in aerobic soil.

In this study, arsenic contents in BF-CS were lower than those in CS, meaning that inoculation of biofertilizer could reduce arsenic accumulation in studied plants. However, the efficiency of biofertilizer in alleviating arsenic accumulation was different among cultivating plants. For lettuce, As concentrations in shoots and roots of the BF-CS group (1.29 mg/kg and 27.86 mg/kg) were slightly lower than those of the CS group (1.56 mg/kg and 38.18 mg/kg). Accordingly, the application of biofertilizer appeared to bring little benefit in alleviating As accumulation of lettuce. For sweet basil, inoculation of biofertilizer reduced 34.1% of As uptake in edible parts of sweet basil, from 5.16 mg/kg (CS group) to 3.40 mg/kg (BF-CS group). The presence of biofertilizer in water spinach shared the same efficiency as sweet basil, reducing arsenic content in shoots from 5.07 mg/kg (CS group) to 3.34 mg/kg (BF-CS group). Although the introduction of biofertilizer into cultivating soil did diminish up to one-third of the arsenic level in edible parts of these plants, it was unable to meet the standard for safe consumption. Therefore, screening for more desirable carriers for better bacterial survival support and arsenic adsorption should be carried out to raise the efficiency of biofertilizer.

Conclusion

Strain DE12, which was identified as *Bacillus megaterium*, showed great abilities to resist and accumulate arsenite among strains isolated from arsenic-contaminated soil in South Korea. Results of adsorption capacity determination revealed that rice straw was able to adsorb the highest amount of arsenite than other studied adsorbents. The combination of this carrier and strain DE12 was able to reduce arsenic contents in edible parts of lettuce, water spinach, and sweet basil compared to the control group. Application of this biofertilizer has the potential to produce biofertilizer that can help reduce arsenic accumulation in other crop plants cultivated in paddy soil with elevated arsenic levels. However, further studies are needed to determine interactions among bacteria, carrier, and As species in both submerged and aerobic soil as well as to improve the performance of carrier. Different As species in plants show different toxicity; therefore, the accumulation of different species of As in soil and plants should also be determined to demonstrate uptake metabolism and evaluate their potential toxicity. In addition, investigations in cultivation especially in field trials are required to evaluate the efficiency of the biofertilizer.

Author contribution Material preparation, data collection, and analysis were performed by A.T.P.H. The first draft of the manuscript was written by A.T.P.H. K-W.K. reviewed the manuscript. All authors read and approved the final manuscript.

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Data availability The data and materials used in this research are available from the corresponding author on request.

Declarations

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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