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SAUDI BIOLOGICAL SOCIETY

Original article

Identification of arsenic oxidizing genes fragment in *Microbacterium* sp. strain 1S1 and its cloning in *E. coli* (DH5*a*)

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ARTICLE INFO

Keywords:
Arsenic
Oxidation
Cluster of genes
Cloning
E. coli

ABSTRACT

Microbacterium sp. strain 1S1, an arsenic-resistant bacterial strain, was isolated with 75 mM MIC against arsenite. Brownish precipitation with silver nitrate appeared, which confirmed its oxidizing ability against arsenite. The bacterial genomic DNA underwent Illumina and Nanopore sequencing, revealing a distinctive cluster of genes spanning 9.6 kb associated with arsenite oxidation. These genes were identified within an isolated bacterial strain. Notably, the smaller subunit (*aioB*) of the arsenite oxidizing gene at the chromosomal DNA locus (Prokka_01508) was pinpointed. This gene, *aioB*, is pivotal in arsenite oxidation, a process crucial for energy metabolism. Upon thorough sequencing analysis, only a singular megaplasmid was detected within the isolated bacterial strain. Strikingly, this megaplasmid did not harbor any genes responsible for arsenic resistance or detoxification. This intriguingly indicates that the bacterial strain relies on the arsenic oxidizing genes present for its efficient arsenic oxidation capability. This is especially true for *Microbacterium* sp. strain 1S1. Subsequently, a segment of genes linked to arsenic resistance was successfully cloned into *E. coli* (DH5a). The fragment of arsenic-resistant genes was cloned in *E. coli* (DH5*a*), further confirmed by the $AgNO_3$ method. This genetically engineered *E. coli* (DH5*a*) can decontaminate arsenic-contaminated sites.

1. Introduction

Metalloid arsenic is prevalent in various natural environments (Garuba, 2023). It is found in every kind of environment, including aquatic and terrestrial, which is due to natural and manmade activities and anthropogenic activities (Saleem et al., 2022). Due to oxidation and reduction, it comes in groundwater from sub-surface environments (Kanel et al., 2023). The wastewater from industry origin comes into the environment, which is already untreated and contains both organic and inorganic lethal compounds, especially ions of heavy metals (Ali et al., 2013), which later on contaminate the background or make it polluted.

Community is receiving a massive benefit from industrial sectors, but on the other end, it releases wastewater, which is laden with toxic compounds or heavy metals. These heavy metals including chromium, cadmium, nickel, zinc, cobalt, mercury, lead, iron, and arsenic are commonly found in industrial wastes (Mishra et al., 2018; Sher and Rehman, 2019). All these heavy metals are toxic to living organisms including human beings, but the nature of arsenic is more poisonous and can cause cancer (Kumar et al., 2023).

When living organisms are exposed to these heavy metal ions, they produce oxidative stress. Hydrogen peroxide, hydroxyl radicals, and superoxide radicals are reactive oxygen species, which are the consequences of metal exposure in living cells (Parida and Patel, 2023). In the list of hazardous substances, arsenic is at the top due to its toxicity (Hughes et al., 2011). Arsenic could be part of organic and inorganic in soil, water, and food (Jobby et al., 2016). There are two forms of arsenic one is oxidized (arsenite) and the other is reduced (arsenate), the latter one is a hundred times less toxic than the first one (Jaafarzadeh et al., 2023; Mujawar et al., 2019). Microorganisms like bacteria are present in every kind of environment, and metal toxicity can be decreased using these microorganisms.

The *Ars* operon comprises three primary genes: *arsR,* responsible for encoding the repressor gene; *arsB*, which encodes an arsenic efflux pump aiding in cellular defense; and *arsC*, encoding the arsenate reductase

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https://doi.org/10.1016/j.sjbs.2023.103846
Received 24 August 2023; Received in revised form 9 October 2023; Accepted 19 October 2023
Available online 20 October 2023

enzyme critical for detoxification. However, the operon's composition can expand to encompass *arsA* and *arsD*. As delineated by (Ahmad et al., 2023), the complete gene sequence is "*arsRDABC*." Notably, *ArsA*, an intracellular ATPase enzyme with 583 units, exhibits distinct A1 and A2 components connected by a linker. This enzyme serves a dual purpose, contributing to the operon and arsenic oxidation through the *aoxA* and *aoxB* genes. The operon houses other significant players, including respiratory arsenate oxidase, reductase, and cytoplasmic arsenate reductase, collectively shaping arsenic-related cellular mechanisms.

The arsenic resistance operon, commonly referred to as *Ars*, constitutes a set of distinct genes with specific functions. Key constituents encompass *arsR* (encoding a repressor gene), *arsB* (responsible for producing an arsenic efflux pump), and *arsC* (which encodes an enzyme facilitating As^{+5} reduction). Notably, the operon might also encompass additional genes like *ArsA* and *ArsD*. The comprehensive genetic sequence of this operon is denoted as (*arsRDABC*) (Ahmad et al., 2023). Particularly, the enzyme *ArsA*, recognized as an ATPase protein, resides within cells and features a composition of 583 units partitioned into A1 and A2 segments, linked via an interconnecting segment. This enzyme fulfills a crucial role in the oxidation of arsenic, intimately associated with the *aoxA* and *aoxB* genes (Silver and Phung, 2005). In bacterial systems, key components encompass respiratory arsenate oxidase, reductase, and cytoplasmic arsenate reductase, each serving distinct roles in orchestrating transformations associated with arsenic.

A range of conventional techniques is employed for mitigating arsenic contamination. These methods consist of phytoremediation (utilizing plants), membrane filtration, coagulation, ion exchange, nanoparticle-based strategies, and chemical interventions involving substances such as alum to purify or detoxify wastewater (Singh et al., 2021). However, due to the emergence of secondary toxic byproducts and the high costs associated with these procedures, their continued application is constrained (Tariq et al., 2019). A more viable recourse is bioremediation, a sustainable and cost-efficient approach that circumvents the generation of secondary toxic substances. In this paradigm, bacteria and other microorganisms exploit arsenic as an energy source within metabolic pathways, effectively mitigating its toxicity (Mazumder et al., 2020).

In this study, the arsenic-resistant bacterium, *Microbacterium* strain 1S1, was isolated and subjected to comprehensive whole genome sequencing using Illumina and nanopore technologies. Surprisingly, no arsenic resistance genes were detected on the megaplasmid following genome analysis. Notably, a 9.6 kb DNA fragment housing the arsenite oxidizing gene *aioB* was pinpointed within the genomic DNA and subsequently cloned into an *E. coli* plasmid. Confirmation of the *E. coli* (DH5a) strain's arsenite oxidizing capability was achieved through the formation of distinctive brown precipitation upon interaction with $AgNO_3$. This innovative genetically engineered *E. coli* strain can be used to ameliorate the wastewater laden with toxic metal ions.

2. Materials and methods

2.1. Isolation of bacteria and its MIC against arsenite

The bacterium was sourced from a wastewater sample in Pakistan and meticulously purified using successive streaking techniques on LB-agar plates that were pre-supplemented with arsenite. It was denoted as "1S1″ for reference. Following this, the minimum inhibitory concentration (MIC) of the isolated bacterial strain against arsenite was determined (Kumar et al., 2021). Furthermore, the bacterium's proficiency in arsenic oxidation was confirmed using the $AgNO_3$ method (Krumova et al., 2008).

2.2. Isolation/cleaning of genomic DNA

The genomic DNA of arsenic-resistant bacteria was obtained by using the Genomic Mini AX Bacteria kit, following the methodology detailed by Trojańska et al. (2022). Enabling favorable bacterial growth, ideal conditions were created by culturing the cells in LB broth overnight. The resultant DNA pellet was then suspended in an appropriate volume of Tris buffer to facilitate subsequent experimental steps. For genomic DNA purification, the DNA clean and concentrator TM −5 kit from Zymo Research was utilized, meticulously following the guidelines provided by the manufacturer.

2.3. Agarose gel electrophoresis

To prepare a 0.8 % agarose gel, a TBE buffer with a pH of 8.0 was employed. This buffer composition consisted of 5 % tris base, 74 % H_3BO_3, and 0.5 M EDTA. The gel was subjected to visualization using a UV transilluminator, (Mode MD 20, Waltec Corp USA) and the resulting image was captured using the Gel Doc system.

2.4. Qualification/quantification of DNA

Nano-drop 2000c spectrophotometer was used to check DNA purity. Then, 1 µl of DNA sample was placed on the detection surface that held the sample because of surface tension. The ratio of 260/280 was measured for the samples to check DNA purity and the ratio of 260/230 was measured for the samples to check protein, phenol, and EDTA contamination. DNA of the sample was quantified with Qubit. Two standards were used. Standard 1 was used with zero concentration of DNA and standard 2 was used with maximum concentration of DNA. A working solution (Qubit™ 1 X ds DNA HS) was used for standards and DNA samples. 10 µl for each standard was mixed in a PCR tube already containing 190 µl working solution, mixed a bit, and placed in the cuvette of Qubit for measurement. Then working solution (198 µl) was mixed in a 2 µl sample of DNA and again measured the value against the standards.

2.5. DNA library preparation for whole genome sequencing

The isolated DNA quantification was done by using the Qubit method and then genomic libraries were generated utilizing a customized Nextera XT protocol. This was accomplished by employing the Nextera XT DNA library prep kit, strictly adhering to the manufacturer's provided guidelines (Stawski, 2013).

2.6. PCR clean-up DNA libraries

DNA libraries with minimal size were effectively eliminated through the application of AMPure XP beads, following established protocols (Rodrigue et al., 2010).

2.7. Sequencing through Illumina (NextSeq system)

Following the preparation of genomic DNA libraries, denaturation was performed, and the libraries were subsequently diluted in accordance with the standardized normalization protocol. A freshly prepared solution of NaOH (0.2 N) was meticulously prepared in distilled water, within an Eppendorf tube. With both the genomic and PhiX libraries denatured and diluted, these were methodically loaded onto the reagent cartridge. The sequencing run was then initiated with precision, meticulously following the instructions detailed in the NextSeq 500 system guide.

2.8. Genomic sequencing using oxford nanopore technologies

Nuclease-free water served as the medium for DNA preparation, involving 400 ng of DNA in 7.5 µl of water (Laszlo et al., 2014). The integrity of the flow cell was verified by connecting it to the host computer, confirming its readiness with the soft glow of light and the gentle whir of a functioning fan. The library assembly commenced with

thawing all kit components at room temperature, followed by the careful combination of 7.5 µl (DNA template; 400 ng) and 2.5 µl (fragmentation Mix RB01-02) within a 0.2 ml thin PCR tube. The resulting sample volume was meticulously added to the SpotON sample port drop by drop. Eventually, the flow cell was linked to the computer, and the process was initiated by double-clicking on the MinKION icon on the desktop. The progression of MinKNOW was consistently monitored, while the base-called data from MinKION underwent comprehensive analysis.

2.9. Genome sequencing and assembly

In the field of microbial genome sequencing, a variety of methodologies exist for assembly and processing of raw sequence data. For research teams engaged in the genomic analysis of microbial isolates, the adoption of consistent treatment and assembly approaches for their sequence data proves advantageous, if not imperative. This approach facilitates the attainment of exceptionally refined assembled genomes and ensures comparability across projects. The conventional genome assembly process comprises an initial step of quality filtering, followed by the elimination of sequencing artifacts like contamination from sequencing adapters and primer dimers, merging of overlapping paired-end reads, addressing the alignment of corresponding sequences, correction of sequencing errors, and the final assembly of reads into contiguous sequences called contigs.

2.10. Sequencing analysis and submission to GenBank

The genes for arsenic resistance and 16S rRNA for phylogenetic analysis were obtained after the sequencing technology such as sequences obtained from Illumina or Nanopore. NCBI site was used for the blast of isolated bacterium sequence with the sequence that was already available on NCBI as a reference sequence. In the end accession number was obtained after the submission to GenBank.

2.11. Isolation of plasmid from E. coli

For the isolation of plasmid DNA with varying copy numbers, the Plasmid Midi AX kit was employed. *E. coli* cells were cultivated in 100 ml of LB broth overnight at 37 °C and 100 rpm in a shaker. Subsequently, 50 ml of the bacterial culture underwent centrifugation at 6000 rpm for 6 min, and the resulting pellet was re-suspended in 5 ml of L1 solution. This mixture was further combined with 5 ml of L2 solution, gently inverting the tube around 3–4 times, and then left at room temperature for 5 min. Following this, 5 ml of L3 solution was introduced and the tube was gently inverted once more. The resulting mixture was loaded onto a filtration tube and subjected to centrifugation at 1500 rpm for 5 min. The filtrate was carefully discarded, and the clear lysate flow was directed onto a plasmid 200 column situated within a 50 ml tube, allowing it to flow via gravity.

To cleanse the column, a wash with 20 ml of K2 washing solution was performed, and once the K2 solution had passed through, the column was transferred to a 15 ml precipitation tube. The plasmid DNA was subsequently eluted by applying K3 elution solution. A mixture of 25 µl of precipitation enhancer and 5 ml of isopropanol was then combined with the eluted plasmid, followed by centrifugation at 11,000*g* for 10 min. The supernatant was carefully removed, revealing a light blue pellet of plasmid DNA. This pellet was washed with 2.5 ml of 70 % ethanol, then centrifuged again at 11,000*g* for 3 min. After discarding the supernatant, the tube containing the plasmid pellet was inverted and left at room temperature for 5–10 min. Finally, the plasmid DNA was dissolved in the desired volume of TE buffer.

2.12. Cloning of aioB gene fragment into vector

The *aioB* gene fragment was incorporated into an *E. coli* vector using a partial digestion method, following the procedure elucidated by (Bolivar and Backman, 1979). This multi-step process encompassed five crucial stages: (1) initiation of a partial Sau3A Digest, (2) Electrophoresis, (3) vector digestion and DE phosphorylation, (4) Ligation, and (5) Electroporation. The first step, the partial Sau3A digest, started with 200 µl of DNA, with a concentration of over 0.1 µg/µl. In 200 µl DNA, 20 µl 10 X Sau3A buffer and 2 µl BSA at 10 mg/ml were added. Using ten Eppendorf vials labeled from 1 to 10, a master mix was prepared by adding 40 µl to the first vial and 20 µl to each subsequent vial (2–10). In the first vial, 1 µl of Sau3A enzyme (10 units/µl) was mixed, and then 20 µl from the first vial was transferred to the next, continuing sequentially up to the ninth vial. The tenth vial was maintained as a non-digest control. All vials were incubated at 37 °C for an hour and then heat-inactivated at 65 °C for 20 min. Following this, electrophoresis was carried out on a 0.8 % gel with at least 12 lanes. The first lane contained a marker, while the content of tubes 3–12 was loaded into wells 3 to 12, with the second lane being skipped. After gel electrophoresis, ethidium bromide staining was performed, and the *aioB* gene fragment was observed in lane 8 of the gel. Approximately 5–10 µg of the cloning vector was subjected to digestion with the *BamHI* enzyme in a 20 µl reaction, adhering to the standard protocol. Subsequently, the ligation of the *aioB* gene fragment into the cloning vector was carried out in a 10 µl reaction mixture. This mixture contained 2 µl of 10X ligation buffer, 1 µl of T4 DNA ligase (200 units/µl), 3 µl of partially digested DNA, and 3 µl of the cut vector. The reaction mixture was then incubated at 16 °C overnight. Phenol extraction was conducted once, followed by two rounds of butanol extraction. The extracted DNA was precipitated using ethanol. The resulting DNA was re-dissolved in 10 µl of water. The recombinant DNA was introduced into *E. coli* (DH5a) via electroporation. The transformed *E. coli* cells were then plated on agar plates already supplemented with arsenite.

3. Results

3.1. MIC and arsenic oxidizing ability

The isolated bacterial strain showed 75 mM MIC against arsenite and upon arsenite oxidizing it gave brownish color precipitation with interaction of silver nitrate. The bacterial colonies and their screening for arsenite oxidizing ability are shown in Figs. S1 and S2.

3.2. Genomic DNA and its libraries

Following the isolation of DNA from an overnight bacterial culture, the resultant DNA sample was subjected to visualization on a 0.8 % gel under ultraviolet light, as illustrated in Fig. 1. Notably, the bacterial isolate yielded high molecular weight DNA. Running in parallel with the genomic DNA, a 100 bp marker was included. Specifically, the marker exhibited distinct bands, with the first at 2 kb, the second at 1.5 kb, the third at 1.2 kb, and the lowermost band at 100 bp. The Nextera libraries encompassed a range spanning from 0.4 kb to 1 kb. Fig. 1 further highlights the genomic DNA alongside its respective libraries.

3.3. Bacterial characterization

The 16S rRNA sequence derived from the whole genome sequence was subjected to a BLAST search on NCBI, leading to the identification of the bacterial strain as 1S1 *Microbacterium* sp. Subsequently, the comprehensive genome sequence was officially submitted to GenBank, bearing the accession number (CP043430), as well as the Bio-project number (PRJNA562413). Additionally, this complete genome sequence was deposited in the First Culture Bank of Pakistan (FCBP), assigned the accession number (FCBP-B-731), within the Faculty of Agricultural Sciences at the University of the Punjab, Lahore, Pakistan (Fig. 2).

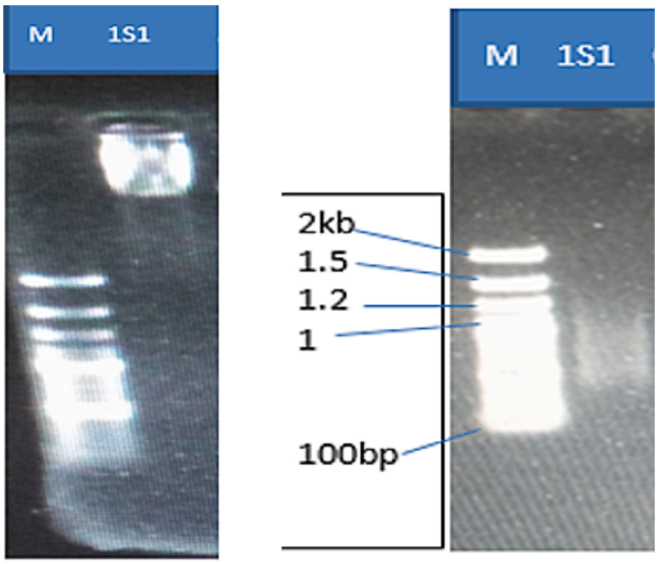


Fig. 1. Agarose gel (0.8 %) showing genomic DNA (left gel) and its Nextera genomic libraries along with marker (M) of *Microbacterium* sp. strain 1S1 (right gel).

3.4. Genetic determinants regarding arsenic oxidation

The arsenic-oxidizing *Microbacterium* sp. strain 1S1 exhibited a distinctive gene cluster spanning 9.6 kb, which is closely associated with arsenite oxidation. The individual gene fragments implicated in the process of arsenic oxidation are meticulously depicted in Fig. 3. Furthermore, comprehensive details concerning the genes, including their respective positions, encoded products, functional roles, and the accession numbers of the most closely related sequences, have been comprehensively documented in Table S1.

3.5. Mega plasmid

After the whole genome sequencing the megaplasmid was found in the isolated bacterial strain as shown in Fig. 4. No one gene on mega-plasmid is related to arsenic reduction or oxidation mechanism.

3.6. Cloning of arsenite oxidizing fragment

The pUC19 vector was used for *aioB* cloning. Cleavage of the pUC19 vector was carried out utilizing the *BamH1* restriction enzyme, which led to the creation of sticky ends akin to those generated by Sau3a. The cleaved plasmid resulting from the *BamH1* digestion is visually illustrated in Fig. 5a. Following the successful cloning of the *aioB* gene fragment through cloning, *E. coli* cultures were grown on specialized LB agar plates supplemented with sodium arsenite, providing a selective environment for growth. The subsequent observation revealed the emergence of distinct *E. coli* colonies on the selective media. In order to serve as a control, LB plates containing ampicillin were utilized, as pUC19 harbors an ampicillin resistance gene regulated by a potent promoter.

The fragment responsible for arsenite oxidation, measuring 9.6 kb in size, was cloned into the pUC19 vector and subsequently introduced into *E. coli* through electroporation. Subsequently, genomic DNA sourced from *Microbacterium* sp. strain 1S1, encompassing the 9.6 kb fragment, was subjected to digestion using the Sau3a restriction enzyme (Fig. 5b). Notably, the initial five wells of the gel displayed heavily degraded gDNA, attributed to the elevated concentration of the restriction enzyme. From the ensemble of fragments, the DNA originating from the eighth well was selected for the cloning of the gene of interest, as it held the pivotal 9.6 kb fragment.

3.7. Confirmation of arsenite oxidizing fragment in E. coli

E. coli gave a brownish precipitate while interacting with $AgNO_3$ after 48 h of incubation on LB-agar plates already supplemented with 10 mM arsenite stress.

4. Discussion

Due to the anthropogenic activities of humans which include agricultural practices and industrialization are the major stakeholders for increasing arsenic and other heavy metals conc in our environment

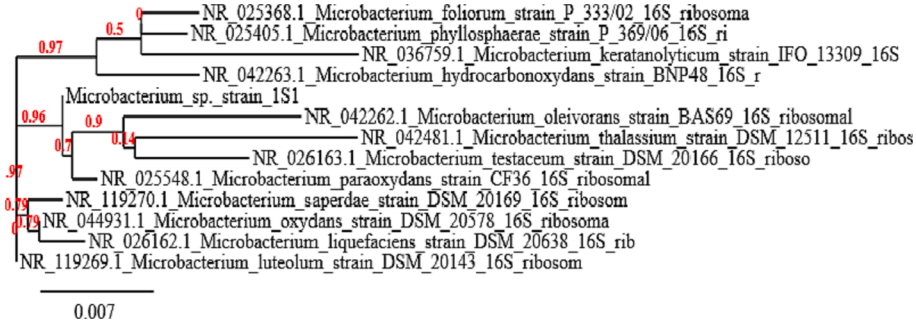


Fig. 2. Phylogenetic analysis of isolated *Microbacterium* sp. strain 1S1.

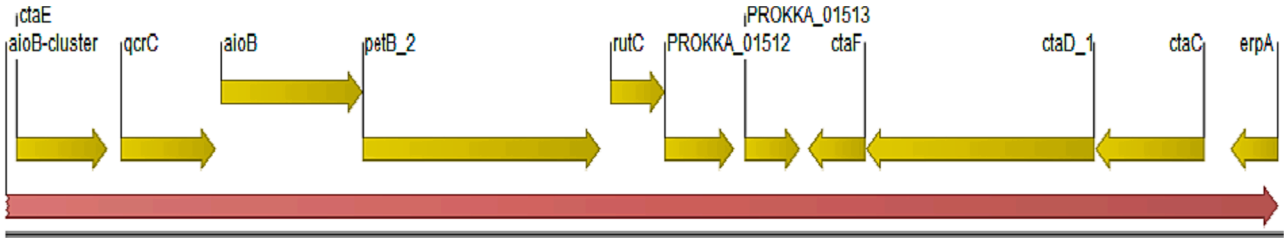


Fig. 3. Arsenite oxidizing genes cluster found in Microbacterium sp. strain 1S1 (Sher et al., 2023).

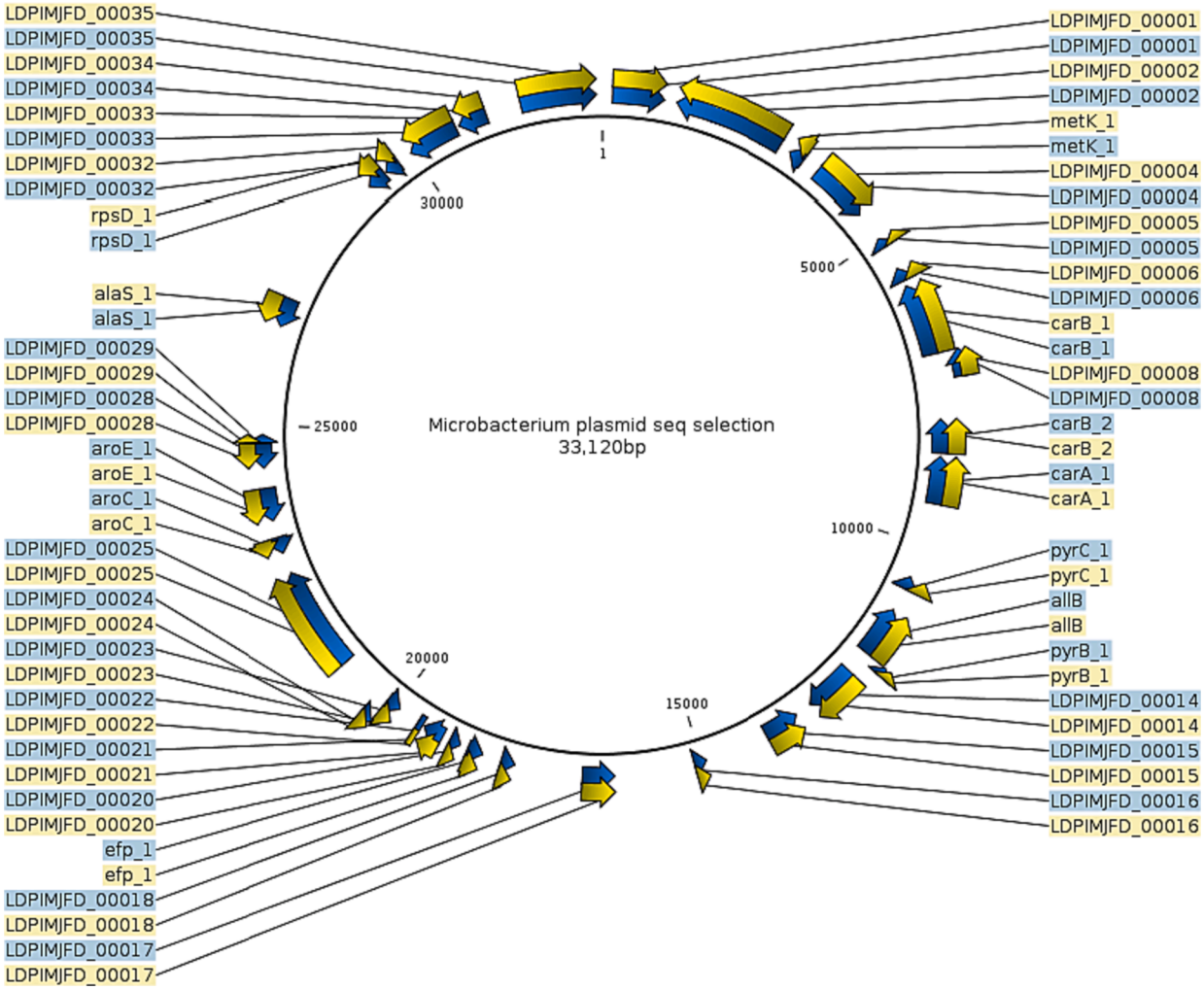


Fig. 4. Presence of mega plasmid in *Microbacterium* sp. strain 1S1 indicating no gene regarding arsenic resistance.

(Kumari et al., 2018). The waste of industrial origin which is being released into our surroundings cannot be ignored when it is not treated before release (Wu, 2019). Various kinds of metals such as chromium, cadmium, nickel, arsenic, and cobalt are a portion of industrial wastewater (Singh and Kumar, 2023). Besides the contamination of surroundings, these are affecting human beings at the same time. However, arsenic is at the top in toxicity and carcinogenicity because of its ubiquitous nature and severe health effects on the human body (Sher et al., 2020).

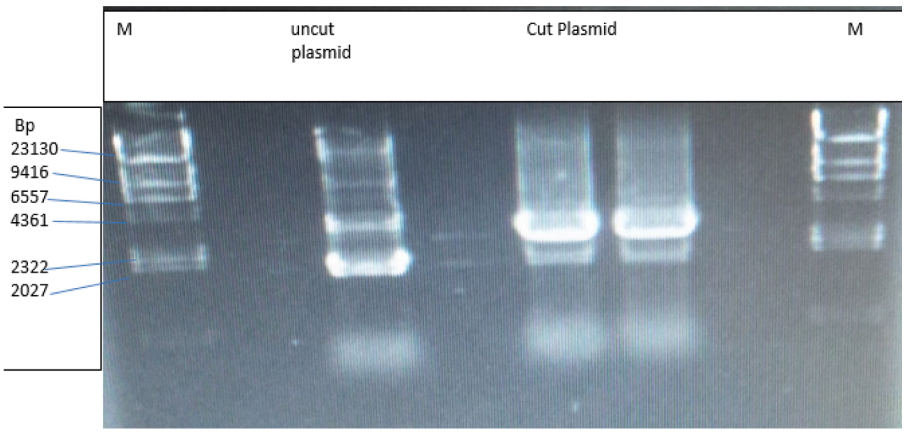
In the current investigation, an arsenic-resistant *Microbacterium* sp. strain 1S1 was successfully screened from industrial wastewater within the Pakistani locale. Notably, this strain exhibited a robust resistance of 75 mM against arsenite. Previous research has documented distinct levels of arsenic resistance; for instance, the AS2 strain of *M. luteus* demonstrated resistances of 50 mM and 275 mM against arsenite and arsenate, respectively (Sher et al., 2019). Likewise, research has highlighted the arsenite resistance of 21 mM in *Klebsiella pneumoniae* (Mujawar et al., 2019).

The isolated strain notably displayed a distinctive brownish precipitate upon interaction with $AgNO_3$, a phenomenon consistent with findings observed by Naureen and Rehman (2019). Ultimately, it's crucial to acknowledge that all arsenite-oxidizing bacteria engage in the conversion of As^{3+} into As^{5+}. The subsequent interaction between arsenate ions and $AgNO_3$ resulted in a precipitate consistently exhibiting a distinct brownish hue (Lett et al., 2001).

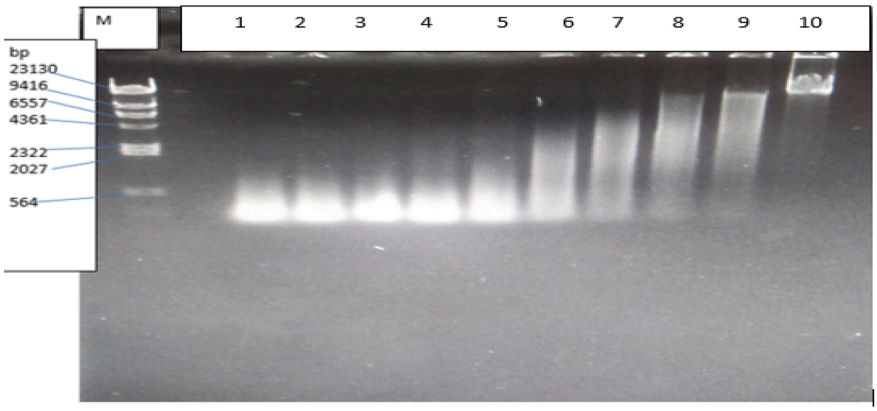
It is determined that all the genes associated with arsenic were found on the chromosomal portion of the bacterium. The minor subunit of the arsenite oxidase gene (*aioB*) is responsible for the protein (Riseke). Which is basically iron and sulfur protein and takes part in the uptakes of electrons. The other portion of the gene section is associated with the molybdopterin biosynthesis protein and the gene name is *MoeB.* cytochrome *c* subunits are also present on the fragments of genes.

The arrangement of genes associated with arsenic oxidation, including *aioB*, *MoeB*, and specific cytochrome *c* genes, follows distinctive patterns (Fig. 3). Sher et al. (2023) reported a similar arrangement of genes, related to arsenic oxidation, in *Brevibacterium* sp. strain CS2. This gene organization underscores the adaptive gene expression observed in challenging or adverse environmental conditions. Notably, the role of cytochrome *c* within the aox system of the bacterium *Ochrobacterium tritici* SC1124 holds significant importance in facilitating arsenic oxidation, as detailed by Branco et al. (2009). Within *Ochrobacterium tritici* SC1124, the orderly arrangement of genes encompasses *aoxAB*, cytC encoding cytochrome *c*, and *MoeA* encoding a molybdenum-like cofactor, as illustrated in Fig. S3. The arsenivorans strain of *Thiomonas delicata* is able to be employed arsenite as a resource of energy because of the existence of aioAB gene responsible for arsenite oxidizing (Michel et al., 2020). Similarly, the AS1 strain of *Bosea sp.* showcases proficiency in arsenic oxidation, attributed to the presence of genes crucial to this intricate process.

Various arsenic resistance genes, such as *arsR*, *arsC*, *ACR3*, and *arsB*, have been identified in all isolated arsenic-resistant bacteria through the application of genome sequencing technology. The presence of the acr3



(a)



(b)

Fig. 5. (a) The pUC19 vector uncut and cut with BamH1, (b) Genomic DNA of *Microbacterium* sp. strain 1S1 subjected to digestion using the sau3a enzyme.

gene within the isolated *Microbacterium* strain 1S1 implies its capacity to accumulate intracellular arsenite, facilitated by the ACR3 protein's role in arsenite transportation. In the genome of strain 1S1, genes associated with arsenic resistance, including *aioB*, *arsR*, and *arsC1*, were detected, alongside genes responsible for resistance to other heavy metals (Lu et al., 2018).

Analysis of the draft genome and gene annotation in *Bacillus strain* PVR-YHB1-1 revealed the presence of two *ars* operons: *arsRacr3arsCDA* and *arsRKacr3arsC* (Jia et al., 2019). Noteworthy arsenic-resistant bacteria, including *Achromobacter, Agrobacterium*, and *Pseudomonas*, showcase genes intricately linked to arsenic resistance, such as *aoxB*, *acr3*, and *arsB* (Cai et al., 2009). Impressively, the chromosomal DNA of all isolated bacterial strains houses genes associated with arsenic resistance, whereas the plasmid within *Microbacterium* sp. 1S1 lacks metal resistance genes, as visualized in Fig. 4. It's notable that the arsenic resistance-related genes within *T. ferrooxidans* are located on the chromosomal DNA (Butcher et al.,2000).

The successful cloning of the 9.6 kb fragment comprised of the arsenite oxidizing gene was effectively achieved in *E. coli* through a partial digestion process using the sau3A enzyme. Subsequent to the *aioB* gene fragment's successful integration, discernible *E. coli* colonies were observed on specialized LB agar plates supplemented with sodium arsenite. As a control, *E. coli* colonies resistant to ampicillin were also observed on LB plates, owing to the presence of the ampicillin resistance gene in pUC19 under a strong promoter. The 9.6 kb arsenite oxidizing fragment was cloned into the pUC19 vector and introduced into *E. coli* through the process of electroporation. Notably, in a separate study, the *ars* gene (spanning 7.4 kb) responsible for conferring arsenic resistance in *T. ferrooxidans* was similarly cloned into *E. coli* utilizing a sau3A partial digestion method, followed by colony selection on LB agar plates supplemented with 0.5 mM arsenite stress (Butcher et al., 2000).

Metals, including arsenic, exert toxic effects on the environment. Unlike organic pollutants, heavy metals do not degrade naturally, posing unique challenges for remediation. Microorganisms are omnipresent in arsenic geochemical surroundings, exerting a profound influence on the biochemical progression of arsenic. Through their activities, arsenic undergoes transformations into diverse forms characterized by unique solubility, mobility, bioavailability, and toxicity attributes. The presence of hazardous compounds in industrial waste contributes to environmental pollution. Bioremediation, which employs microorganisms to cleanse the environment, offers a solution to these issues.

5. Conclusion

A severe metal-resistant *Microbacterium* sp. strain 1S1, isolated from industrial wastewater, was capable of resisting arsenite 75 mM. The appearance of brownish precipitation with $AgNO^3$ interaction confirmed its ability to oxidize arsenite which was later confirmed by the determination of genes regarding arsenic oxidation after Nectseq and nanopore sequencing technology. A 9.6 kb gene cluster, related to

As^{3+} oxidation was found on chromosomal DNA while plasmid DNA showed no resistance genes. The fragments of arsenic-associated genes were cloned in *E. coli* which was further confirmed by the *E. coli* growth appearance on LB plates already supplemented with arsenite stress. The arsenic oxidizing genes occurrence and the arsenic processing potential of the bacterium make it a possible nominee for arsenic cleansing from arsenic-contaminated wastewater. However, the most important and interesting experiment regarding the cloning of gene fragments in *E. coli* made it resistant to arsenite so the genetically engineered *E. coli* can be used for the decontamination of arsenite from the arsenic-contaminated site.

Funding

No funding was obtained for the current work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103846.

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