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Biomineralization based remediation of As(III) contaminated soil by *Sporosarcina ginsengisoli*

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ABSTRACT

Arsenic is a highly toxic metalloid and has posed high risk to the environment. As(III) is highly mobile in soil and leached easily into groundwater. The current remediation techniques are not sufficient to immobilize this toxic element. In the present study, an As(III) tolerant bacterium *Sporosarcina ginsengisoli* CR5 was isolated from As contaminated soil of Urumqi, China. We investigated the role of microbial calcite precipitated by this bacterium to remediate soil contaminated with As(III). The bacterium was able to grow at high As(III) concentration of 50 mM. In order to obtain arsenic distribution pattern, five stage soil sequential extraction was carried out. Arsenic mobility was found to significantly decrease in the exchangeable fraction of soil and subsequently the arsenic concentration was markedly increased in carbonated fraction after bioremediation. Microbially induced calcite precipitation (MICP) process in bioremediation was further confirmed by ATR-FTIR and XRD analyses. XRD spectra showed presence of various biomineralization products such as calcite, gwihabaite, aragonite and vaterite in bioremediated soil samples. The results from this study have implications that MICP based bioremediation by *S. ginsengisoli* is a viable, environmental friendly technology for remediation of the arsenic contaminated sites.

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1. Introduction

Arsenic (As) is a highly toxic semi-metallic element and of global concern. The most common and toxic arsenic species observed in the environment is the trivalent form arsenite [As(III)]. Common sources of arsenic in nature include volcanic activity, rock weathering, marine sedimentary rocks and fossil fuels, including coal and petroleum, application of pesticides and wood preservatives [1,2]. The deleterious effects of arsenic to human health resulting from environmental contamination have been reported worldwide [3,4]. Arsenic is a human carcinogen and its concentration at high levels has been found to increase the risk of skin cancer and tumors of the bladder, kidneys, liver and lungs [5].

Elevated levels of arsenic have been reported in soils and groundwater worldwide especially in Bangladesh, India, Vietnam and Argentina [6]. Urban enterprises and development of mining, smelting and processing of heavy metals or metalloid such as arsenic, has caused serious contamination of soils in China [7]. High concentrations of As are frequently found in area around mining tailings in many parts of China such as Guizhou, Chenzhou, Xi'an and Xinjiang ranging from 11 to 1217 mg kg^{-1} As in contaminated soil [8–10]. Especially during the rainy season, drainages of such mining industries often flooded into nearby farmlands and aquatic systems that lead to huge amount of arsenic deposited in the surrounding areas. In 1983, first endemic arsenic area was found in Kuitun reclamation area of Xinjiang, China. Up to now, the population exposed to arsenic has exceeded 2 million in China. Owing to the rapid industrial growth in the past two decades, serious soil contamination has been found in Urumqi, Xinjiang, China. This city was ranked as fourth heavily polluted cities over the world by the Word Health Organization in 1998 [11].

The released arsenic from different sources can be immobilized in tailings or soil and also it can be easily spread into other regions through the transport of arsenic-contaminated solids and arsenic dissolution occurred by changes in the geo-chemical environment to a reductive condition. Therefore, before arsenic reaches a waterbody or groundwater, remediation of arsenic contaminated soils is highly desired. Arsenic is, therefore, a common pollutant of concern in environmental cleanup, and there is an urgent need to find biological based cost-effective and environmentally friendly techniques.

Biological treatment especially phytoremediation utilizing *Pteris vittata* (Chinese brake fern) is widely used as a cost effective method for removal of arsenic from soils [12,13]. Phytoremediation has limitation that it cannot be used successfully in arid area such as north-western China where climate is harsh and dry. It is

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dependant on the growing conditions required by the plant such as climate, geology, altitude and temperature. Beside phytoremediation, a variety of bacteria including sulfate reducing bacteria and other species such as *Paenibacillus*, *Pseudomonas*, *Haemophilus*, *Micrococcus*, and *Bacillus* may be involved to remediate arsenic from contaminated environments [14–16]. The basic principle governing bioremediation is a change in redox reactions, increasing or decreasing the solubility using different complexation reactions, changing pH, and adsorption or uptake of a substance from the environment [17]. Still, the current method of bioremediation is not so effective, as heavy metal ions immobilized or adsorbed are being released again. Arsenic readily changes valence state and reacts to form species with varying toxicity and mobility, its effective treatment is difficult. The redox potential of soil varies greatly that affects the bioremediation process. Due to the sensitivity to redox potential change microbes such as sulfate-reducing bacteria used in immobilization, are not highly effective in the stabilization of heavy or trace metals in contaminated soil. Also, there is still considerable interest in the metabolic capacity of bacteria to remove arsenic within indigenous microbial consortia in various ecosystems. Briefly, the current bioremediation techniques fail mainly because of limitation of phytoremediation in arid area, re-release of immobilized or adsorbed heavy metals by some bacteria in environment, microbial sensitivity to redox potential change and changes into the valence state of particular toxic metal. So, the drawbacks of even current bioremediation technologies have made necessary to seek economic, effective and green technology for *in situ* remediation of contaminated soil.

Biomineralization based microbially induced calcite precipitation (MICP) seems to be a promising technique to remediate arsenic from contaminated environments with additional advantages on current bioremediation techniques [18,19]. This process is active in almost every environment on earth. The microorganisms secrete one or more metabolic products that react with ions or compounds in the environment resulting in the subsequent deposition of mineral particles. Calcite, a biomineralization product, can strongly adsorb on its surfaces and incorporate this metalloid ion into its crystal structure [20]. Considerable research on bio-calcite precipitation has been performed using ureolytic bacteria [21,22]. These bacteria are able to influence the precipitation of calcium carbonate by the production of an enzyme, urease. Calcium carbonate precipitation occurs as a consequence of bacterial metabolic activity. Omnipresence of MICP and the ability of its products to trap toxic elements may provide a new *in situ* remediation method for contaminated soil. Most of the studies to remediate heavy metals such as Sr and Pb by MICP have been performed in groundwater [23]. To our knowledge no study has been performed in past for bioremediation of arsenic contaminated soil based on MICP.

In the present study the ability of a novel indigenous bacterium *Sporosarcina ginsengisoli* has been investigated in the remediation of As(III) contaminated soil. A five-step Tessier sequential extraction procedure to analyze the geochemical speciation of As and investigate the bioremediation efficiency by bacterial isolate was adopted. To measure the efficiency of microbially induced calcite precipitation, the biomineralization products were characterized by Attenuated Total Reflectance Fourier Transform-Infra Red (ATR-FTIR) spectroscopy and X-Ray Diffraction (XRD) analyses. For the first time MICP based remediation of As contaminated soil has been studied applying *S. ginsengisoli*.

2. Materials and methods

2.1. Isolation of As(III) resistant bacteria

Calcifying arsenic resistant bacteria were isolated from soil collected in screw capped sterilized bottles from As contaminated site near Urumqi, China. One gram of soil was inoculated in 50 mL Nutrient Broth (NB) (pH 8.0) containing 100 mg L^{-1} arsenic solution (As_2O_3) and incubated at 30 °C for 48 h under shaking condition (130 rpm). The composition of NB (in g L^{-1}) was peptone, 10; beef extract, 1.5; yeast extract, 1.5; and NaCl, 5. Bacteria were enumerated using serial dilution technique by total plate count method on arsenic containing nutrient agar plates. Arsenic oxide (As_2O_3) was filter sterilized and added to the medium for the initial concentration of 5, 10, 25 and 50 mM. The plates were incubated at 30 °C overnight. Bacterial isolates that could tolerate the highest arsenite concentration were selected. Subsequently, the colonies were transferred onto urea agar base, urease selective medium, to check the production of urease (as urease is key indicator for microorganisms precipitating calcite). One isolate designated as CR5 was finally selected for further studies based on their ability to produce higher urease qualitatively.

2.2. Identification based on molecular characterization

Genomic DNA was extracted from overnight grown bacterial cells by alkaline lysis. The 16S rRNA gene from the genomic DNA was amplified by PCR using the following primers: 5′-AGA GTT TGA TCC TGG CTC AG-3′ and 5′-AAG GAG GTG ATC CAG CCG CA-3′ corresponding to the forward and reverse primers of 16S rDNA, respectively. PCR amplification was performed using a thermocycler as described by Achal and Pan [24]. 16S rRNA amplicon was gel eluted and ligated into the pTZ57R/T vector as per manufacturer's instruction (Fermentas, USA). The sequences were generated by chain termination method using an Applied Biosystem automated sequencer. A BLAST search was performed to find the possible sister groups of the newly sequenced taxa. Sequences retrieved from GenBank were added to the alignments. The sequences were edited with BioEdit 5.0.6 and aligned using MAFFT v 6.240 with other sequences obtained from GenBank.

Bayesian phylogenetic analysis was done in MrBayes v. 3.1.2. Bayesian analyses utilized the Metropolis-coupled Markov Chains Monte Carlo search algorithm as implemented in the program MrBayes v 3.1.2. Two simultaneous independent replicates of five were run for 5 million generations with sampling at every 100th generation, and the convergence of the runs visualized using Tracer ver. 1.4.

2.3. Effect of As(III) on bacterial growth and urease activity

The selected bacterial strain CR5 was inoculated into NB (nutrient broth) media containing 2% urea and 25 mM $CaCl_2$ (hereafter name is used as NBU media) supplemented with 50 mM As_2O_3 and then incubated at 30 °C, 130 rpm for 7 days. The final pH of NBU media was adjusted to 8.0. Growth was determined by recording the *cfu* (colony *f*orming *u*nit) count at regular time interval. Determination of arsenic concentration in culture medium was performed at regular time interval by atomic florescence spectroscopy (AFS) (Jitian AFS-820, Beijing, China).

The urease activity was also determined at regular time interval by measuring the amount of ammonia released from urea according to the phenol-hypochlorite assay method at different time intervals as described in Achal et al. [22]. One unit of urease is defined as the amount of enzyme hydrolyzing 1 μmol urea min^{-1}. The effect of urease activity was shown by extrapolating urease production and arsenic concentration data together.

2.4. Arsenic bioremediation from soil

The soil was collected from nearby farmland, Urumqi, China. The arsenic remediation studies were performed at 30 °C in a beaker containing 100 g of arsenic contaminated soil supplemented with

500 mg kg^{-1} As(III) (As_2O_3) and 200 mL of over night grown *S. ginsengisoli* CR5 (equivalent to 10^7 cfu ml^{-1}) in NBU media. Control remediation studies were also simulated in similar way without addition of bacterial cells. As(III) was mixed thoroughly into the soil in solution form. The experiment was terminated in 10 days and soil samples were analyzed for arsenic concentration.

2.5. Arsenic sequential extraction procedure

The five-stage Tessier sequential extraction method was used for arsenic fractionation in soil samples treated with bacteria and results were compared with control samples. The following arsenic fractionation was obtained: exchangeable, carbonate bound, Fe–Mn oxides bound, organic matter bound and residual fractions [25].

2.6. Arsenic analysis

Arsenic was analyzed from different fractions using the hydride generation atomic florescence spectroscopy (AFS) (Jitian AFS-820, Beijing, China). The AFS utilizes a continuous flow hydride generation system for the detection of arsenic. Briefly, an aliquot of 5 mL sample was transferred into sample bottles, to which 1 mL of ultrapure HCl (37%) and 1 mL of preliminary reductant (10% mV^{-1} KI + 2% mV^{-1} ascorbic acid) was added. The samples were made up to 10 mL volume with Milli-Q water and left for 30 min at room temperature prior to analysis using the Jitian AFS-820 (Beijing, China) spectrometer. The optimized AFS instrumental parameters for As determination were as follows: lamp current (50 mA), negative high voltage of photomultiplier (280 V), carrier argon flow (400 mL min^{-1}), atomizer height (10 mm). The As calibration curve demonstrated good linearity ($r > 0.999$). All the reagents used were analytical grade or higher purity. The results were expressed in mg kg^{-1} dry weight.

2.7. ATR-FTIR spectroscopy

ATR-FTIR experiments were performed for direct monitoring of the effect of bacterial cells used in treatment of arsenic contaminated soil on As(III) reaction to calcite. To confirm calcite products in bioremediation process based on biomineralization, ATR-FTIR spectral measurements were performed using a Bruker Tensor 27 spectrometer equipped with a single reflection diamond ATR crystal with an incidence angle of 45°. Transmission spectra were obtained using KRS-5 windows. For each spectrum, the average of 100 successive scans, over the range of 400–4000 cm^{-1} with a resolution of 2 cm^{-1} was recorded for soils samples treated with *S. ginsengisoli* CR5 and spectra were also compared with control.

2.8. XRD analysis

The bioremediated soil samples treated with *S. ginsengisoli* CR5 were also analyzed by XRD to confirm biomineralization. XRD spectra were obtained using Bruker D8 diffractometer with a Cu anode (40 kV and 30 mA) and scanning from 5 to 80° 2θ. After natural drying, the samples were crushed and grinded using motor pestle before mounting on to a glass fiber filter using a tubular aerosol suspension chamber. The components of the samples were identified by comparing them with standards established by the International Center for Diffraction data.

2.9. Statistical analysis

All the experiments were performed in triplicates. Error bars on graphs show the standard deviation. The data were analyzed by analysis of variance (ANOVA) and the means were compared by Tukey's test ($p < 0.05$) using GraphPad Prism software (version 5.0).

3. Results and discussion

3.1. Isolation and identification of As(III) resistant bacteria

Indigenous bacteria from arsenic contaminated soils were firstly enriched in nutrient broth with increased As_2O_3 concentrations of 5, 10, 25 and 50 mM. Among the different bacteria isolated, one bacterial strain designated as CR5 was selected based on higher urease production and ability to grow luxuriously in media amended with higher concentrations of arsenic. This result suggested that the isolate CR5 may have developed metal resistance systems in an attempt to protect sensitive cellular components. In general, microbial ability to grow at high metal concentration is found coupled with a variety of specific mechanisms of resistance and environmental factors [26].

The nucleotide BLAST and RDP-II analysis showed that the isolate CR5 belongs to the phylum Firmicutes and the family Planococcaceae. Phylogenetic analyses of the 16S rDNA region showed a reasonable degree of correlation with the morphological classification schemes of species within the genus. Seven sequences were included in the dataset, *Sporolactobacillus inulinus* was used as outgroup taxon for rooting purposes. Phylogenetic analysis revealed that CR5 showed 100% similarity with *S. ginsengisoli* (Fig. 1). The 16S rRNA gene sequence for CR5 determined in this study, identified as *S. ginsengisoli*, was deposited in GenBank of NCBI under the accession number HQ331532.

3.2. Effect of As(III) on bacterial growth

Growth comparisons of the bacterial cells grown in arsenite-free media and arsenite-containing media revealed a decrease in growth following arsenite treatment as compared to the cells grown in arsenite-free media. *S. ginsengisoli* CR5 was able to grow luxuriously in NBU media although the growth was relatively slow when grown in the same media amended with 50 mM As(III). This was probably due to the effect of arsenite which retarded bacterial growth [26]. The growth pattern was found to be similar in both

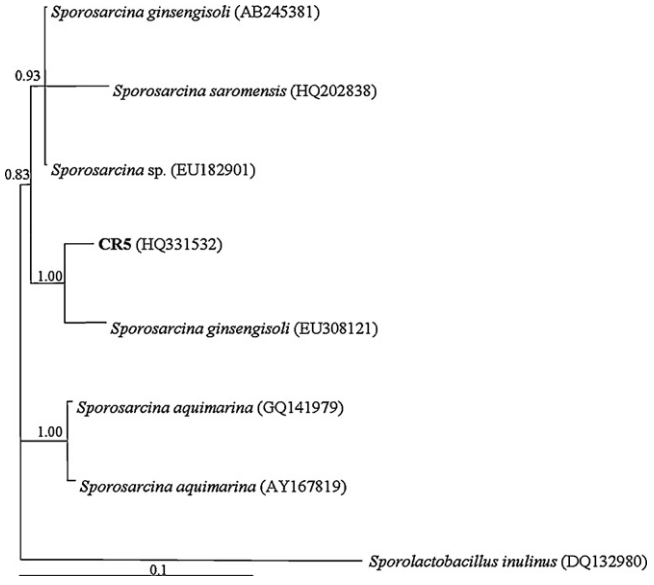

Fig. 1. Phylogeny of CR5 generated from Bayesian analysis of 16S rDNA sequences rooted with *Sporolactobacillus inulinus*. Bayesian posterior probability (PP) values, >50%, are given at the internodes (BS/PP).

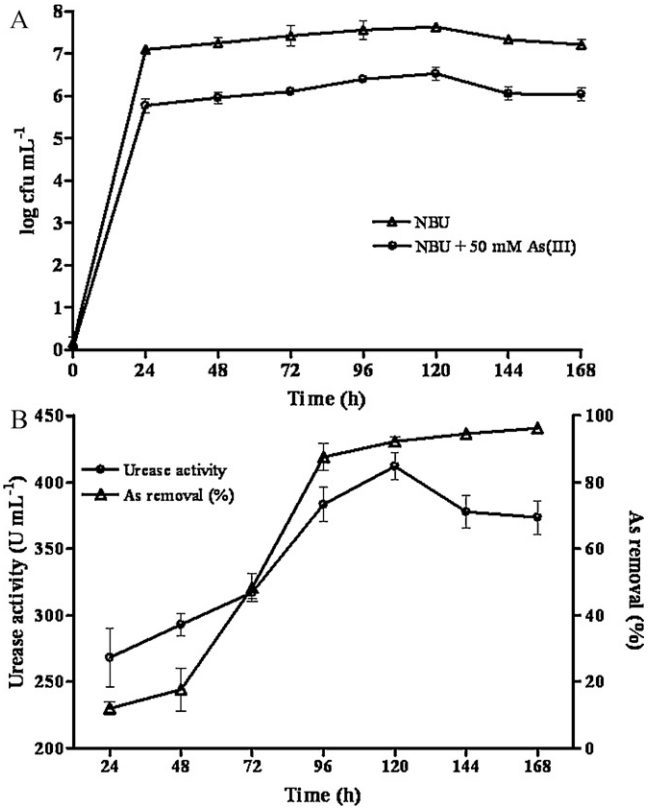

Fig. 2. (a) Time course of growth of isolate CR5 in NBU and NBU media supplemented with 50 mM As(III), and (b) urease productions and As removal by CR5 in NBU media supplemented with 50 mM As(III). Error bars represent the standard deviation.

conditions where long exponential phase was observed (Fig. 2a). The decrease in the bacterial cell growth in the presence of arsenite also may be due to the association of this ion with the membrane fraction, resulting in an expanded membrane, which may increase the number of binding sites and make it less effective at transporting materials needed for growth [27]. Several bacteria belonging to the genera *Acidithiobacillus*, *Bacillus*, *Deinococcus*, *Desulfitobacterium* and *Pseudomonas* have also been reported to be resistant to high concentrations of As(III) and other arsenic species [28–30]. *S. ginsengisoli* CR5 of present study was found to resist higher concentrations of As(III) compared to other reports [31–34].

S. ginsengisoli CR5 produced significant amount of urease in NBU media amended with 50 mM As(III). The urease production profile was recorded for 7 days. Maximum urease activity was measured at 120 h where *S. ginsengisoli* CR5 produced 412 U mL^{-1} urease. After 120 h, urease productions were relatively low and *S. ginsengisoli* CR5 produced 378 and 373 U mL^{-1} urease at 144 and 168 h, respectively (Fig. 2b). The results were supported by previous studies [22,24]. Urease is a key enzyme that leads to calcite precipitation and has been reported to produce significantly in the media containing urea and calcium source [22,35,36]. Ureolytic bacteria such as *Sporosarcina pasteurii* and *Bacillus megaterium* couple calcification to their metabolic assimilation processes to scavenge protons [37]. The presence of ammonium ions and the additional release of CO_2 into the surrounding medium increase the pH that accelerate the rate of the urease induced calcite precipitation.

The ability of *S. ginsengisoli* CR5 to remediate arsenic was first investigated at flask level in NBU media containing 50 mM As(III). The data showed that the isolate of present study was able to remediate 96.3% of arsenic at the end of 7 days (Fig. 2b). *Xanthomonas* sp. B13 has been reported to remediate 96.9% of arsenic in aqueous media containing 40 mM of As(III) [26]. Nagvenkar and Ramaiah reported 92% removal of arsenic by Enterobacteriaceae at the end of 5 days [38].

The results of present study were in the correlation with urease productions, as maximum amount of arsenic was remediated with increased urease productions. Further it may suggest that calcite precipitation by ureolytic bacteria like *S. ginsengisoli* CR5 does sequester dissolved arsenic. Remediation studies by Rouff and Reeder observed significant removal of a Pb(II) from aqueous solution by sorption onto calcite [39]. It confirms the importance of MICP in the process of heavy metal or metalloid bioremediation.

3.3. Arsenic bioremediation studies in soil

The bioremediation efficiency of *S. ginsengisoli* CR5 was also tested in soil supplemented with additional 500 mg kg^{-1} As(III). A growth curve of *S. ginsengisoli* CR5 in bioremediated soil for different time intervals has been shown in Supplementary Fig. 1. The measured concentration of total arsenic can be used as a general index for soil pollution, but it does not provide enough information about the bioavailability, mobility and the exposed risk by this metalloid in contaminated soils. To provide a comprehensive picture of arsenic bioavailability and other potential risks, the arsenic concentration in different soil fractions was determined by sequential extraction. Soil arsenic distributions pattern in five fractions of exchangeable, carbonate bound, Fe–Mn oxides bound, organic matter bound, and residual were obtained. The fractionation results of control contaminated soil other than residual fraction showed the following arsenic distribution: Residual > Exchangeable > Carbonated > Fe–Mn oxides > Organic matter, while in bioremediated soil the distribution was as follow: Residual > Carbonated > Fe–Mn oxides > Organic matter > Exchangeable.

The results indicate that *S. ginsengisoli* CR5 can reduce the exchangeable fraction of As significantly. The arsenic concentration in the exchangeable fraction of bioremediated soil samples was only 0.88 mg kg^{-1}. When bacterial cells were not added into the soil, the exchangeable fraction of soil contained 25.85 mg kg^{-1} arsenic which was significantly higher than bioremediated samples (Fig. 3). The exchangeable fraction is most likely to cause a release into the soil solution due to ion exchange. When exchanged with other cations, it goes into solution. Thus arsenic can be bioavailable and mobile in the soil, so it is very important to reduce its content [40]. Our results demonstrated excellent bioremediation efficiency by *S. ginsengisoli* CR5.

The residual fraction of As did not changed significantly in control and bioremediated soil samples. Arsenic concentration in the residual fraction was 62% in control while 66% in bacterial treated

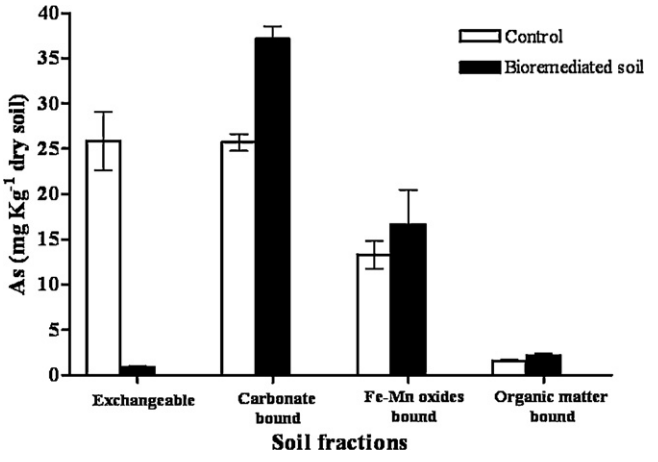

Fig. 3. Arsenic concentration in soil fractions for the control and bioremediated As contaminated soil samples.

soil. The heavy or trace metals in the residual fraction are tightly bound and would not be expected to be released under natural conditions [41,42].

The species of As associated with organic matter was found to be only 1.6 mg kg^{-1} in control soil samples while bioremediated soils samples contained slightly higher content *i.e.*, 2.2 mg kg^{-1} (Fig. 3). Arsenic species associated with organic matter are either complex or adsorbed. Thus they are tightly held and their release into the soil solution is slow. Organic acids such as humic acid and fluvic acid may compete strongly with As(III) for active adsorption sites on mineral surfaces influencing the mobility of As. The competition for active binding sites on mineral surfaces between organic acids and As species may result in lowering the levels of As retention [2].The concentrations of the carbonate fraction of As increased significantly in bioremediated soil samples compared to control (Fig. 3). Bioremediated samples enhanced the As content in carbonate fraction from 14.7% to 22.3%. The carbonate bound fraction results implicate the possibility of calcite precipitation based on ureolytic activity by bacteria. The strong arsenic–calcite complex precipitation leads to obstruction in As release in to the soil. The uptake of arsenic in solid phases can remove this metalloid from solution, thus retarding its transport and decreasing its bio-availability [43–45]. Recently, Román-Ross et al. have demonstrated that it is possible, under laboratory conditions, to synthesize calcite in the presence of high As(III) concentrations [46]. The metalloid is incorporated in calcite by a solid solution mechanism, with CO_3^{2-} ions being replaced by $HAsO_3^{2-}$ ions, offering a more stable trap than superficial adsorption. The possible role of calcite in sequestering As has been considered in a number of studies [46–48]. The consequences of As uptake by calcite formed due to ureolytic bacteria action could be of considerable relevance because of its stability in a variety of geologic environments; therefore, calcite could represent an effective agent for As immobilization. The feasibility of the $CO_3^{2-} \Leftrightarrow AsO_3^{2-}$ replacement mechanism by calcite further support the As bioremediation in soil [20].

3.4. FTIR analysis

FTIR spectra of biomineralization products based on treatment of soils by *S. ginsengisoli* CR5 and control are presented in Fig. 4. The spectra based on the bacterial cells and As binding revealed that the presence of calcium carbonate located at 1550 cm^{-1} as strong intense band which was confirmed by pure calcium carbonate

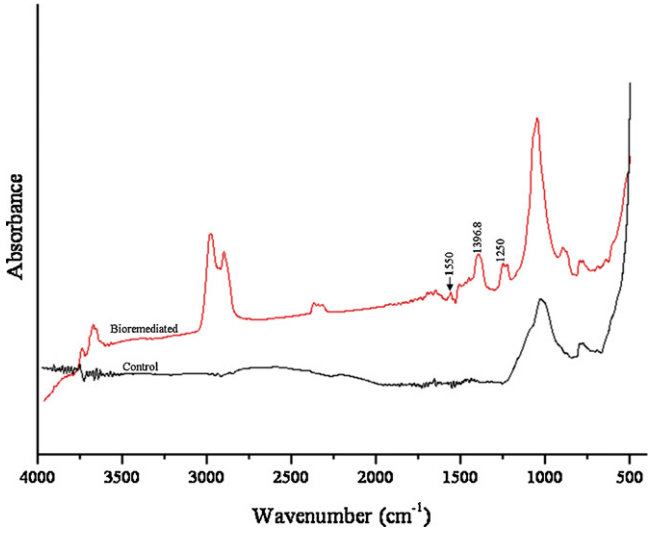

Fig. 4. FTIR analysis for the bioremediated As contaminated soil samples by *S. ginsengisoli* CR5 and comparison with control.

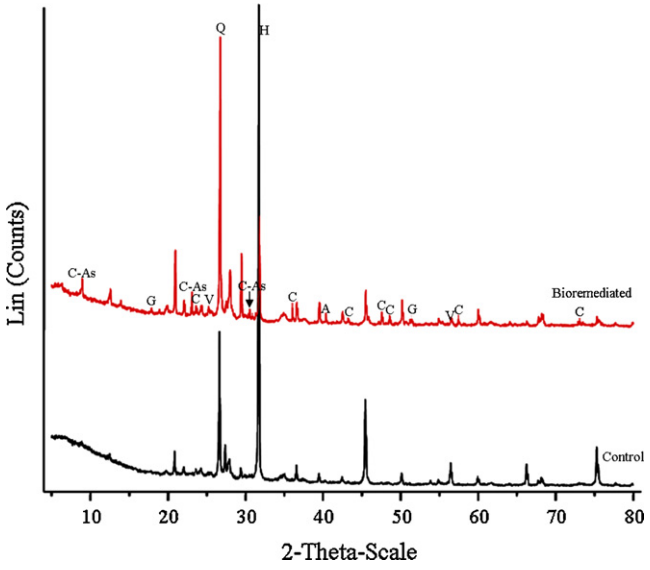

Fig. 5. XRD spectra conforming biomineralization products in soil samples induced by *S. ginsengisoli* CR5 and comparison with control (C, calcite; A, aragonite; C-As, calcite–arsenite precipitate; V, vaterite; G, gwihabaite; Q, quartz; H, halite).

spectra (data not shown). The three peaks located from 1250 to 1550 cm^{-1} can be attributed to the C–O bonding of $CaCO_3$ [49]. The functional groups present on the surfaces of microorganisms act as binding sites for a variety of chemical species, particularly trace metals [50]. These groups also mediate adhesion between microbial cells and mineral surfaces [51]. The microorganisms secrete one or more metabolic products that react with ions or compounds in the environment resulting in the subsequent deposition of mineral particles [52].

3.5. XRD analysis

FTIR analysis was not sufficient enough to confirm the role of calcite products in all As bioremediated soil samples, so detailed XRD patterns were investigated. XRD analysis confirmed the presence of various minerals such as gwihabaite, calcite, vaterite and aragonite crystals in bioremediated As contaminated soil samples (Fig. 5). Quartz and halite dominate the mineralogy in the control soil samples. An important identified mineral in bioremediated soil samples was gwihabaite [$(NH_4,K)NO_3$]. Gwihabaite has been reported previously formed due to bacterial action [53]. During urease activity the released ammonia might form a precipitate as gwihabaite that could be helpful in co-precipitation of As.

XRD analysis showed the majority of carbonate deposits were present as calcite crystals in bioremediated soil samples (Fig. 5). Beside calcite, aragonite and vaterite crystals were also observed in XRD spectra. These are one of the less abundant crystalline polymorphs of calcium carbonate. It was reported that As oxyanions may substitute for the carbonate group in the calcite structure [46,47]. This replacement should be possible in spite of the remarkable size and geometry difference between the carbonate group (C–O distance ~1.3 Å, planar shape) and As oxyanion (As–O ~1.8 Å in arsenite, pyramidal shape), because of the known flexibility of the calcite structure [54].

XRD spectra also showed the presence of As(III)–calcite co-precipitated products in bioremediated soil sample formed due to possible indirect action of urease. During coprecipitation, arsenic species are incorporated sparingly into calcite without discernible change in its tetrahedral geometry or oxidation state [47]. Cheng et al. examined As(III) interaction with the calcite surface, showing that arsenite groups exchange dominantly at surface carbonate

sites [48]. The presence of As(III)–calcite co-precipitated products further confirms the role of calcite as an effective scavenger of a variety of trace elements, and is capable of retaining such trace elements via adsorption (surface) reactions as well as through coprecipitation reactions [55].

Natural calcite has received little attention as a mineralogical trap for arsenic, despite its abundance and widespread distribution on the Earth's surface [48]. Thus, microbially induced calcite is an alternative option to trap arsenic from soil. Calcite has been implicated as playing a possible role in the retention and solubility of arsenic in soils and various other environments in the presence of carbonates.

4. Conclusion

The results of present study clearly demonstrated the versatility of *S. ginsengisoli* CR5 as it was capable of tolerating even very high concentrations of As(III). The bacterial isolate produced significant amount of urease, a calcite precipitating enzyme. Further effectiveness of microbially induced calcite precipitation was demonstrated for successful bioremediation of arsenic. Bacterial treatment significantly reduced the exchangeable arsenic fraction in soil but significantly increased the carbonate bound fraction. FTIR and XRD analyses confirmed the presence of MICP products such as calcite, vaterite, gwihabaite and aragonite and their role in overall bioremediation process. Calcite is known to be more stable at alkaline pH so that it can trap arsenic that cannot be leached again from the carbonate bound complex. Urease hydrolyzing bacteria have additional advantage of accelerating calcite precipitation by increasing pH and alkalinity along with insensitivity of MICP to soil redox potential change. The results determined *S. ginsengisoli* as a good candidate for the bioremediation of As contaminated soil.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.11.067.

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